Anti-dsDNA Testing Specificity for Systemic Lupus Erythematosus: A Systematic Review

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Background: Autoantibody specificity in autoimmune diseases is variable due to each patient's individual spectrum of autoantibodies and the inherent differences between detection methods and tests. Since false-positive results have downstream consequences, we conducted a comprehensive assessment of anti-double stranded DNA (anti-dsDNA) specificity from published studies of systemic lupus erythematosus (SLE).

Methods: A systematic review (MEDLINE, Embase, Cochrane Central Register of Controlled Trials, and Database of Abstracts of Reviews of Effects) identified cross-sectional or case-control studies published January 2004 to August 2019, reporting anti-dsDNA test accuracy data in SLE. Study quality was assessed using Quality Assessment Tool for Diagnostic Accuracy Studies, version 2. A meta-analysis was conducted to estimate specificity by test method or named test where feasible.

Results: Thirty studies were included covering 43 different tests. The Crithidia luciliae indirect immunofluorescence test (CLIFT) and fluorescence enzyme immunoassay methods are likely to be $\geq$ 90% specific (Euroimmun 97.8% (95% CI 96.2%–98.7%) 4 studies; EliA 94.7% (95% CI 91.7%–96.7%), 6 studies; CLIFT 98.7% (95% CI 96.7%–99.5%), 8 studies/7 tests]. For other test methods, specificity was not fully demonstrated to be $\geq$ 90% and/or the control group included healthy patients possibly overestimating specificity. More studies are required for NOVA Lite [96.0% (95% CI 87.2%–98.9%), 5 studies], chemiluminescence immunoassays [92.3% (95% CI 83.6%–96.6%), 6 studies/4 tests], multiplex immunoassays [89.3% (95% CI 86.1%–91.8%), 4 studies/2 tests], and Farr fluorescent immunoassays (no estimate, 2 studies). Specificity data reported for Farr radioimmunoassays [93.8% (95% CI 85.4–97.5%), 11 studies, 9 tests] and enzyme-linked immunosorbent assays [93.4% (95% CI 89.9%–95.7%), 15 studies/16 tests] lacked consistency.

Conclusion: Anti-dsDNA testing shows considerable variation in test specificity, with potential impact on the management of SLE patients. This review may help laboratory specialists and clinicians choose and interpret the appropriate anti-dsDNA test for their setting.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous disease (1). With positivity for antinuclear antibodies (ANA) as a major entry criterion, the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) SLE classification criteria stipulate higher specificity compared

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IMPACT STATEMENT

Specific anti-double stranded DNA (anti-dsDNA) testing is crucial for accurate classification and diagnosis of systemic lupus erythematosus, a potentially life-threatening systemic autoimmune disease and might help in disease activity assessment. Currently, different anti-dsDNA test options are available with predicted quality differences in test performance, affecting test specificity. Our comprehensive assessment of available anti-dsDNA tests, based on the systematic review of relevant accuracy studies, indeed confirms markedly variable test performance, potentially resulting in > 10% false-positive rates. The overview presented can guide laboratory specialists in the optimization of anti-dsDNA test strategy, improving patient management and disease outcomes.

to the previous Systemic Lupus International Collaborating Clinics (SLICC) criteria developed in 2012 (2, 3). Although classification criteria are primarily intended for patient selection in clinical trials (4), ANA testing is also a routine tool in the general diagnostic workup for SLE due to its high sensitivity (2, 5). As double-stranded DNA (dsDNA) antibodies are among the most commonly detected ANAs in SLE, reported to correlate also with disease activity and especially renal involvement (6–9), a positive ANA test is routinely followed up with testing for these antibodies (2, 10).

Consequently, anti-dsDNA testing is an essential part of the diagnosis, classification, and management of SLE (2, 3, 11–13) and highly avid anti-dsDNA antibodies of the IgG class are commonly considered more clinically relevant and specific for SLE compared to antibodies of other immunoglobulin classes or of lower affinity/avidity (6, 14, 15). However, testing is complicated by the expression of anti-dsDNA since it comprises a (polyclonal) mixture of antibodies. Subpopulations of this mixture may have different fine specificities [e.g., anti-dsDNA only (double helix) (16), antisingle-stranded DNA and dsDNA combined (backbone) (16), antisingle-stranded DNA only (bases) (17–19)], and these fine specificities may differ in their associations with disease or clinical symptoms (6, 16, 20–22).

Many test methods are available and anti-dsDNA tests, by design, detect different subpopulations of anti-dsDNA with distinct fine specificities (16, 20, 22–24). One of the original methods is the Farr radioimmunoassay (Farr-RIA), first described in 1969 and sometimes still referred to as the gold standard (25). The technique relies on immunoprecipitation with radiolabeled dsDNA under high salt conditions to select for highly avid anti-dsDNA antibodies (26). To circumvent safety issues related to handling of radioactive reagents, Farr fluorescent immunoassays (Farr-FIA) use fluorescence as a readout (27). Another established, fluorescence-based method used for almost 5 decades is the Crithidia luciliae indirect immunofluorescence test (CLIFT), which makes use of the compact arrangement of dsDNA in the mitochondrion of the Crithidia cell, the so-called kinetoplast (28, 29). This method is laborious and requires skilled technicians, and its routine use in laboratories varies widely (30). Enzyme immunoassays have helped to overcome limitations such as user-dependent variability, processing time, and safety concerns and have led to partial or even full automation. ELISAs are typically performed in a microtiter well with the antigen of interest immobilized to a solid surface, with quantification of bound antibodies through a colorimetric signal generated by an enzyme coupled to a detection

antibody. Fluorescence enzyme immunoassays (FEIA) and chemiluminescence immunoassays (CLIA) are variations of this principle using fluorescence or luminescence as a readout resulting in a higher analytical measurement range and a reduced need for sample dilutions. CLIA typically use paramagnetic beads as the solid phase for antigen immobilization. Multiplex immunoassays (MIA) combine the bead format with multiple fluorescent dyes to enable the simultaneous detection of antibodies to multiple autoantigens. To limit the binding of antibodies that may be of little or no clinical relevance, manufacturers apply different approaches (31). Despite this, there is still a risk of false positives with any anti-dsDNA test, potentially leading to further investigations to rule out SLE.

When developing the 2019 classification criteria for SLE, the EULAR/ACR noted that some anti-dsDNA tests used in practice have relatively low specificity (2). Hence the EULAR/ACR criteria set out a benchmark for the specificity of anti-dsDNA testing, that is "an immunoassay with demonstrated ≥ 90% specificity for SLE against relevant disease controls" (2). This statement has 2 implications: (i) "with **demonstrated** ≥ 90% specificity" implies there is a need to account for all the evidence available for each test, and (ii) "against **relevant** disease controls" implies that the specificity should be measured in a range of controls that is representative of the population that would receive the test in practice.

On this basis, we set out to conduct a systematic literature review that would identify all published studies evaluating the diagnostic accuracy of anti-dsDNA tests for SLE. This provides a comprehensive evidence base upon which the discussion in this paper is based. We provide an overview of the specificity data reported in these studies and a formal assessment of the quality of the studies, including an appraisal of the control group in which specificity is measured. In addition, we conducted a meta-analysis to pool data across studies to assess whether anti-dsDNA tests are likely to meet a benchmark ≥ 90% specificity in practice.

MATERIALS AND METHODS

Systematic Literature Review Process

A structured literature search and systematic literature review were conducted as per the Cochrane Collaboration recommendations, for a review of diagnostic test accuracy studies (32). The search strategy combined filters for "systemic lupus erythematosus" and "double-stranded DNA antibodies" with key terms for diagnostic accuracy test studies using Emtree/MeSH terms and free text strings, plus an additional search filter for commercially available dsDNA tests (test name or manufacturer in title or abstract, device name, or device manufacturer). Using these filters, we conducted an electronic search for studies published from 2004 to August 2019 in MEDLINE, Embase, Cochrane Central Register of Controlled Trials, and Database of Abstracts of Reviews of Effects. The original database search was conducted on March 4, 2015, to capture all studies published since 2004. The literature search was updated on August 21, 2019, and studies published from January 2004 to August 2019 are included in this review.

Screening of Studies for Inclusion in the Review

All citations retrieved from the electronic search were screened against the prespecified study inclusion criteria (see Supplemental Table 1 on the online Supplemental Data for details). Eligible studies were diagnostic test accuracy studies with a cross-sectional or case-control design and at least 10 patients enrolled. The study populations needed to include an SLE cohort (cases) and a non-SLE group (controls) in which specificity was measured. All patients had a definitive classification of SLE (for

cases) or had SLE ruled out or an alternative diagnosis was confirmed (controls). The reference standard for classification of SLE needed to be the accepted criteria at the time of the study, namely ACR 1982 (33), ACR 1997 (13), or SLICC 2012 (3). Studies needed to include a commercially available anti-dsDNA test, with a named manufacturer and test results reported at the manufacturer's recommended threshold for positivity. Modified or generic in-house tests were excluded to standardize test parameters across studies.

The initial screening of the studies was based on the citation title and abstract, with a second screening using full-text papers to confirm their eligibility for inclusion in the review. The literature search citation flow is reported as per the Preferred Reporting Items for Systematic reviews and Meta-analyses of Diagnostic Test Accuracy studies statement (34) and is provided in Supplemental Fig. 2. A summary of the included studies and test information is provided in Supplemental Table 4.

Study Data and Summary Estimates of Specificity

Each study needed to report the total number of true positives, true negatives, false positives, and false negatives for each test. Specificity data from each study are grouped by test method and presented in forest plots (Figs. 1 and 2) with a 95% CI calculated using the exact (Clopper-Pearson) method (35). If studies reported data separately for healthy and diseased controls, the calculation of specificity was based on results from disease controls only (see section Relevance of Disease Controls).

In addition, hierarchical summary ROC (HSROC) curve plots of sensitivity vs specificity (expressed as 1 – specificity) were generated after fitting the test count data (36, 37). The plots show the individual study data and a summary (pooled) estimate taking into account all studies as well as the HSROC curve, a 95% confidence region around the summary estimate (based on the study observations), and a 95% prediction region [to allow for potential unobserved heterogeneity: if a new study was conducted we would expect the "true" sensitivity and specificity to lie within the prediction region with a 95% confidence level (32, 37, 38)]. The summary HSROC graphs are presented in Supplemental Figs. 6 to 8. All analyses were conducted in STATA MP v16.1. Note that a minimum of 4 studies are required for the HSROC plot.

Relevance of Disease Controls

In this paper, the assessment of whether studies measured specificity in a relevant disease control group was guided by the derivation and validation cohorts used to develop the 2019 EULAR/ACR classification criteria (see Supplemental Table 3) (2). International SLE experts contributed control samples from patients evaluated at their centers, with a range of medical conditions mimicking SLE including other connective tissue diseases (Sjögren syndrome, systemic sclerosis, inflammatory myopathies, undifferentiated connective tissue disease, or mixed connective tissue disease) and other rheumatic diseases (rheumatoid arthritis, psoriatic arthritis, spondyloarthritis, and osteoarthritis). Healthy controls (blood donors or volunteers with no relevant medical condition) do not represent patients who would receive anti-dsDNA testing in practice, and their inclusion in the control group could lead to an overestimation of anti-dsDNA test specificity (39).

Quality Assessment

The study quality was assessed using the Quality Assessment Tool for Diagnostic Accuracy Studies, version 2 (QUADAS-2) checklist (40) to assess the potential for bias in patient selection, attrition, flow, and timing of the tests and conduct and interpretation of the index tests and reference standard. The QUADAS-2 assessment for each study included in the review is given in

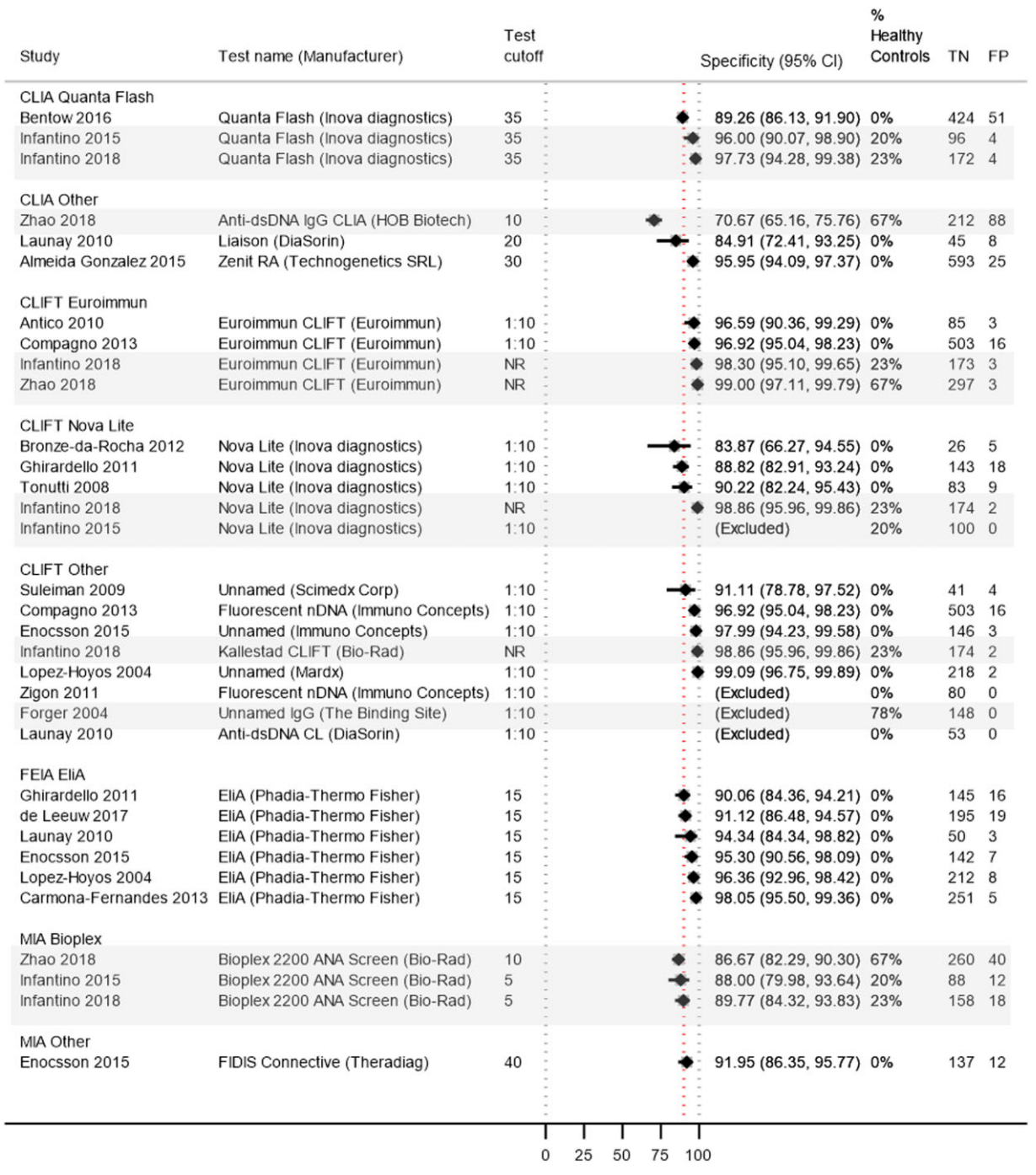

Fig. 1. Forest plot of anti-dsDNA test specificity by test method (CLIA, CLIFT, FEIA, or MIA). Red line 90% specificity, grey box = study includes healthy controls (specificity may be overestimated in this study). Excluded = 95% CI could not be estimated using the exact method as FP = 0. TN, true negatives; FP, false positives.

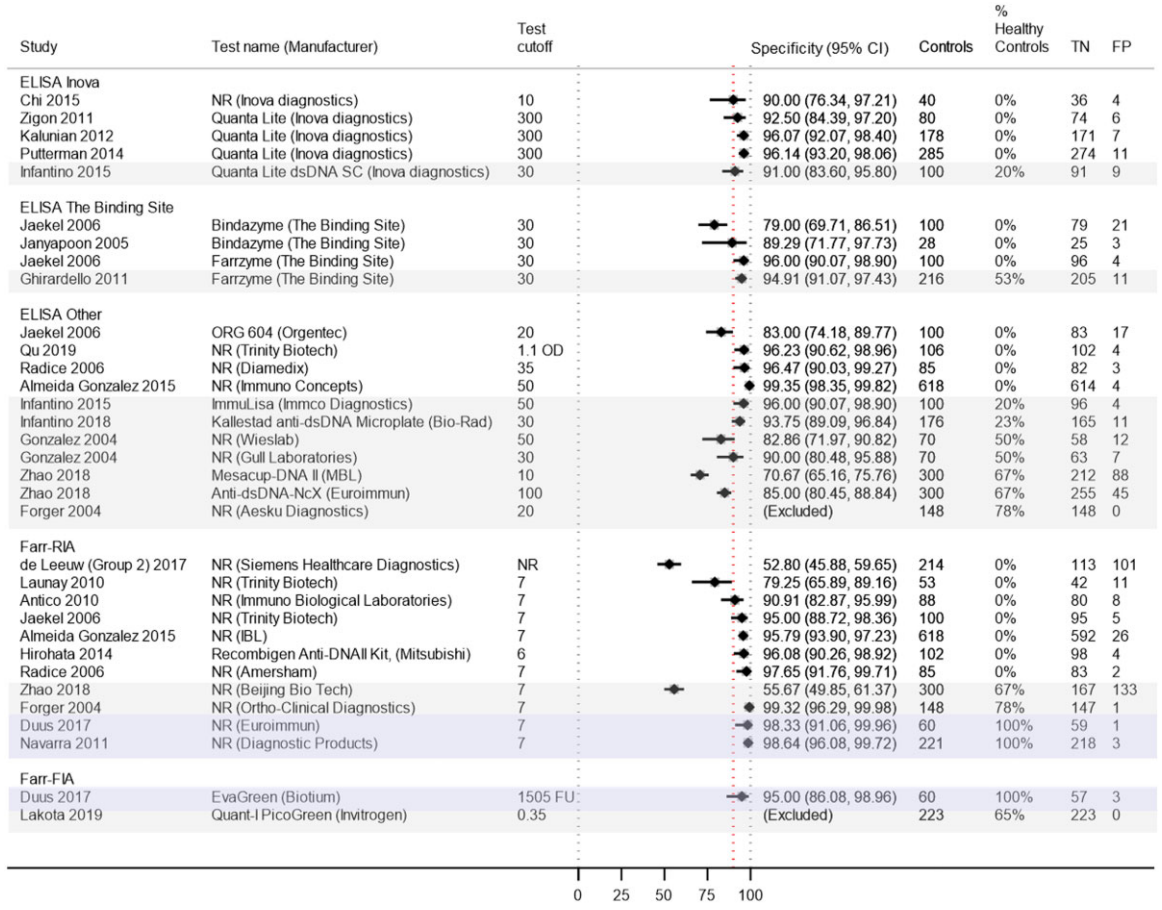

Fig. 2. Forest plot of anti-dsDNA test specificity by using the ELISA, Farr-RIA, or Farr-FIA method. Red line 90% specificity, grey box = study includes healthy controls (specificity may be overestimated in this study); purple box = study includes only healthy controls (specificity may be overestimated in this study). Excluded = 95% CI could not be estimated using the exact method as FP = 0. TN, true negatives; FP, false positives.

Supplemental Table 5, and Fig. 3 provides an overview of the quality of the studies.

RESULTS

Studies Included in the Review

The literature search (up to August 2019) identified 30 studies (27, 41–69) that met the review inclusion criteria (see Supplemental Table 1) from which there were 66 sets of results for 43 different anti-dsDNA tests (Supplemental Table 4). For the main part of this review we have divided the study data into groups by test method (CLIA, CLIFT, ELISA, Farr-FIA, Farr-RIA, FEIA, MIA) with further subdivision for individual named tests where feasible.

Specificity estimates for CLIA are reported in 6 of the studies included in the review (41, 43, 55, 56, 60, 68), covering 4 different CLIA tests, with 3 studies reporting data for the QUANTA Flash CLIA test (43, 55, 56). Specificity estimates for CLIFT test are reported in 14 studies (42, 44, 47, 50–52, 55, 56, 60, 61, 66–69), covering 9 different CLIFT tests, with 4 studies reporting data for Euroimmun

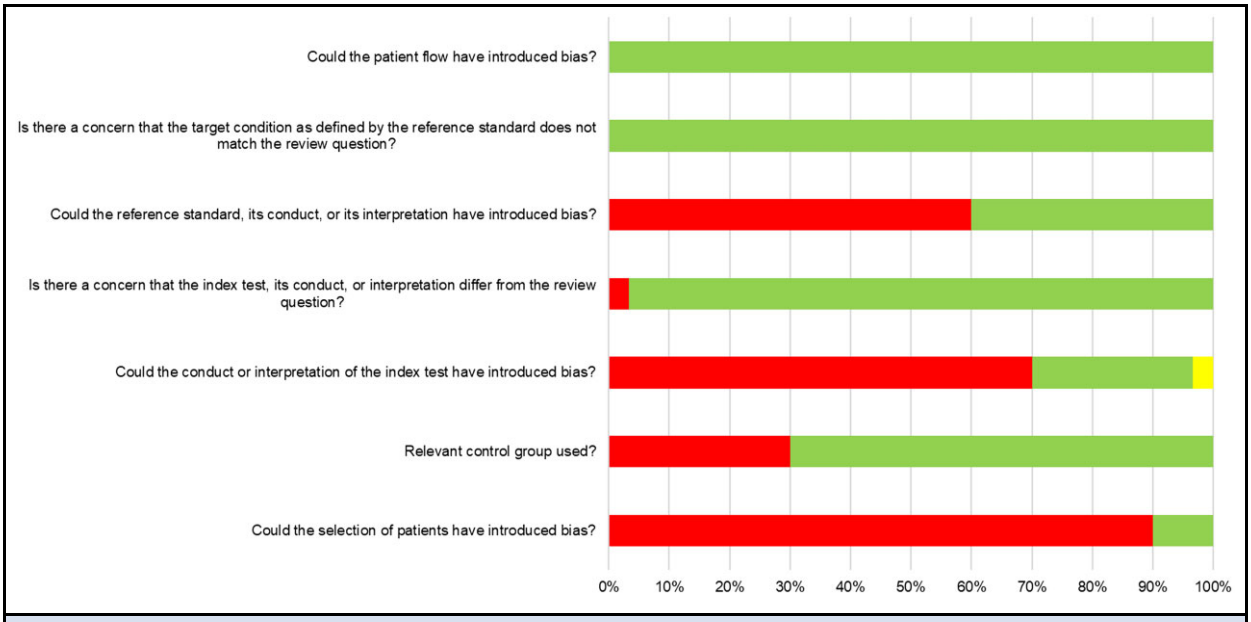

Fig. 3. Overview of the QUADAS-2 assessment: summary risk of bias and applicability questions. Green = low risk of bias; red = high risk of bias; yellow = unclear/information not reported.

CLIFT (42, 47, 55, 68) and 5 studies for NOVA Lite CLIFT (44, 52, 55, 56, 67). Specificity estimates for FEIA tests are reported in 6 studies (45, 48, 50, 52, 60, 61), all of which were for the EliA dsDNA test. Specificity estimates for MIA tests are reported in 4 studies (50, 55, 56, 68), with 2 different MIA tests. In addition there were 15 studies (41, 46, 51–53, 55–59, 63–65, 68, 69) with test accuracy data for 16 different ELISA tests, with 11 studies (41, 42, 48, 49, 51, 54, 57, 60, 62, 65, 68) covering 9 different Farr-RIA tests and 2 studies (27, 49) covering 2 different Farr-FIA tests. Forest plots showing the specificity data reported by each study are provided in Fig. 1, grouped by CLIA, CLIFT, FEIA, or MIA method and by named test where feasible, and in Fig. 2 for ELISA, Farr-RIA, and Farr-FIA tests.

Summary of Anti-dsDNA Specificity by Test Method/Named Test

Table 1 summarizes the key characteristics of the studies reporting data for each set of tests, alongside estimates of test specificity from the meta-analysis, wherever this was feasible (see HSROC plots in Supplemental Figs. 6–8).

CLIA. Three out of the six studies with data for CLIA tests reported a specificity point estimate less than 90% (range 89%–98% for QUANTA Flash; 71%—96% for other CLIAs). Based on the data available for the four different CLIA tests at the time of this review, the summary estimate for specificity is 92.3% (95% CI 83.6%, 96.6%), (see Supplemental Fig. 7, A). It is noted that 15.1% of control subjects were healthy controls, and for two studies the disease controls may not be representative of controls in practice (in comparison to the EULAR/ACR 2019 derivation and validation set).

CLIFT (Euroimmun at cutoff of 1:10). All four studies reported specificity above 90% although two studies included healthy controls in the specificity estimates: these studies reported higher specificity compared to the two studies that included

Table 1. Summary of anti-dsDNA specificity data by test method/named test.

Method	Anti-dsDNA test	Cutoff	Studies	Subjects	% SLE	Study design	Test setting	Reference standard for SLE	Relevant disease controls	% HC[a]	Specificity estimate	
CLIA	QUANTA Flash	35 IU/mL	3 (43, 55, 56)	1794	58%	3 case-control	3 secondary/ hospital	1 ACR 1997 2 ACR 1982 or SLICC 2012	1 study includes a similar mix to 2019 EULAR/ACR dvalidatio/validation cohort; 1 study includes only treated inflammatory artdritis or autoimmune tdyroiditis, 1 study includes rheumatoid artdritis or autoimmune tdyroiditis 53% RA,[b] SpA,[c] PsA[d] 11% otder CTD[e] (SjS,[f] SSc,[g] DM[h]/PM[i])	8% (2 studies)	• Min: 89% (in DCs[j]) • Max 98% (mixed DC/HCs)	• HSROC: 92.3% (95% CI 83.6%, 96.6%) • Supplemental Fig. 7, A
	3 tests: Liaison-DiaSorin, Zenit-Technogenentics, HOB BioCLIA	Various	3 (41, 60, 68)	1063	14%	2 case control; 1 cross-sectional	3 secondary/ hospital	3 ACR 1997	3 studies include a similar mix to 2019 EULAR/ACR dvalidatio/validation cohort 63.6% from cross-sectional study (consecutive referrals for dsDNA testing in practice) 5% RA; 11% otder CTD (SjS, SSc, IM,[k] MCTD[l])	20.68% (1 study)	Min: 71% (mixed DC/HCs), Max 96% (in DCs)	
• Three out of the 6 studies reporting specificity for CLIA report a point estimate less than 90%. • Specificity of the QUANTA Flash CLIA test is not currently demonstrated to be ≥ 90% nor demonstrated against relevant disease controls; more studies are required to conduct a meta-analysis for this named test and 2 out of 3 studies included healthy controls. • Specificity of tests using the CLIA method in general is not demonstrated to be ≥ 90%: HSROC summary estimate incorporating 6 studies, lower 95% CI is less than 90%. • Not demonstrated against relevant disease controls: 11% of control subjects were healthy controls and for 2 studies the disease controls may not be representative of controls in practice (does not include the broad range of patients with an alternative diagnosis as seen in the EULAR/ACR 2019 derivation and validation set). • Caveats: all of the studies were in secondary/tertiary care settings and most were of a case-control design											
CLIFT	Euroimmun CLIFT	01:10	4 (42, 47, 55, 68)	1389	22%	3 case control; 1 cross-sectional	4 secondary/ hospital	1 ACR 1982; 3 ACR 1997	3 studies include a similar mix to 2019 EULAR/ACR derivation/validation cohort 1 study includes only treated inflammatory arthritis or autoimmune thyroiditis 32% RA, OA; inflammatory arthritis (treated) 14% other CTD (SjS, SSc, IM, MCTD)	22.2% (2 studies)	• Min: 97% (in DCs) • Max 99% (mixed DC/HC)	• HSROC: • 97.8% (95% CI 96.2%–98.7%) • Supplemental Fig. 6, A
• None of the 4 studies reporting specificity for CLIFT Euroimmun report a point estimate less than 90%. • 22% of control subjects were healthy controls, and for 1 study the disease controls may not be representative of controls in practice (does not include the broad range of patients with an alternative diagnosis as seen in the EULAR/ACR 2019 derivation and validation set). • Specificity of the Euroimmun CLIFT is likely to be ≥ 90% based on HSROC summary estimate and 95% CI but is not fully demonstrated against relevant disease controls. Specificity may be lower in practice than that observed in the studies. • Caveats: all of the studies were in secondary/tertiary care settings, and most of the studies were of a case-control design.											
CLIFT	NOVA Lite	01:10	5[m] (44, 52, 55, 56, 67)	1129	50%	5 case control	5 secondary/ hospital	3 ACR 1982 or 1997 1 ACR 1997 1 ACR 1997 or SLICC 2012	3 studies include a mix of diseases that overlap with the 2019 EULAR/ACR derivation/validation cohort; 1 study	10.7% (2 studies)	• Min: 84% (in DCs) • Max 100% (mixed DC/HCs)	• HSROC: • 96.0% (95% CI 87.2%–98.9%) • Supplemental Fig. 6, B

Continued

Table 1. (continued)

Method	Anti-dsDNA test	Cutoff	Studies	Subjects	% SLE	Study design	Test setting	Reference standard for SLE	Relevant disease controls	% HC[a]	Specificity estimate	
									includes only treated inflammatory arthritis or autoimmune thyroiditis, 1 study includes rheumatoid arthritis or autoimmune thyroiditis 32.1% rheumatic, infectious or neoplastic diseases (unspecified) 30% RA, inflammatory arthritis (treated) 8% UCTD[n] 5.4% viral infections (EBV, CMV, HCV)			
• Two out of the 5 studies reporting specificity for CLIFT NOVA Lite report a point estimate less than 90% • Specificity of the NOVA Lite CLIFT is not demonstrated to be ≥ 90% based on the HSROC summary estimate incorporating 5 studies (lower 95% CI is less than 90%) nor is specificity demonstrated against relevant disease controls: 11% of control subjects were healthy controls and for 2 studies the disease controls may not be representative of controls in practice. • Caveats: all of the studies were in secondary/tertiary care settings and were of a case-control design.												
CLIFT	• Immuno Concepts: 2 tests • Fluorescent nDNA • 1 unnamed test	1:10	3 (47, 50, 69)	1067	30%	1 case control; 2 cross-sectional	3 secondary/ hospital	• 1 ACR 1982 • 1 ACR 1982 or SLICC 2011 • 1 ACR 1982 or 1997	3 studies include a similar mix to 2019 EULAR/ACR derivation/validation cohort 46% RA, SpA, PsA, OA; 19% other CTD (SjS or not specified)	0% (0 studies)	• Min: 97% (in DCs) • Max 100% (in DCs)	• HSROC: • 98.7% (95% CI 96.7%–99.5%) • Supplemental Fig. 7, B
	5 tests: DiaSorin, BioRad, Mardx, Scimedx, The Binding Site	1:10	5 (51, 55, 60, 61, 66)	2275	39%	5 case control	5 secondary/ hospital	• 2 ACR 1982; • 3 ACR 1997	4 studies include a similar mix to 2019 EULAR/ACR derivation/validation cohort; 1 study includes only treated inflammatory arthritis or autoimmune thyroiditis 30% RA, SpA; 27% other CTD (SjS, SSc, PM, UCTD, or not specified)	24.1% (2 studies)	• Min: 91% (in DCs) • Max 100% (in DCs/mixed DC/HC)	
• None of the 3 studies reporting specificity for CLIFT ImmunoConcepts† report a point estimate less than 90%. • Specificity of the Immuno Concepts CLIFT test is likely to be ≥ 90% but more studies are required to conduct a meta-analysis for this named test. • Specificity for other CLIFTs likely to be ≥ 90% based on the HSROC summary estimate incorporating 8studies and 95% CI, but most tests only have data from 1 study data, and specificity is not fully demonstrated against relevant disease controls: 17% of control subjects were healthy controls, and for 1 study, the disease controls may not be representative of controls in practice. • Caveats: All of the studies were in secondary/tertiary care settings, and most of the studies were of a case-control design.												
ELISA	• Inova diagnostics: 3 tests • QUANTA Lite, • QUANTA Lite SC • 1 unnamed	• 300 IU/mL, 30 IU/mL, • 10 AU/mL	5 (46, 56, 59, 63, 69)°	1429	52%	5 case control	5 secondary/ hospital	• 1 ACR 1982 • 3 ACR 1982 or 1997 • 1 ACR 1982 or SLICC 2012	4 studies includes a similar mix to 2019 EULAR/ACR derivation/validation cohort; 1 study includes rheumatoid arthritis or autoimmune thyroiditis	3% (1 study)	Min: 90% (in DCs); max 96% (in DCs)	• HSROC: • 93.4% (95% CI 89.9%–95.7%) • Supplemental Fig. 8, A
	The Binding Site: Bindazyme, Farrzyme	30 IU/mL	3 (52, 57, 58)°	695	51%	3 case control	3 secondary/ hospital	1 ACR 1982, 2 ACR 1982 or 1997	3 studies include a similar mix to 2019 EULAR/ACR	33.4% (1 study)	Min: 79% (in DCs), Max 96% (in DCs)	

Continued

Table 1. (continued)

Method	Anti-dsDNA test	Cutoff	Studies	Subjects	% SLE	Study design	Test setting	Reference standard for SLE	Relevant disease controls	% HC[a]	Specificity estimate	
									derivation/validation cohort			
	11 tests: Aesku, Bio-Rad (Kallestad), Diamedix, Euroimmun (NcX), Gull, Immco (ImmuLisa), Immuno Concepts, MBL (Mesacup), Orgentec (ORG 604), Trinity, Wieslab	Various	9 (41, 51, 53, 55–57, 64, 65, 68)[o]	2613	35%	8 case-control; 1 cross-sectional	8 secondary/hospital	• 2 ACR 1982 • 3 ACR 1997 • 3 ACR 1982 or 1997 • 1 ACR 1982 or SLICC 2012	5 studies includes a similar mix to 2019 EULAR/ACR derivation/validation cohort, 1 study includes rheumatoid arthritis or autoimmune thyroiditis, 1 study includes only treated inflammatory arthritis or autoimmune thyroiditis, 1 study includes non-representative controls (ANA positive but excluding systemic autoimmune diseases)	24.1% (5 studies)	Min: 71% (mixed DC/HCs), Max 100% (in mixed DCs/HCs)	
• Four studies (covering 6 ELISA tests) reported a point estimate less than 90% for specificity. • Specificity of the Inova QUANTA Lite test is likely to be ≥ 90% but not demonstrated: more studies are required to conduct a meta-analysis for this named test. • Specificity for ELISAs may not to be ≥ 90% based on the HSROC summary estimate incorporating 15 studies. Most tests only have data from 1 study data, and specificity is not fully demonstrated against relevant disease controls: 20.8% of control subjects were healthy controls, and for 3 studies, the disease controls may not be representative of controls in practice. • Caveats: All of the studies were in secondary/tertiary care settings, and most of the studies were of a case-control design.												
Farr-FIA	EvaGreen (Biotium), Quant-I PicoGreen (Invitrogen)	1505 FU, 0.35	2 (27, 49)	410	31%	1 case control; 1 cross-sectional	1 clinical research blood biobank	• 1 ACR 1997 • 1 SLICC 2012	1 study did not include any disease controls (100% healthy controls); 1 study included patients with antiphospholipid syndrome, Sjögren syndrome, and rheumatoid arthritis alongside healthy blood donors	72.4% (2 studies)	95%–100% (in mixed DC/HC)	–
• More studies are required to conduct a mixed-effect bivariate meta-analysis for Farr-FIA • Specificity estimates are not measured in relevant disease controls. The majority of control patients were healthy controls												
Farr-RIA	9 tests: Amersham, Beijing North Institute Biological Technology, Diagnostic Products, Euroimmun, Immuno Biological Laboratories, Ortho-Clinical, Siemens, Trinity, Mitsubishi	6 or 7 IU/mL	11 (41, 42, 48, 49, 51, 54, 57, 60, 62, 65, 68)	3238	39%	9 case control; 2 cross-sectional	10 secondary/hospital, 1 clinical research blood biobank	• 5 ACR 1982 • 4 ACR 1997 • 1 ACR 1982 or 1997 • 1 ACR 1997 or SLICC 2012	8 studies include a similar mix to 2019 EULAR/ACR derivation/validation cohort 2 studies do not include any disease controls (100% healthy controls) 2 studies include > 60% healthy controls	30.0% (4 studies)	• Min: 52.8% (in DCs) • Max 99.3% (in mixed DCs/HCs)	• Meta-analysis 93.8% (95% CI 85.4–97.5%) • (Fig. Supplemental 8, B)
• Three of the 11 studies reporting specificity for Farr-RIA report a point estimate less than 90%, with 1 study reporting a specificity of 53%. • Specificity is not demonstrated to be ≥ 90% based on the HSROC summary estimate incorporating 11 studies. Most tests only have data from 1 study data, and specificity is not fully demonstrated against relevant disease controls with 4 studies including 60% to 100% healthy controls. • Caveats: Most of the studies were in secondary/tertiary care settings, and most of the studies were of a case-control design.												
FEIA	EliA dsDNA	15 IU/ml		1977	47%			• 2 ACR 1982 • 3 ACR 1997	All studies include similar mix to 2019			

Continued

Table 1. (continued)

Method	Anti-dsDNA test	Cutoff	Studies	Subjects	% SLE	Study design	Test setting	Reference standard for SLE	Relevant disease controls	% HC[a]	Specificity estimate	
			6 (45, 48, 50, 52, 60, 61)			5 case control; 1 cross-sectional	5 secondary/hospital; 1 university research department blood bank	• 1 ACR 1997 or SLICC 2012	EULAR/ACR derivation/validation cohort 44% RA, JIA,[p] SpA PsA 24% other CTD (SjS, SSc, UCTD, MCTD, PM, Raynaud's)	0% (0 studies)	Min: 90% (in DCs) Max: 98% (in DCs)	HSROC: 94.7% (95% CI 91.7%–96.7%) Supplemental Fig. 6, C
• None of the 6 studies reporting specificity for FEIA EliA dsDNA report a point estimate less than 90%. • Specificity of the FEIA EliA dsDNA test is likely to be ≥ 90% based on the HSROC summary estimate incorporating 6 studies and 95% CI and is demonstrated against relevant disease controls: 0% of control subjects were healthy controls, and controls included a broad range of patients with an alternative diagnosis. • Caveats: Most of the studies were in secondary/tertiary care settings, and most of the studies were of a case-control design.												
MIA	Bioplex 2200	5 or 10 IU/mL	3 (55, 56, 68)	895	36%	3 case control	3 secondary/hospital	1 ACR 1997 or SLICC 2012 2 ACR 1997	1 study includes a similar mix to 2019 EULAR/ACR derivation/validation cohort; 1 study includes only treated inflammatory arthritis or autoimmune thyroiditis; 1 study includes rheumatoid arthritis or autoimmune thyroiditis 30% RA; 11% other CTD (SjS, SSc, IM, MCTD)	45.1% (3 studies)	• Min 86.7% (mixed DCs/HCs); • Max 89.8% (mixed DCs/HCs)	• HSROC: • 89.3% (95% CI 86.1%–91.8%) • Supplemental Fig. 7, C
	FIDIS Connective	40 IU/mL	1 (50)	327	54%	1 case control	1 secondary/hospital	1 ACR 1997 or SLICC 2012	SjS (36%) or RA (64%)	0%	91% (95% CI 86%, 96%) study estimate	
• All 3 studies reporting specificity for MIA Bioplex report a point estimate less than 90%. • Specificity of the Bioplex 2200 MIA test is not demonstrated to be ≥ 90%. More studies are required to conduct a meta-analysis for this named test. All studies included healthy controls (45% of controls), and for 2 studies the disease controls may not be representative of controls in practice. For 1 study, the cutoff was set at 10 IU/mL. If the cutoff had been set at 5 IU/mL (current manufacturers' recommended cutoff) it could be expected that specificity would be reduced (possibly more false positives if there are results in the 5–10 IU/mL range). • Specificity of tests using the MIA method in general is not demonstrated to be ≥ 90% based on HSROC summary estimate incorporating 4 studies (95% CI is less than 90%) nor demonstrated against relevant disease controls: 11% of control subjects were healthy controls and for 2 studies the disease controls may not be representative of controls in practice. • Caveats: All of the studies were in secondary/tertiary care settings, and all were of a case-control design.												

[a] Healthy controls.
[b] Rheumatoid arthritis.
[c] Spondyloarthritis.
[d] Psoriatic arthritis.
[e] Connective tissue disease.
[f] Sjögren syndrome.
[g] Systemic sclerosis.
[h] Dermatomyositis.
[i] Polymyositis.
[j] Disease controls.
[k] Inflammatory myopathy.
[l] Mixed connective tissue disease.
[m] Data from 1 study was excluded: specificity was reported to be 44%, and the authors agreed this was not representative of CLIFT specificity in practice.
[n] Undifferentiated connective tissue disease.
[o] 15 studies, 16 different ELISA tests.
[p] Juvenile idiopathic arthritis.

disease controls only (Fig. 1 and HSROC in Supplemental Fig. 6, A). One study estimated specificity in a limited range of disease control patients (patients with treated inflammatory arthritis or autoimmune thyroiditis). For the CLIFT Euroimmun anti-dsDNA test at cutoff of 1:10, the summary estimate for specificity is 97.8% (95% CI 96.2%, 98.7%) (see Supplemental Fig. 6, A). Euroimmun CLIFT is likely to have a specificity ≥ 90% although further studies in a representative range of disease controls would be required to confirm this.

CLIFT (NOVA Lite at cutoff of 1:10). Two out of the five studies with data for CLIFT NOVA Lite tests reported a specificity point estimate less than 90% (range 84%–100%). Based on five studies with data for CLIFT NOVA Lite at cutoff of 1:10, the summary estimate for specificity is 96.0% (95% CI 87.2%, 98.9%) (see Supplemental Fig. 6, B). It was noted that 11% of control subjects were healthy controls. Studies including healthy controls reported higher specificity compared to the studies that included disease controls only (Fig. 1 and HSROC in Supplemental Fig. 6, B). For two studies the disease controls may not be representative of controls in practice (in comparison to the EULAR/ACR 2019 derivation and validation set).

CLIFT (other tests). Eight studies reported data for seven other CLIFT tests, all of which had specificity above 90%. Specificity of the Immuno Concepts CLIFT tests (Fluorescent nDNA and one unnamed) is likely to be ≥ 90% (reported range 97%–100% in disease controls with similar range of patients to EULAR/ACR 2019 derivation and validation set): more studies are required to conduct a meta-analysis for this test. For the other CLIFT tests, two studies included healthy controls in the specificity estimates. Studies including healthy controls tended to report higher specificity compared to the studies that included disease controls only (Fig. 1 and HSROC in Supplemental Fig. 7, B). In one study the disease controls may not be representative of controls in practice (in comparison to the EULAR/ACR 2019 derivation and validation set). Based on the data from eight studies covering seven different CLIFT tests at a cutoff 1:10, the summary estimate for specificity is 98.7% (95% CI 96.7%–99.5%) (see Supplemental Fig. 7, B).

ELISA. Specificity of the ELISA tests is not demonstrated to be ≥ 90% based on the lack of consistent data for this test method. Most of the ELISA tests only had data from one study, and there is a wide variation in test characteristics making comparisons difficult. Reported specificity ranged from 71% to 100%. Four studies (covering six ELISA tests) reported a point estimate less than 90% for specificity. Based on the data from 15 studies covering 16 different ELISA tests at various cutoffs, the summary estimate for specificity is 93.4% (95% CI 89.9%–95.7%) (Supplemental Fig. 8, A). Specificity was not always measured against relevant disease controls: 20.8% of control subjects were healthy controls, and for two studies, the disease controls may not be representative of controls in practice. Five studies reported data for an Inova ELISA (specificity range 90%–96%); however, these data were not compared in a separate meta-analysis given the mixed test characteristics.

Farr-FIA. Data were available from two studies. More studies are required to conduct a meta-analysis for Farr-FIA. Specificity was reported to be 95% to 100%; however, this could be an overestimate given that the majority of control patients were healthy controls (72.4% healthy blood donors).

Farr-RIA. Three out of the 11 studies reporting specificity for Farr-RIA report a point estimate less than 90% with one study reporting a specificity as low as 53%. Specificity is not demonstrated to be ≥ 90% based on the HSROC summary estimate incorporating 11 studies [93.8% (95% CI 85.4%–97.5%)] (see Supplemental Fig. 8, B). This is based

on the 95% CI (also the large 95% prediction region) and the lack of consistency in test characteristics. Most tests only have data from one study, and specificity is not fully demonstrated against relevant disease controls with four studies including 67% to 100% healthy controls.

FEIA (EliA dsDNA at cutoff of 15 IU/mL). All six studies reported specificity above 90%, and healthy controls were excluded from the specificity estimates. All studies included a range of patients similar to the 2019 EULAR/ACR derivation/validation cohort: 44% rheumatoid arthritis, juvenile idiopathic arthritis, spondyloarthritis, psoriatic arthritis and 24% other connective tissue diseases (Sjögren syndrome, systemic sclerosis, undifferentiated connective tissue diseases, mixed connective tissue diseases, polymyositis, Raynaud's syndrome). For the EliA dsDNA test at cutoff of 15 IU/mL, the summary estimate for specificity is 94.7% (95% CI 91.7%, 96.7%) [see Supplemental Fig. 6, C (70)]. EliA dsDNA is likely to have a specificity $\geq$ 90% in practice based on the 95% CI, although further cross-sectional studies could be conducted including studies of patients referred from primary care.

MIA. The three studies reporting specificity for MIA Bioplex reported a point estimate less than 90% (range 86.7%–89.8%). All three studies included healthy controls. More studies are required to conduct a meta-analysis for MIA Bioplex. One additional study reported data for FIDIS (specificity 91.9%). Combining the data from these four studies of MIA anti-dsDNA tests, the summary estimate for specificity is 89.3% (95% CI 86.1%, 91.8%) (see Supplemental Fig. 7, C). More studies are required to demonstrate the specificity of MIA especially studies in relevant disease controls.

Summary of Study Quality Assessment

Figure 3 provides an overview of the QUADAS-2 assessment and indicates the key areas of potential bias that may impact on the estimates of specificity.

Selection of the patients. It was noted that most of the studies (90%) were a case-control design, and studies with this design are downgraded in the QUADAS-2 assessment. Whilst this may lead to selection bias, some case-control studies enrolled consecutive patients for each group or avoided overly restrictive exclusions.

For this review we assessed whether the disease control patients enrolled in the study represented a relevant control group for measuring test specificity. The control group was deemed to be representative to the review question in 70% of studies. Nine studies included healthy controls or did not include a range of relevant disease controls (see Supplemental Table 4). It was noted that patients were selected from patients presenting for anti-dsDNA testing in secondary/tertiary care settings in most studies. The majority of studies were conducted in European centers (21 studies), with 3 studies in China, 4 studies in other Asian countries, and 3 United States/Canada studies.

Index test. There were 2 areas where the conduct or interpretation of the index test results may bias the results. One of the QUADAS-2 signaling questions is whether the index test results are interpreted without knowledge of the reference standard (not known if patient is in the SLE cohort or control group). Case-control studies have been assessed to have a high risk of bias for this domain unless the publication mentioned that index test results were interpreted without knowledge of the reference standard. The second signaling question is whether the test threshold was prespecified. For 1 study, the test threshold was based on maximizing the ROC area under the curve; otherwise, the test cutoff was set before the test was conducted. We note that the results from anti-dsDNA tests are (semi-)quantitative, and we did not note any particular issues with

uninterpreted or borderline test results (see flow and timing). Although some studies have been marked down in the QUADAS-2 assessment, it is unlikely that interpretation bias is an issue. Note that 1 test result was excluded from the analysis of CLIFT tests. In this study the specificity of CLIFT NOVA Lite was reported to be 44% (69), which is an outlier compared with other CLIFT anti-dsDNA test data (42, 44, 47, 50–52, 55, 56, 60, 61, 66–69).

Reference standard. The included studies used ACR 1982 (33), ACR 1997 (13), SLICC 2012 (3), or a combination of these classification criteria to define the SLE group. We therefore deemed all studies to have used an acceptable reference standard to determine the target condition. Similarly, the control group of patients had a definitive diagnosis other than SLE. One of the signaling questions relates to the incorporation of the index test into the reference standard. This potential form of confirmation bias was noted in 60% of studies where it was assumed that an anti-dsDNA test was part of the diagnostic workup. For other studies the index test was a different test to that used for diagnosis, or the publication indicated that the reference standard was independent of the index testing.

Flow and timing. For the final domain the signaling questions relate to the time interval between the index test (or more accurately the time when serum sample was taken) and reference standard, differential or partial diagnosis, and handling of missing results (unexplained exclusion of patients or test results, or uninterpretable or equivocal test results). All studies were judged to have a low risk of bias for this domain.

To summarize, the key area of potential bias arises from the selection of the patients, in particular the use of case-control designs and range of disease controls included in the studies.

DISCUSSION

This review includes 30 studies covering 43 different anti-dsDNA tests, which we have grouped by method for comparison purposes. It should be noted that systematic reviews are designed to include all the published evidence available for each test that meets the inclusion criteria. This evidence base, and the resultant meta-analysis of the data confirms that there is a wide variation in anti-dsDNA test performance and effectiveness for classification and diagnosis of SLE. Some studies report false-positive rates above 10% (out of 30 studies: 2 CLIA, 2 NOVA Lite CLIFT, 4 ELISA, 3 Farr-RIA, 3 MIA). For some methods more data are needed (such as the newer methods CLIA, MIA, and Farr-FIA). The current data indicates that FEIA and some CLIFT tests are likely to have a specificity $\geq$ 90%: for these named tests the 95% CI from the meta-analysis was above 90%, and the disease control group was judged to be sufficiently relevant. It was noted that for all tests the 95% prediction region shown in the HSROC plots included values where specificity $<$ 90%. If a new study was conducted, we would expect the "true" specificity to lie somewhere within the prediction region with a 95% confidence level. Despite the number of studies reporting test performance for Farr-RIA and ELISA, there is a wide range of test characteristics and a lack of consistency the data reported, such that we are unable to determine whether these tests are likely to have a specificity $\geq$ 90%. The Farr assay, first described for the detection of anti-dsDNA in 1969, is sometimes still regarded as the gold standard because of its high specificity for SLE. In addition, the Farr assay is the method that is included in the widely used composite index SLE Disease Activity Index 2000 (71). Our review of the data for Farr assays does not support the assay's status as a reference method for anti-dsDNA testing. It should be noted that there were too few data to allow estimates of individual tests

for CLIA, MIA, Farr-RIA, and ELISA. The summary estimates in the paper are grouped according to analytical principle of the dsDNA method, although specificities clearly vary across assay manufacturer.

Eight studies in our review include between 20% and 100% of healthy subjects in their control groups, which can lead to an underestimation of the false-positive rate (39). Furthermore, it is important that diagnostic test accuracy studies include a relevant set of disease controls. This is particularly apt for antibody-specificity testing in the context of autoimmune disease since the expression of anti-dsDNA comprises a mixture of antibodies and fine specificities (6, 72). As part of our review, we contrasted the controls groups included in the studies against the validation set used for the EULAR/ACR 2019 classification criteria since this cohort included patients evaluated in practice, with a wide range of medical conditions mimicking SLE. More studies with a cross-sectional design would ensure that a broad range of relevant controls will be represented.

Anti-dsDNA tests of differing design may lead to the differential classification of patients, as is the case for other autoantibodies (e.g., rheumatoid factor and anti-CCP antibodies) (72–74). Furthermore, false-positive results have direct and indirect downstream consequences (75) and can result in a delay in obtaining the correct diagnosis and potentially inappropriate or no treatment. While the differences in the tests could hamper efforts toward test harmonization in autoimmune diagnostics (72), this is also an opportunity to adopt complimentary diagnostic approaches depending on the setting, such as a combination of a sensitive and a highly specific test (6).

The 95% CI estimates provided indicate the extent of variation in the specificity estimates reported in the studies. These are from a mixed-effect meta-analysis model that allows for variation within studies and between studies. The studies included the use of a single measurement with each test and for each patient. A different model would be needed to analyze the variance resulting from repeated testing of the same sera, although individual patient data are unlikely to be available to make this feasible. We noted that most of the studies included in our review were conducted in patients from secondary/tertiary care settings and mostly European countries. This may have an impact on the generalization of the results, for example, to the primary care setting where there is a low pretest probability of SLE and a need for a highly specific test. If there is a high suspicion of SLE, a more sensitive test may be required to support diagnosis. Our review was not designed to assess the sensitivity, and further work is required to assess sensitivity of anti-dsDNA testing to support the diagnosis of SLE, and separately anti-dsDNA testing to monitor disease activity.

In conclusion, this comprehensive assessment of anti-dsDNA test specificity has allowed us to estimate the likely variation in false-positive rates across tests. Some studies report false-positive rates > 10% for some of the tests, and since specificity is not always measured in an appropriate control group, this may be an underestimate. Given the complexity of antibody-specificity testing in autoimmune diseases, the evidence presented can help lab specialists identify the most appropriate anti-dsDNA tests for their setting. The review also identifies gaps in the evidence where more studies are required.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: SLE, systemic lupus erythematosus; ANA, antinuclear antibody; EULAR, European League Against Rheumatism; ACR, American College of Rheumatology; SLICC, Systemic Lupus International Collaborating Clinics; dsDNA, double-stranded DNA; Farr-RIA, Farr radioimmunoassay; Farr-FIA, Farr fluorescent immunoassay; CLIFT, Crithidia luciliae indirect immunofluorescence test; FEIA, fluorescence enzyme immunoassay; CLIA, chemiluminescence immunoassay; MIA, multiplex immunoassay; HSROC, hierarchical summary receiver operating characteristic; QUADAS-2, Quality Assessment Tool for Diagnostic Accuracy Studies, version 2.

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