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Recommendations for the use of anti-dsDNA autoantibodies in the diagnosis and follow-up of systemic lupus erythematosus – A proposal from an expert panel

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ABSTRACT

Anti-dsDNA autoantibodies are listed as one of the classification criteria for systemic lupus erythematosus (SLE) and are relatively effective indicators for monitoring disease activity and treatment response. Therefore, clinicians rely on them to diagnose and adjust medication and treatment strategies for SLE patients. However, the use of anti-dsDNA antibodies is not free from controversy. Part of this controversy stems from the fact that anti-dsDNA antibodies are found in several disorders, besides SLE. In addition to this, anti-dsDNA antibodies are a heterogeneous group of antibodies, and their determination still lacks proper standardization. Moreover, anti-dsDNA testing specificity and diagnostic performance change depending on the population under study. These and other issues result in inconsistency and encumber the clinical use of anti-dsDNA antibodies.

A panel of medical laboratory and clinical experts on SLE discussed such issues based on their clinical experience in a first meeting, establishing a series of recommendations. The proceedings of this first meeting, plus an exhaustive review of the literature, were used to compose a paper draft. The panel subsequently discussed and refined this draft in a second meeting, the result of which is this paper. This document is relevant to clinical laboratories as it guides to improving diagnosis and monitoring of SLE. Simultaneously, it will help laboratories compile more informative reports, not limited to a mere number. It is also relevant to clinical doctors who wish to better understand laboratory methods so that they can do a more efficient, better-aimed laboratory test ordering.

1. Introduction

Systemic lupus erythematosus (SLE) is considered a chronic, potentially lethal autoimmune disorder with multiple manifestations: cardiovascular, dermatologic, gastrointestinal, immune-hematologic, musculoskeletal, neuropsychiatric, pulmonary, and renal. Delays in the diagnosis and treatment lead to increased damage in affected organs [1]. Establishing the aetiology and suggesting a precise definition of SLE has been impossible [2]. Autoantibodies, especially when forming immune complexes, are considered pathogenic, as they are present in virtually all SLE patients. However other mechanisms are also being studied, and there is no unique correspondence between autoantibody targets and

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clinical manifestations. For instance, autoantibodies against the C1q complement protein (anti-C1q) are associated to lupus nephritis [3], as also are autoantibodies anti-double-stranded DNA (anti-dsDNA) [4]; however, of these two, only anti-dsDNA are associated to leukopenia.

Lack of a reliable diagnostic test for SLE necessarily led to using classification criteria to describe relatively homogenous populations from which patients are included in clinical trials assessing efficacy and safety of developing therapies [5–7]. The last one of these initiatives to come to light has been the SLE classification criteria jointly published in 2019 by the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) (EULAR/ACR 2019) [8]. This new criteria system constitutes a simple, direct, and precise classification method that can be easily implemented and even integrated in computer applications [8]. It has been developed through a balanced use of both expert-based and data-driven methods, and its sensitivity and specificity are superior to all classification criteria previously published [8].

However, the multifactorial nature of SLE potentially generates a very large number of different phenotypes for which there is no evidence of a common aetiology or a pathogenic association [9]. In fact, it is theoretically possible that the signs and symptoms considered in the EULAR/ACR 2019 criteria result from different pathogenic mechanisms [9]. Moreover, this classification conflicts with the concept of SLE as a syndrome, as it is based on signs and symptoms that can coincide or appear at separate times in the history of a particular patient. In medicine, the term syndrome refers to a set of signs and symptoms that, when occurring at the same time, indicate a specific disease or disorder. This term originates from the Greek word *syndromē*, which means concurrence [9].

Along with the search for an exact and precise definition of the classification criteria, interpretation of laboratory test results should be harmonized across assays. This is especially difficult when different test systems use different scales and arbitrary units for a given parameter. This problem is addressed using international units (IU) and likelihood ratios, which, strictly speaking, is only possible when international standards are available. International standards are reference sera that have been assigned units of activity following extensive studies involving multiple international laboratories. These standard sera are needed to calibrate quantitative anti-dsDNA testing methods and so make them comparable. A standard serum always produces the same IU reading no matter what assay is used (10,11).

In 1985, the subcommittee for standardization of anti-dsDNA, appointed by the International Union of Immunological Societies, developed the Wo/80 serum, which was used as an international standard in anti-dsDNA testing until its exhaustion more than a decade ago [10,11]. Recently, the National Institute for Biological Standards and Control (NIBSC) prepared a reference serum, coded 15/174, and evaluated it in an international collaborative study including 36 laboratories from 17 countries. However, discrepancies in the readings obtained with 15/174 across these laboratories have prevented it from being established as a replacement standard universally accepted [11].

In conclusion, the current lack of standardization of autoantibody assays, including antinuclear antibodies (ANA) and anti-dsDNA, increases the importance of selecting the most adequate assay and an appropriate interpretation of results. Algorithms in many routine clinical laboratories automatically add testing for anti-dsDNA if ANA are positive, even in the absence of clinical suspicion of SLE. As regards disease-activity monitoring, prediction of clinical relapses continues to be challenging as currently available anti-dsDNA tests are not marketed for this purpose, but clinicians keep on requesting these tests for follow-up. In addition to this, so long as defined diagnostic criteria for SLE continue to be lacking, maximum attention must be put into the correct application of the classification criteria [12]. All these issues are impacting the diagnostic work-up of patients suspected of SLE, and the subsequent monitoring of those with an established diagnosis.

The overall goal of this project was to analyze the needs mentioned in this introduction and to compile a document of recommendations to optimize the activity of healthcare providers, including those clinicians involved in managing patients with SLE and the immunology laboratories that participate in diagnosing and monitoring these patients. The objectives that we set out to fulfil with this document were:

- To recommend best practices for the immunology laboratory to collaborate in the diagnosis and follow-up of SLE, emphasizing the optimization of the determination of anti-dsDNA antibodies.
- To provide clinicians working with SLE patients with a greater understanding of the methods used in the immunology laboratory, and to facilitate the management of test requests.

To that end, nine independent experts, widely experienced in autoimmunity and working at different first-order Spanish institutions, completed a survey conducted by an independent consultancy to detect gaps, problems and areas of improvement in SLE management. Later, this panel met to examine and discuss all issues detected at the survey. At this meeting, a first draft with recommendations was produced, based on scientific evidence and the experience and opinions of the panel members, to help in SLE diagnosis and follow-up. Later, an exhaustive non-systematic literature search was conducted to support and complete these recommendations. In a second meeting, the draft was reviewed and amended, and the recommendations were refined. Lastly, the manuscript was reviewed again by the panel members and final comments and suggestions were included.

2. Results and discussion

2.1. The immunology laboratory in SLE diagnosis

2.1.1. Antinuclear autoantibodies

One of the main changes in the EULAR/ACR 2019 classification criteria compared to previous SLE classification systems is introducing a positive result in the ANA detection test as a required entry criterion in the SLE classification [8]. This has been described as the most relevant modification introduced by the EULAR/ACR 2019 [13] and has even been considered strategic by some authors [12,14]. In previous SLE classifications, positive ANA was not an entry criterion, and had the same level of importance as anti-dsDNA and other autoantibodies. Moreover, the terms in which this criterion was expressed in the past were clearly arbitrary, as it just required ANA levels to be above the laboratory reference interval [5], and did not establish a standard cut-off point. In the EULAR/ACR 2019 classification, ANA are considered positive when they are detected at a titer $\geq$1:80 by the indirect immunofluorescence test on HEp-2 cells (IFI). This change has improved the performance of the criteria as a classification system, but it makes caution necessary when they are used in the diagnosis of SLE because, with the 1:80 cut-off point, ANA detection specificity for SLE is 74.7% (IC 95%: 66.7% - 81.3%) [15], which is much lower than the 93.4% specificity observed when the EULAR/ACR 2019 criteria are used as a whole [8]. This relatively low specificity reflects the fact that ANA are associated with a variety of systemic rheumatologic disorders and are relatively frequent in healthy individuals [16]. Moreover, in clinical practice, ANA tests are requested in a variety of scenarios, such as diagnosis (SLE), clinical algorithms to rule out autoimmune disorders and treatment evaluation unrelated to SLE. It is precisely the high sensitivity and relatively low specificity what make ANA at titers $\geq$1:80 an appropriate entry criterion for the EULAR/ACR 2019 classification, as such criterion ensures the selection of a heterogeneous group of patients that will be subclassified in the next step by means of a screening algorithm using more specific criteria [17]. It is in this sense that using ANA detection as an entry criterion in the SLE classification can be considered a strategic move. However, it is necessary to insist that this is a classification criterion of limited use in diagnosing or screening. In a clinical context, with relatively low SLE prevalence (low pre-test

probability), positive ANA results should be used to select additional assays to assess other parameters. These additional assays should be determined by the clinical context; however, a detailed account of what parameters should be considered is out of the scope of this paper. Great caution must be exercised in interpreting the results obtained using the 1:80 cut-off point as it is associated with a low positive likelihood ratio, i.e., the correct diagnosis of patients will be primarily based on individual clinical manifestations or characteristics [12,18]. To increase the ANA pre-test probability, patients should be preselected using clinical and analytical criteria, which may only sometimes be feasible. At any rate, our recommendation is to accompany the analytical request with as much pertinent clinical information as possible so that the laboratory can assess the results and decide on subsequent studies. Here it is worth noting that the positive likelihood ratio, and therefore the diagnostic performance, of ANA detection in unselected populations increases when using the 1:160 dilution as the cut-off point, the reason being that specificity reaches 86.2% (IC 95%: 80,4% - 90,5%) while maintaining an appropriate sensitivity (95,8%; IC 95%: 94,1% - 97,1%) [15]. Incidentally, cases with ANA titers between 1:80 and 1:160 may be further elucidated by testing anti-ENA, specifically anti-Ro autoantibodies, as the Ro ribonucleoprotein has been shown to be a clinically important target of autoantibodies in autoimmune diseases such as SLE, Sjogren's syndrome and others [19].

We are fully aware that our recommendation to include pertinent clinical information in the requests of autoimmunity tests will have different implications depending on the care level of origin:

- Primary care specialists, familiarized with the more general aspects of diagnosing SLE and other autoimmune disorders, and applying a low referral threshold, should be able to add ANA tests to their routine laboratory requests when there is a high degree of clinical suspicion of SLE.
- Secondary care specialists can use the EULAR/ACR 2019 criteria to facilitate standardization of the diagnostic process (bearing in mind that these criteria are not diagnostic per se) so that diagnosis is not merely based on observing some unspecified symptoms and a few serologic tests. In collaboration, secondary care specialists and laboratory professionals may establish standard testing protocols.
- Specialized clinics and centers of excellence provide care for a more selected population (greater pre-test probability) where there may be a high index of suspicion, so ANA detection at titers $\geq$ 1:80 in this context has a high positive predictive value.
- The immunology laboratory professionals must know the level of care where analytical requests are generated to develop realistic expectations about the available clinical information and the most appropriate assays and cut-off points.

In addition to establishing a cut-off point for ANA positiveness, the EULAR/ACR 2019 classification recommends ANA detection using the IFI or a solid-phase ANA screening immunoassay (SPA) with at least equivalent performance. This recommendation has been questioned at two levels. First, there is a remarkable diversity of IFI methods in the market. Some of these are entirely dependent on the operator's expertise, and others, although they can be automatized, still need to be validated by an experienced technician as they do not recognize or do not precisely distinguish all the ANA fluorescence patterns [18]. Second, it has been pointed out that the lack of precision in the concept "equivalent performance" can lead to the use of SPAs with insufficient specificity in SLE screening [20]. In many cases, clinicians need to be made aware of the differences between methods used to detect ANA as can wrongly attribute the characteristics of the IFI assay to other methods [21]. Undoubtedly, introducing other ANA detection techniques will facilitate the use of the classification criteria in sites where the IFI assay is unavailable; this, although not the gold standard, could add to other well-known advantages of using SPAs, such as quantification, greater specificity, and automatization. On the other hand, it means that an effort should be made to find a reference method for standardization. The laboratory report should always detail which method was used; when several methods are used, we recommend reporting the results obtained with each method [21].

2.1.2. Anti-dsDNA autoantibodies

Once the positive ANA entry criterion is met, the anti-dsDNA autoantibodies constitute the most prominent immunological criterion in the EULAR/ACR 2019 classification [8]. However, their use as a biomarker and pathogenic factor of SLE and lupus nephritis seems to have given rise to a general misconception according to which the anti-dsDNA is viewed as a well-defined SLE-specific entity. This conception is far from reality. In the first place, anti-dsDNA can be observed in healthy individuals [22–24] as well as in other autoimmune syndromes [23], bacterial [25–27], viral [28–31] and parasitic infections [32], and cancer [33]. In the second place, the term anti-dsDNA encompasses a great diversity of antibodies with different antigenic specificities, i.e., anti-dsDNA antibodies exhibit the typical heterogeneity of polyclonal responses. Additionally, it has been proved that they target multiple and different DNA structures [2], such as single-stranded DNA (ssDNA) [34], left-handed dsDNA (Z dsDNA) [35–37], elongated and bent right-handed dsDNA (B dsDNA) [38,39], single-stranded RNA (ssRNA), double-stranded RNA (dsRNA) and double-stranded hybrids RNA-DNA [40,41], folded and unfolded cruciform DNA [42,43], bacterial DNA [44,45], several forms of viral dsDNA [46–48], plus a long list of proteins and phospholipids. Therefore, the conception of anti-dsDNA as a uniform group of antibodies that work as a highly specific SLE biomarker targeting a unique form of dsDNA with great sensitivity is incorrect.

It has been well known since long ago that anti-dsDNA sera detected in clinical practice differ in their binding capacity to the different assays in the market. Traditionally, this has been explained in terms of variability in anti-dsDNA avidity or affinity [49,50]. However, according to the model we just outlined, the term anti-dsDNA refers to a heterogeneous set of antibodies with mature affinity (so high avidity is possible), with a whole spectrum of unique specificities for the different DNA structures present in chromatin. Therefore, differences in binding capacity to each assay observed in a patient's serum should be interpreted as a result of the antigenic specificities present in that particular serum, as the antigenic material used in each assay varies (native DNA, plasmid DNA, purified tissue, recombinant DNA or synthetic DNA) (Table 1). This opens the door to a potential role of anti-dsDNA specificity in SLE and lupus nephritis pathogenesis and in SLE classification [2].

The prominent position of anti-dsDNA in the EULAR/ACR 2019 classification may have contributed to creating that misconception of these autoantibodies as a homogeneous entity involved in SLE aetiology. Although it is justified to attribute autoimmune anti-dsDNA-mediated pathogenesis to some criteria, such as nephritis [51–56], skin involvement [57] or some neuropsychiatric disorders [58], an etiologic role has not been demonstrated. Moreover, other classification criteria are independent of the effects of anti-dsDNA. A more homogenous definition of SLE would be desirable. For example, it has been suggested that, restricting the definition to the presence of proteinuria and anti-dsDNA, a homogenous group of patients with lupus nephritis would be selected in whom the pathogenesis of the other signs and symptoms could be studied [9].

The above leads to the conclusion that lupus nephritis, with or without anti-dsDNA, might reflect different pathophysiological situations and, therefore, different syndromes. A deeper knowledge of the etiopathogenic role of anti-dsDNA is necessary. For instance, it should be clarified why some patients remain seropositive and asymptomatic, and the clinical relevance of the different types of anti-dsDNA should be established in extensive studies of homogenous cohorts, using standardized assays, to determine the predictive value of anti-dsDNA with specificity and avidity for well-defined dsDNA structures. This would facilitate defining the different SLE phenotypes and their

Table 1
Main characteristics of anti-dsDNA antibody testing techniques [59].

	RIA		SPA			
	Farr	CLIFT	ELISA	FEIA	CLIA	MPIA
Avidity	High	High	Low and high	Medium and high	Medium and high	NA
Detection	Quantitative	Semiquantitative	Quantitative	Quantitative	Quantitative	Quantitative
Antibody class	IgG, IgM	IgG	IgG	IgG	IgG	IgG
Antigenic source	Native, plasmid	Native	Purified tissue, eukaryotic cells, recombinant, synthetic	Plasmid	Synthetic	Recombinant, Synthetic
Specificity (%)	93	95–100	92,5	94,2	87,4	90,9
Sensitivity (%)	68,9	5,7–83	61,8	54,5	66,5	51,9
Activity correlation	Yes	Positive results suggest disease activity	Variable	Yes	Yes	Yes
User-friendliness	Labor-intensive, radioactivity use	Experienced user	Easy, automated	Easy, automated	Easy, automated	Easy, automated

CIA: Chemiluminescence immunoassay; CLIFT: *Crithidia luciliae* immunofluorescence test; ELISA: Enzyme linked immune sorbent assay; FEIA: fluorometric enzyme-linked immunoassay; IgG: Immunoglobulin G; IgM: Immunoglobulin M; MPIA: multiplex immunoassay; NA: Not available; RIA: Radioimmunoassay; SPA: solid-phase assay.

epidemiological weight. At the same time, studying these phenotypes' non-nephrological signs and symptoms would help clarify anti-dsDNA cross-reactivity's pathogenic relevance. This effort would result in the obtention of better biomarkers and the design of a superior SLE theoretical model, which would ultimately contribute to an improved diagnosis and the development of more effective target therapies.

The current situations of anti-dsDNA and ANAs are similar in that there are critical gaps in knowledge of their role in SLE and their usefulness as biomarkers. Still, clinical practice must go on and the immunology laboratory must provide the best possible answer to the needs of patients. The Fig. 1 shows an algorithm for the interpretation of the lab tests used in SLE diagnosis.

Anti-dsDNA detection is conducted using different assays that vary in methodology, sensitivity, specificity, avidity, and type of antigen (Table 1); therefore, inter-assay correlation can be low [59]. A recent comparative study has shown that harmonization and commutability of

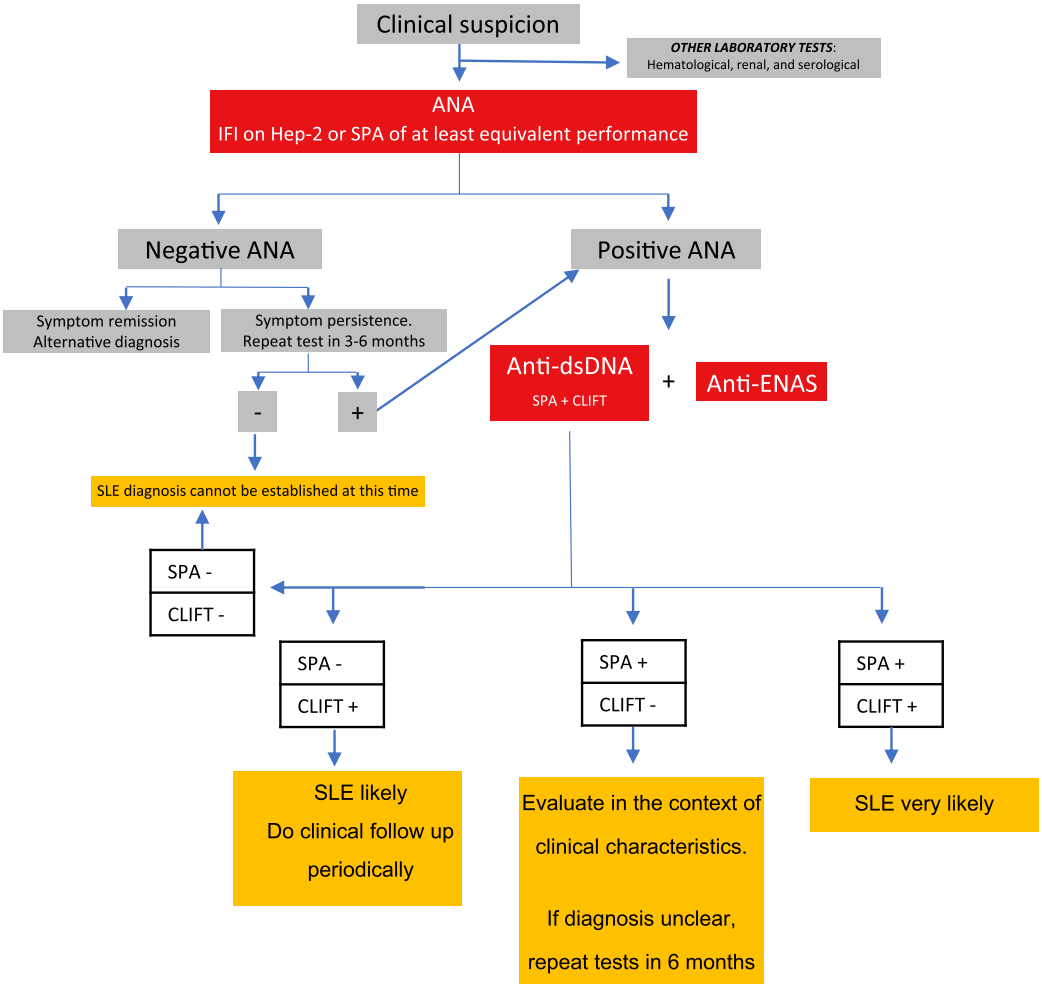

Fig. 1. Algorithm for the interpretation of lab tests in the management of patients with suspected SLE.

anti-dsDNA antibody testing remains an unmet need because of the high inter-method variability [60].

The guidelines recommend testing the presence of anti-dsDNA in all ANA-positive cases with clinical suspicion of SLE (Fig. 1). When ANA are negative, investigating the presence of anti-dsDNA is not recommended except when there is a high degree of clinical suspicion [21,61,62]. If the laboratory receives the pertinent clinical information, or if this is provided under request, anti-dsDNA can be recommended or initiated, especially if the IFI pattern highly suggests anti-dsDNA.

The reference method for anti-dsDNA detection is considered the Farr assay; however, this assay is currently not used in clinical practice because it is a radioimmunoassay (RIA) that cannot be automatized. Instead, the immunology laboratory can use the CLIFT, considered pathognomonic, but with very low sensitivity, and the SPAs (FEIA, CLIA, ELISA and multiplex assay), which are less specific and have varying sensitivity [12] (Table 1).

Our recommendation for anti-dsDNA testing is a double-screening strategy using a last-generation SPA in the first place and, subsequently, the CLIFT as the confirmation test. A summary of the advantages and disadvantages of each technique, using FEIA as an example of SPA, is shown in Table 2.

A summary of the four potential results in anti-dsDNA double screening is shown in Table 3. Although some laboratories routinely perform both steps of the double screening simultaneously, a sequential strategy, doing the most sensitive assay (SPA) first ensures minimization of false positive results obtained with the SPA. At any rate, guideline recommendations establish that results obtained with each method should be reported explaining their significance and clinical relevance [21].

In the event of a negative SPA result, our recommendation is to do a confirmatory CLIFT only when other signs of clinical suspicion are present. Without signs of clinical suspicion, the result can be reported directly as negative anti-dsDNA, and the SLE diagnosis would be ruled out. However, if the CLIFT assay was done, and produced a positive result, as opposed to the SPA, this inconsistency should be explained. The laboratory may recommend repeating the anti-dsDNA assays in a new sample. If the inconsistency continues, the diagnosis will fundamentally depend on the patient's clinical characteristics. The patient should undergo clinical follow-up periodically as anti-dsDNA antibodies may be detected long before the development of clear clinical signs. The follow-up interval should be determined by the clinical findings and applying the clinician's individual judgement.

The situation is also complex, although relatively frequent, when the most sensitive test (SPA) is positive, and the most specific test (CLIFT) is negative. This result neither calls for an unreserved SLE diagnosis, nor does it constitute a sufficiently solid argument to rule it out. There are cases with persisting clinical suspicion, and positive ANA, that have revealed positive anti-nucleosome tests. This is likely down to the anti-nucleosomes preceding the ANA in the SLE pathogenesis. In a recent report, IgG anti-nucleosome antibodies showed 83.33% sensitivity and 96.67% specificity for SLE [63]. In addition, when the clinical findings are consistent with SLE, detection of antiphospholipid antibodies (anticardiolipin, anti-β2GP1, and/or lupus anticoagulant or antibodies against a mixture of phosphatidylserine, phosphatidic acid and β2GP1) increases the likelihood of SLE as 30–40% of SLE patients are positive for antiphospholipid antibodies [64].

Table 2
Comparison of the FEIA and CLIFT assays in SLE diagnosis [59].

	FEIA	CLIFT
Advantages	No need of trained staff Fast Quantitative: it would facilitate the implementation of the ISO 15189 norm Can be automatized. Sensitive: facilitates diagnosis and is more adequate for screening	More specific Greater clinical correlation Semiquantitative Can be automatized* Lower cost
Disadvantages	Unspecific (especially at low titers) Lacking clinical correlation in some instances	Variability between *Chritidia luciliae* substrates Inter-observer variability Requires specialized staff to get the reading

CLIFT: *Crithidia luciliae* immunofluorescence test; FEIA: fluorometric enzyme-linked immunoassay.

* There are methods that provide a digitalized image, but results must always be validated.

Table 3
Potential combinations of results in anti-dsDNA double screening.

FEIA	CLIFT	Report*	Interpretation
+	+	anti-dsDNA+	SLE very likely
+	–	Inconclusive	Dependent on the clinical characteristics, IFI pattern of ANA and other tests (proteinuria, complement, antiphospholipid, anti-nucleosome, anti-ENA)
–	–	anti-dsDNA-	No SLE diagnosis can be established at this time
–	+	anti-dsDNA+	SLE likely Patient should be followed up periodically.

ANA: antinuclear autoantibodies; anti-dsDNA: anti double-stranded DNA autoantibodies; anti-ENAs: anti-extractable nuclear antigens; CLIFT: *Crithidia luciliae* immunofluorescence test; FEIA: fluorometric enzyme-linked immunoassay; IIF: indirect immunofluorescence on HEp-2 cells; SLE: systemic lupus erythematosus.

* Both the FEIA and CLIFT results must be included in the lab report.

2.1.3. Anti-ENA autoantibodies

When ANAs turn out positive during diagnosis, confirmatory testing of anti-extractable nuclear antigens autoantibodies (anti-ENA) is recommended. If anti-ENA and ANA results or the clinical presentation are contradictory, international detection methods, reports, and discrepancy resolution recommendations should be followed [21].

The most common targets of the autoantibodies detected in SLE are [16]: chromatin, ribosomal P protein, spliceosome small nuclear ribonucleoproteins (Smith), Sjögren syndrome type A antigen (Ro), Sjögren syndrome type B antigen (La), and U1-ribonucleoprotein.

2.2. The immunology laboratory in SLE monitoring

Long-term management of SLE diagnosed patients requires periodic follow-up. Using ANA to monitor disease activity and progression is not recommended in the guidelines [61], so repeating the ANA assay when monitoring patients after a positive result is neither appropriate nor cost-effective [65,66]. Anti-dsDNA can be used to monitor disease activity. A quantitative assay should be used, preferably with the same method used in the diagnosis and performed by the same laboratory [21]. The CLIFT assay may be used for confirmation purposes and to ascertain a potential clinical correlation.

It should be noted that there are patients that lack a correlation between serological results and clinical characteristics. These are patients with quiescent, but serologically active, SLE [67], and patients with diagnosis of lupus nephritis (membranous), but negative anti-dsDNA. Both situations can be maintained in the long-term [68–70]. Anti-nucleosome antibodies can be used to monitor the disease activity of patients with lupus nephritis who remain anti-dsDNA negative [71,72]. Furthermore, anti-histone antibodies (H1, H2A, H2B, H3 and H4) are more prevalent in patients with lupus nephritis than in patients without kidney disease [73,74]; however, as they have the disadvantage of being more frequently found in drug-induced SLE [75–77], anti-histone antibodies can play a role in disease monitoring when lupus nephritis is confirmed to be not secondary to any drug treatment.

Additionally, low levels of the complement protein C1q have been

associated with SLE flare-ups and the observation of anti-C1q antibodies [78,79]. Prevalence of anti-C1q antibodies in patients with SLE varies between 30% and 60%, and they are found in almost 100% of patients with active lupus nephritis [80,81]; therefore, it has been suggested that the anti-C1q could be disease-activity biomarkers [82]. The critical negative predictive value of these antibodies must be emphasized, i.e., patients with lupus nephritis are doubtful to experience a flare-up in the absence of anti-C1q [83–85]. Also, it has been speculated that anti-C1q antibodies could play an active role in SLE pathogenesis [85].

Table 4 summarizes the advantages and disadvantages of the FEIA and CLIFT assays in SLE follow-up. We recommend always using anti-dsDNA and complement levels in patient follow-up, even if they were negative/normal in previous monitoring visits.

The heterogeneity of SLE clinical manifestations and outcomes has hindered the development of disease-assessing tools that show good clinical correlation and help make comparisons, study disease progression, and evaluate treatment response, especially when using treat-to-target strategies [86]. Different types of indexes have been proposed for SLE:

i. Clinical indexes of overall activity, such as the Physician Global Assessment (PGA) [87], the Systemic Lupus Erythematosus Disease Activity Score (SLE-DAS) [86], and the SLE Disease Activity Index (SLEDAI) [88], and its modifications SLEDAI-2 K [89], SLEDAI-2KG [90], which include steroid doses in the evaluation of disease activity and SLEDAI-2 K RI-50, or SELENA-SLEDAI [86]. The index designed by the British Isles Lupus Activity Group (BILAG) is also in this group of tools [91].
ii. Indexes that measure accumulated damage, such as the Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index (SDI) [92].
iii. Composite indexes based on activity indexes, such as the SLE Responder Index (SRI), which combines items from the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI, PGA and BILAG [93]; and the BILAG-Based Composite Lupus Assessment (BICLA) [94].

Clinical experience with these indexes has been gathered mainly in clinical trials. Several authors have reviewed their advantages and disadvantages, as well as the needs that should be met by optimal activity indexes. Reports highlight the need to develop organ-specific metrics (skin, kidney, and joint involvement) combined with generic disease activity indexes. All analyses are limited to the context of clinical research, emphasizing the challenges of finding the ideal reference method, and concluding that these time-consuming tools are inappropriate for clinical practice [86,93,95]. Nevertheless, international guidelines recommend monitoring SLE activity using indexes that have been validated, such as the SLEDAI and the PGA [96].

Table 4
Comparison of the FEIA and CLIFT assays in SLE monitoring [59].

	FEIA	CLIFT
Advantages	Quantitative monitoring It can predict relapses Quick and objective	Reinforces decision making
Disadvantages	Lack of clinical correlation in some situations	Semiquantitative information Should not be the only method used

CLIFT: *Crithidia luciliae* immunofluorescence test; FEIA: fluorometric enzyme-linked immunoassay.

2.3. Summary of recommendations

2.3.1. SLE diagnosis

- In a clinical context, positive ANA results should be used to select additional assays to assess other parameters.
- Confirmatory testing of anti-ENA is recommended when ANAs are positive in the diagnosis process.
- Analytical requests should include pertinent clinical information so the laboratory can assess the results and decide on subsequent studies.
- In situations with a high degree of clinical suspicion of SLE, primary care specialists should be able to add ANA-tests to their routine lab requests.
- Secondary care specialists and laboratories may establish standard testing protocols based on the EULAR/ACR 2019 criteria.
- Immunology laboratory professionals must make realistic expectations about the available clinical information and the most appropriate assays and cut-off points pertinent to the level of care requesting the study.
- In unselected populations the 1:160 dilution should be the cut-off point for ANA detection.
- New ANA detection methods other than the IFI mean that an effort should be made to produce a reference method for standardization.
- The laboratory should include the method used in their reports; if several methods are used, the results obtained with each method should be reported.
- Anti-dsDNA testing should follow a double-screening strategy using a last-generation SPA in the first place, and subsequently, the CLIFT as the confirmation test.
- In the event of a negative anti-dsDNA SPA result, a confirmatory CLIFT should be done only when other signs of clinical suspicion are present. Without a clinical suspicion, the result should be reported directly as negative anti-dsDNA

2.3.2. SLE monitoring

- A quantitative assay testing anti-dsDNA should be used to monitor disease activity, preferably with the same method used in diagnosis and performed by the same laboratory.
- Anti-dsDNA and complement levels should be measured in patient follow-up, even if they were negative (anti-dsDNA) / within the reference range (complement) in previous monitoring visits.

We conclude our discussion with a brief list of points that, in our view, represent areas of improvement to facilitate the immunology laboratory performance at SLE diagnosing and monitoring:

- There is a need to study the diagnostic performance of the anti-dsDNA detection methods (such as predictive values, and likelihood ratios…) in different populations, and the clinical correlation between methods in each of those populations.
- We need to urge manufacturers to evaluate and improve the specificity of SPAs.
- Lastly, we would like to raise awareness of the need to conduct a homogenous, multicentric, long-term study to assess the outcomes of patients with inconsistent SPA and CLIFT results. Such a study would help clarify the diagnostic value of the CLIFT assay and explain why some patients show a negative CLIFT with a clearly positive SPA.

3. Conclusions

- Observation of anti-dsDNA autoantibodies is associated with SLE plus several other diseases.

- Non-correlation between methods used in anti-dsDNA determination may reflect differences in the antigenic specificity of the autoantibodies and in the antigens used as substrate.
- Receiving relevant clinical information will help laboratories validate results and make valuable recommendations to diagnose and monitor the SLE patient.
- Given the polyclonal character of the SLE autoimmune response, a 100% sensitive and specific diagnostic method may never be achieved.
- Realistic and feasible objectives should be established, such as improving the cost-benefit ratio and applicability of available methods in broader populations, detecting more antigens, with reproducible results in different laboratories.
- More studies are needed to improve the diagnostic value of anti-dsDNA assays in different populations.

4. Take-home messages

- Implementing our recommendations will require fluent communication between clinicians and the laboratory.
- A better understanding of the immunoassays used in anti-dsDNA determination will help clinicians make laboratory requests suited to their patient population and interpret ANA and anti-dsDNA results.
- Further research is needed to consolidate the usefulness of anti-dsDNA determination in SLE diagnosis and follow-up.

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Data availability

No data was used for the research described in the article.

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