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ORIGINAL ARTICLE

Laboratory and clinical practices in antinuclear antibody detection and related antigens: recommendations from a Spanish multicentre survey

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Abstract

Antinuclear antibodies (ANA) are the most widely used immunological test for the diagnosis of autoimmune diseases. Despite the recommendations of experts, there is some variability in performing and interpreting this test in routine practice. In this context, the Spanish Group on Autoimmune Diseases (GEAI) of the Spanish Society of Immunology (SEI) conducted a national survey of 50 autoimmunity laboratories. Here we report the survey results on ANA testing, detection of related antigens, and our recommendations. The survey showed that most of the participating laboratories use a similar approach for most key practices: 84% perform ANA by indirect immunofluorescence (IIF) on HEp-2 cells as the screening methodology while the other laboratories use IIF to confirm positive screens; 90% report ANA test results as either negative or positive with titer and pattern; 86% indicated that the ANA pattern conditioned follow-up testing for specific antigen-related antibodies; and 70% confirm positive anti-dsDNA. However, testing practices were highly heterogeneous for certain items, such as sera dilutions and the minimum time period for repeating ANA and related antigen determinations. Overall, this survey shows that most autoimmune laboratories in Spain use a similar approach but that further standardization of testing and reporting protocols is needed.

Keywords Antinuclear antibodies (ANA) · Indirect immunofluorescence (IIF) · HEp-2 cells · ICAP · Survey

Collaborating members of the GEAI-SEI group are listed in the Acknowledgements.

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Introduction

Antinuclear antibodies (ANA) testing is the most widely used method for the diagnosis of several autoimmune diseases, particularly systemic and autoimmune liver diseases [1, 2]. The clinical value of ANA testing has been well-established [3]. Specifically, the latest 2019 EULAR/ACR classification criteria for systemic lupus erythematosus establish positive ANA as the only obligatory entry criterion [4], which underscores the importance of ANA determination in the study of systemic autoimmune diseases. The most common ANA screening method is indirect immunofluorescence (IIF) on HEp2 cells [5]. However, several variables can influence the results of IIF, including the HEp-2 substrate, the serum screening dilution ratio, and the operator's interpretation. Guidelines on ANA testing by IIF recommend reporting both the titer and the staining patterns observed in HEp-2 cells, including not only nuclear patterns (e.g., homogeneous or speckled), but also cytoplasmic and mitotic patterns [6, 7]. The pattern is important because it helps to determine follow-up testing for specific autoantibodies. It is worth noting that solid phase assays (SPA) are increasingly being used in clinical laboratories to screen for ANA, although they do not provide information on patterns [2]. HEp-2 pattern recognition by IIF has undergone a revolution in recent years, mainly due to the development of automated computer-assisted diagnosis (CAD) platforms. Despite significant advances in this area, we are still far from achieving our ultimate goal of fully automated testing because although CAD-based technology is useful for initial screening, it is still insufficient to detect all the expert-level patterns defined by the International Consensus on ANA Patterns (ICAP) and mixed HEp-2 patterns [8, 9].

In 2015, the ICAP made a comprehensive effort to develop an alphanumeric nomenclature to harmonize the names and descriptions of HEp-2 patterns [10]. This report represents a major milestone in the standardization of this technique. Although the use of this nomenclature has been steadily increasing worldwide, many laboratories have not yet adopted this system.

Despite the routine use of ANA testing for autoimmune disease, no clear, well-established criteria are available on how to perform the technique and interpret the results. For this reason, the Spanish Group on Autoimmune Diseases (GEAI) from the Spanish Society of Immunology (SEI) considers it necessary to determine how autoimmunity laboratories in Spain perform and interpret ANA testing and how they determine related antigen specificities. The GEAI-SEI was created to promote closer contact among healthcare professionals specializing in the diagnosis of autoimmune diseases. This group carries out a range of activities, including workshops on autoimmune diseases, which provide useful information not only to laboratory specialists but also to clinicians, thereby improving the management and interpretation of autoantibody detection [11].

In this context, we surveyed autoimmunity laboratories in Spain. The GEAI committee then met to assess the results of the survey in order to establish consensus-based recommendations for the determination of ANA and antigen specificities.

Materials and methods

Participants

The survey was sent to a total of 65 centres, including members of the GEAI and the autoimmunity group of the Spanish Society of Clinical Chemistry (SEQC). Of these, 50 autoimmunity laboratories, uniformly distributed throughout Spain, completed the survey.

Survey

The survey (Table 1) consisted of 36 multiple choice questions to assess the following:

- ANA testing methodology: screening; substrate; screening, final and estimated dilutions; use or not of an automated computer-assisted system.
- Reporting of results: titer, pattern, and ICAP nomenclature.
- Criteria for performing (or not) ANA-related antigen specificities, such as anti-dsDNA (double-strand DNA), anti-ENA (extractable nuclear antigen antibodies), and anti-nucleolar and anti-cytoplasmic antibodies.
- Frequency of test repetition for ANA and antigen specificities.
- Methods used to identify ANA-related antigen specificities.
- Patterns associated with autoimmune liver diseases.

Statistical analysis

Statistical analysis was performed with the IBM-SPSS statistical software, version 24. For each survey question, consensus was defined when $\geq 75\%$ of all participating laboratories selected the same response option.

Table 1 Survey questions, results and responses

	Question	Results, n (%)	Responses
1	What ANA detection method do you primarily use for initial screening?	38 (76%)	IIF
		4 (8%)	ELISA with cell extracts and recombinant antigens (SPA)
		2 (4%)	ELISA/FEIA/CLIA with antigenic mixtures (SPA)
		2 (4%)	Multiplex bead technology
		2 (4%)	IIF and ELISA with cell extracts and recombinant antigens (SPA)
		2 (4%)	IIF and ELISA/FEIA/CLIA with antigenic mixtures (SPA)
2	What substrate do you use for IIF?	36 (72%)	HEp-2 cells
		9 (18%)	HEp-2000 cells
		4 (8%)	Other variants of HEp-2 cells
		1 (2%)	HEp-2 cells and Rat liver
3	If you use a method other than IIF for the initial screening, do you use IIF in any case?	10 (100%)	Yes
		0 (0%)	No
4	Do you use different ANA methods for initial screening?	10 (20%)	Yes
		40 (80%)	No
5	What criteria do you use to select the screening method?	3 (30%)	According to the origin of requesting physician
		3 (30%)	According to diagnosis
		1 (10%)	According to clinical information
		2 (20%)	According to the origin of requesting physician and diagnosis
		1 (10%)	According to the origin of requesting physician and diagnosis and clinical information
6	What screening dilution ratio do you use?	3 (6%)	1/40
		19 (38%)	1/80
		26 (52%)	1/160
		2 (4%)	1/100
7	Do you dilute the positive sera or estimate the final dilution?	2 (4%)	No dilution
		7 (14%)	Serial (twofold) dilution
		11 (22%)	Alternate (fourfold)) dilution
		23 (46%)	Estimated dilution
		2 (4%)	Serial (twofold) and estimated dilution
		3 (6%)	Alternate (fourfold)) and estimated dilution
		2 (4%)	No response
8	What is the highest reported titer?	2 (4%)	1/100
		4 (8%)	1/320
		11 (22%)	1/640
		20 (40%)	1/1280
		6 (12%)	1/2560
		6 (12%)	1/5120
		1 (2%)	No response
9	Do you use IIF automated computer-assisted interpretation for ANA determination (titer and/or pattern)?	28 (56%)	No
		7 (14%)	Yes, but only screening positive vs. negative
		2 (4%)	Yes, for screening and titer
		11 (22%)	Yes, for screening, titer and pattern
		2 (4%)	No response
10	When you use IIF, how do you report the results?	1 (2%)	Positive/Negative
		45 (90%)	Positive (titer and pattern)/Negative
		1 (2%)	Positive (only titer)/Negative
		3 (6%)	No response
11	Is there any pattern that you do not titer and only report as positive?	10 (20%)	Yes
		39 (78%)	No
		1 (2%)	No response
12	Do you use the ICAP nomenclature for the definition of IIF patterns on HEp-2 cells?	24 (48%)	Yes
		13 (26%)	No
		13 (26%)	No response
13	If you use a screening method other than IIF, how do you report the results?	8 (80%)	Quantitative
		1 (10%)	Positive/Negative
		1 (10%)	Negative and Quantitative when positive

Table 1 (continued)

	Question	Results, n (%)	Responses
14	When the ANA result is negative, do you perform anti-dsDNA or anti-ENA antibody determinations if requested?	9 (18%)	Yes, in any case
		7 (14%)	No, in all cases
		12 (24%)	Only if cytoplasmic specificities are requested
		9 (18%)	According to ENA
		6 (12%)	According to clinical information
		1 (2%)	No, in all cases and according to clinical information
		1 (8%)	Only if cytoplasmic specificities are requested and according to ENA
		1 (2%)	Only if cytoplasmic specificities are requested and other
		1 (2%)	According to ENA and clinical information
15	When the ANA result is positive, do you perform anti-dsDNA or anti-ENA antibody determinations even if not requested?	22 (44%)	Yes
		1 (2%)	No
		21 (42%)	According to pattern/titer
		3 (6%)	According to pattern and clinical information
		1 (2%)	Ye, according to pattern/titer
		1 (2%)	According to pattern/titer and clinical information
		1 (2%)	No response
16	Does the ANA pattern condition the performance of ANA specificities?	43 (86%)	Yes
		6 (12%)	No
		1 (2%)	No response
17	How do you determine ANA specificities?	29 (58%)	Screening with antigenic mixtures (ELISA, CLIA, FEIA)
		12 (24%)	Line blot
		1 (2%)	Antigenic specificities using ELISA/CLIA/FEIA
		4 (8%)	Multiplex bead technology
		4 (8%)	Line blot and antigenic specificities using ELISA/CLIA/FEIA
18	How do you confirm the different positivities?	3 (9%)	FEIA
		4 (12%)	CLIA
		15 (45%)	Line blot
		3 (9%)	Multiplex bead technology
		1 (3%)	ELISA and Line blot
		5 (15%)	FEIA and Line blot
		2 (6%)	CLIA and Line blot
19	Have you established a minimum period of time for the repetition of ANA?	21 (42%)	No
		1 (2%)	1 month
		2 (4%)	2 months
		18 (36%)	3 months
		1 (2%)	4 months
		4 (8%)	6 months
		2 (4%)	12 months
		1 (2%)	No response
20	In the case of a patient whose initial diagnosis is under study, when should the ANA should be repeated?	5 (10%)	Never
		1 (2%)	1 month
		4 (8%)	2 months
		18 (36%)	3 months
		1 (2%)	4 months
		1 (2%)	6 months
		1 (2%)	12 months
		8 (16%)	No response
21	In a patient with a definitive diagnosis, when should ANA testing be repeated?	22 (44%)	Never
		1 (2%)	2 months
		4 (8%)	3 months
		16 (32%)	6 months
		1 (2%)	12 months
		6 (12%)	No response
22	In patients with a well-defined ANA pattern that is clearly associated with a particular specificity, should ANA determination be repeated?	16 (32%)	Yes
		31 (62%)	No
		3 (6%)	No response

Table 1 (continued)

	Question	Results, n (%)	Responses
23	Has your laboratory established a minimum period of time for the repetition of anti-dsDNA antibodies?	6 (12%)	Yes, always
		34 (68%)	No, never
		6 (12%)	Yes, but only if anti-dsDNA antibodies are negative
		2 (4%)	According to clinical information
		2 (4%)	No response
24	Has your laboratory established a minimum period of time for the repetition of ENA?	10 (20%)	Yes, always
		14 (28%)	No, never
		15 (30%)	Yes, but only if ENAs are negative
		3 (6%)	Only if negative
		7 (14%)	According to clinical information
		1 (2%)	No response
25	What is the initial method used to determine anti-dsDNA antibodies?	6 (12%)	CLIFT
		4 (8%)	ELISA
		11 (22%)	FEIA
		1 (2%)	RIA
		2 (4%)	Line blot
		19 (38%)	CLIA
		4 (8%)	Multiplex bead technology
		1 (2%)	CLIFT and CLIA
		1 (2%)	CLIFT and FEIA
		1 (2%)	No response
26	When the initial test result for anti-dsDNA antibodies is positive, do you use another technique to confirm the result?	35 (70%)	Yes
		15 (30%)	No
27	If yes, indicate the technique that you use:	21 (60%)	CLIFT
		1 (3%)	ELISA
		5 (14%)	FEIA
		1 (3%)	RIA
		1 (3%)	Line blot
		2 (6%)	CLIA
		2 (6%)	Multiplex bead technology
		1 (3%)	CLIFT and ELISA
		1 (3%)	ELISA and Line blot
28	When the initial test result for anti-dsDNA antibodies is negative, do you use another technique to confirm the result?	1 (2%)	Yes
		28 (56%)	No
		18 (36%)	Yes, if symptoms are suggestive and/or hypocomplementemia
		3 (6%)	No response
29	How do you determine the presence of anti-centromere antibodies?	12 (24%)	IIF
		27 (54%)	Line blot
		3 (6%)	IIF and confirmation method if pattern is suggestive
		3 (6%)	IIF and Line blot
		1 (2%)	CLIA and Line blot
		1 (2%)	IIF, CLIA and Line blot
		1 (2%)	Multiplex bead technology, IIF, FEIA and Line blot
		2 (4%)	No response
30	With regard to cytoplasmic patterns (AC-19, AC-20), do you perform antigenic specificities related with these patterns even if not requested?	28 (56%)	Yes
		3 (6%)	No
		19 (38%)	Yes, if ENAs are negative and symptoms are suggestive
31	If yes, indicate which specificities you analyze:	10 (20%)	Myositis-specific antigens (including Ro52)
		1 (2%)	Ribosomal-P
		20 (40%)	According to the pattern
		1 (2%)	According to the pattern and clinical information
		15 (30%)	Myositis-specific antigens (including Ro52) and Ribosomal-
		1 (2%)	Myositis-specific antigens (including Ro52), Ribosomal-P and according to the pattern
		1 (2%)	Myositis-specific antigens (including Ro52), Ribosomal-P and according to clinical information

Table 1 (continued)

	Question	Results, n (%)	Responses
32	Which technique do you use?	1 (2%)	IIF
		2 (4%)	ELISA
		32 (64%)	Line blot
		2 (4%)	IIF and Line blot
		2 (4%)	CLIA and Line blot
		5 (10%)	Line blot and Dot blot
		1 (2%)	IIF, ELISA and Line blot
		2 (4%)	Multiplex bead technology, FEIA, Dot blot and Line blot
		3 (6%)	No response
33	With regard to nucleolar patterns (AC-8, AC-9, AC-10), do you perform antigenic specificities related with these patterns even if not requested?	15 (30%)	Yes
		6 (12%)	No
		16 (32%)	According to IIF titer
		10 (20%)	Yes, if ENAs are negative and symptoms are suggestive
		3 (6%)	According to IIF titer and Yes, if ENAs are negative and symptoms are suggestive
34	Which technique do you use?	1 (2%)	IIF
		36 (72%)	Line blot
		2 (4%)	ELISA and Line blot
		2 (4%)	Line blot and Multiplex bead technology
		1 (2%)	CLIA, ELISA and Line blot
		2 (4%)	Multiplex bead technology, FEIA, Dot blot and Line blot
		6 (12%)	No response
35	With regard to nuclear or cytoplasmic patterns associated with autoimmune hepatitis, do you perform antigenic specificities related with these patterns even if not requested?	47 (94%)	Yes
		2 (4%)	No
		1 (2%)	No response
36	Which technique do you use?	3 (6%)	IIF (specific substrate)
		2 (4%)	ELISA
		24 (48%)	Line blot
		13 (26%)	IIF and Line blot
		2 (4%)	IIF, Dot blot and Line blot
		1 (2%)	IIF, CLIA and Line blot
		5 (10%)	No response

Results

The completed survey was returned by 50 of the 65 laboratories (77% response rate). Table 1 shows the 36 multiple choice questions and the number and frequency of each response. For many of the questions, more than one response was possible.

The most common screening methodology to determine ANA was IIF (84% of centres; question [Q]1, Fig. 1A) on HEp-2 cells and their variants (Q2, Fig. 1B). The laboratories that did not use IIF as the primary screening method (16% of laboratories) mainly used SPA (12%), such as ELISA (enzyme-linked immunosorbent assay) with cell extracts and recombinant antigens (8%), or ELISA, FEIA (fluoroenzyme immunoassay), or CLIA (chemiluminescence) with antigenic mixtures. In cases with a positive SPA screening test result, all of the laboratories indicated that they perform IIF on HEp-2 to characterize these ANA (Q3). Only two laboratories (4%) used multiplex bead technology as a screening methodology (Q2).

Most laboratories (80%) do not use different ANA methods for initial screening (Q4). Among the laboratories that do use a different method, the choice is influenced by the requesting physician and/or the patient's diagnosis (Q5). Most of the responding laboratories (90%) use a screening dilution ratio of 1:80 or 1:160 (38% and 52%, respectively; Q6). However, there was no consensus on whether to dilute, estimate, or perform serial twofold dilutions of positive sera (Q7). Similarly, there was no agreement among respondents regarding the maximum dilution ratio (Q8); however, the most commonly reported ratio was 1/1280 (40% of respondents). More than half (56%) of the participating laboratories do not use automated computer-assisted interpretation (Q9). The laboratories that use this methodology do so only to screen for positive vs. negative results (14%), or for screening and titer (4%), or for screening, titer, and pattern (22%).

Most laboratories (90%) report ANA test results as either negative or positive, together with titer and pattern (Q10). However, 20% of these laboratories do not titrate positive samples with specific patterns (Q11). Nearly half of the

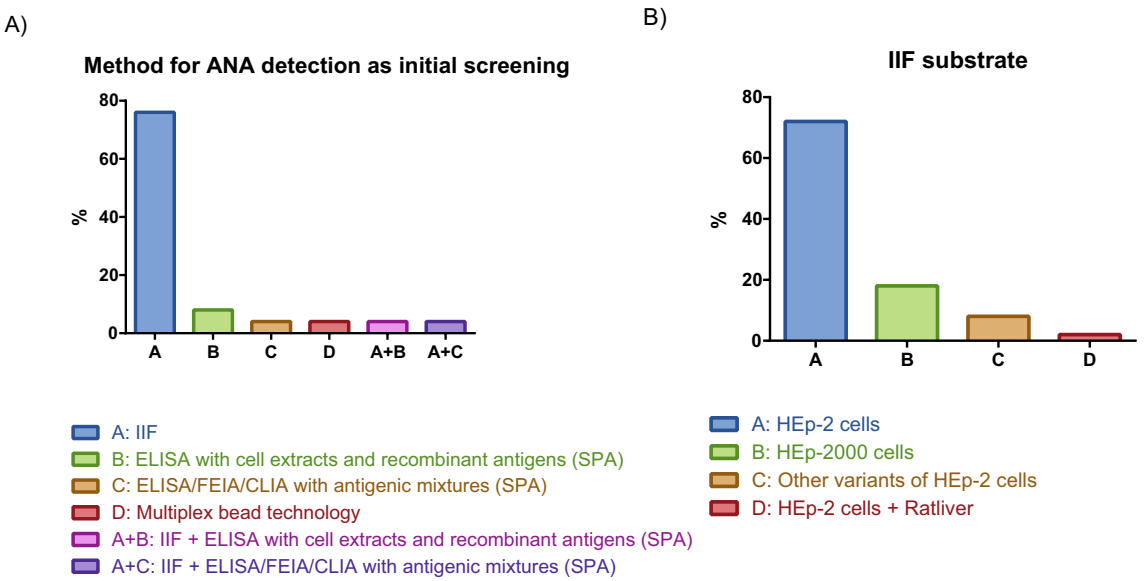

Fig. 1 **A** Initial screening method for antinuclear antibodies (ANA) detection. (**B**) Substrate used for indirect immunofluorescence (IIF) assay

laboratories (48%) use the ICAP nomenclature for pattern designation (Q12). In the laboratories ($n = 10$) that use SPA for screening, 80% (8/10) report quantitative results (Q13).

Given that ANA and related autoantibody tests are often requested at the same time, the laboratories must decide whether to perform the autoantibody tests or not. One of the survey questions (Q14) asked the respondents to indicate whether they performed specificities for related antigens in cases with a negative ANA test. Responses to this question were highly variable, as follows: 18% of respondents perform autoantibody detection in all cases, 14% do not perform antigen specific tests in this case, and the remaining laboratories (68%) base their decision on the requested specificity and on the clinical information.

In the presence of a positive ANA test, laboratory practices were highly variable with regard to testing for related antigen specificities (Q15). However, for most laboratories (86%), the decision to perform these specificities is based on the ANA pattern (Q16). In these cases, the first step for 58% of laboratories is to use a screening test containing mixtures of the clinically relevant antigens (Q17). If this first test is positive, the result is then confirmed by a different methodology (i.e., ELISA, FEIA, CLIA, or line blot) using individualized antigens (Q18).

The minimum time interval for repeating ANA testing (e.g., for the initial or definitive diagnosis) was highly heterogeneous (questions 19 to 22). Similarly, the protocols used to test for related antigens, including anti-dsDNA, anti-ENA, or cytoplasmic antigens, were also highly variable (Q23 and Q24).

The two most commonly used testing methods for anti-dsDNA antibodies were CLIA (42%) and FEIA (22%) (Q25, Fig. 2A). Most laboratories (70%) confirm positive anti-dsDNA results by another technique (Q26, Fig. 2B), mainly IIF in *Crithidia luciliae* cells (CLIFT) (Q27). Even though Farr radioimmunoassay is the reference method for anti-dsDNA antibodies testing, clinical laboratories rarely use this method because it requires radioactive materials, which has numerous drawbacks: it entails risks for personnel and the environment, and it requires custom-built spaces, highly qualified personnel, and high costs. Interestingly, a substantial proportion of the laboratories (38%) verify negative anti-dsDNA results (mainly in patients with suggestive symptomatology or in the presence of hypocomplementemia due to consumption by immune complexes) by performing another test using a different technique (Q28). To detect centromere antibodies, 54% of laboratories use only line blot assays while 24% use only IIF on HEp-2 cells (Q29).

The autoantibodies tested depend on the specific HEp-2 pattern. For cytoplasmic patterns AC-19 and AC-20, most of the laboratories (94%) perform specificities for related antigens, even if not requested by the clinician (Q30). However, 38% of the respondents only determine specificities in cases with a negative anti-ENA screening test and the presence of suggestive symptoms (Fig. 3A). The specificities analysed were highly variable among the laboratories (Q31). In nucleolar patterns (AC-8, 9, 10), determination of specificities is conditioned by the titer (question 33, Fig. 3B). For cytoplasmic and nucleolar specificities, most laboratories use line blot (64% and 72%, respectively; Q32 and 34).

Finally, in cases presenting nuclear or cytoplasmic patterns associated with autoimmune liver diseases, 94% of the laboratories determine the antigenic specificities associated with these patterns, even if not requested (Q35 and 36).

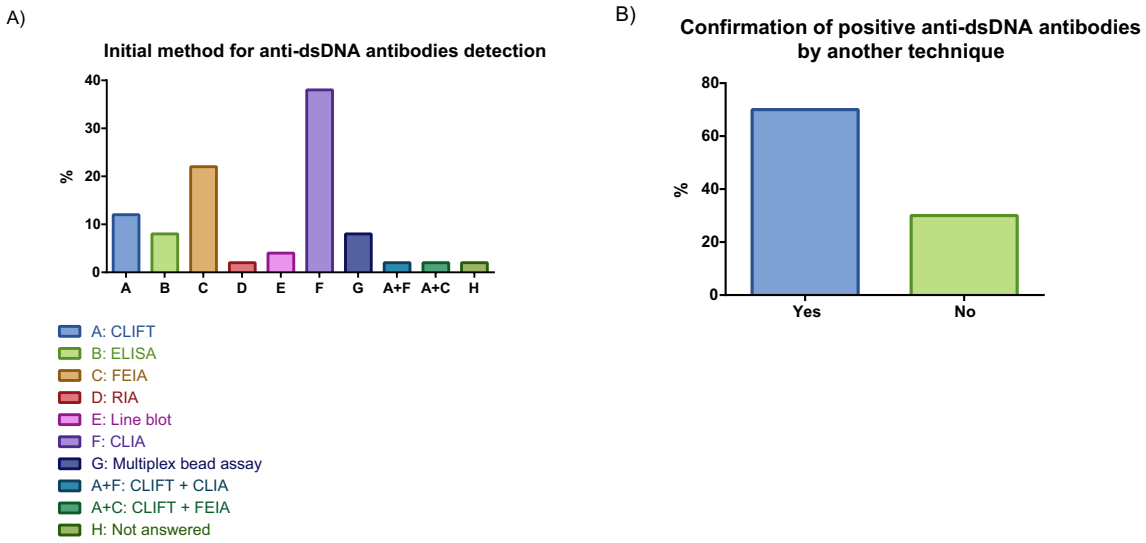

Fig. 2 **A** Initial method for anti-dsDNA antibodies detection. (**B**) Confirmation of positive anti-dsDNA antibodies by another technique

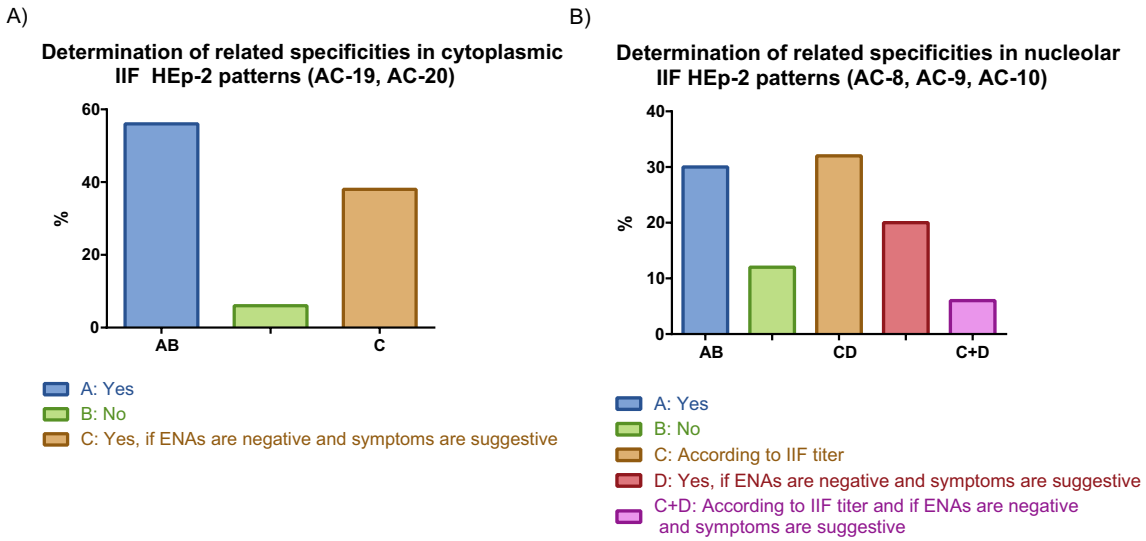

Fig. 3 **A** Determination of related antigenic specificities in cytoplasmic indirect immunofluorescence (IIF) HEp-2 patterns (AC-19, AC-20). (**B**) Determination of related antigenic specificities in nucleolar IIF HEp-2 patterns (AC-8, AC-9, AC-10)

Discussion

ANA autoantibody tests provide highly valuable data for the diagnostic work-up, which is why these are the most commonly requested tests by physicians in patients when autoimmune disease is suspected [12]. Moreover, the combination of antibody levels and patterns can provide valuable information to help establish the diagnosis [13].

The primary aim of this survey was to assess the procedures and practices utilised by laboratories in Spain to detect ANA and related antigens. A secondary aim was to disseminate the survey findings, as well as recommendations based on those results, to promote greater harmonization among laboratories in Spain.

Overall, we found that, for most of the key items in this survey, the participating laboratories generally follow the same testing procedures. The screening methodology for ANA used by most of the laboratories (84%) is HEp-2 IIF (Fig. 1A). In addition, 90% of the laboratories also report the titer and ANA pattern together with the test results, as recommended by international guidelines and expert consensus statements (Fig. 2B) [6, 14, 15]. Similarly, the substrates used for IIF were HEp-2 cells and their variants (Fig. 1B). Although only a minority

(12%) of laboratories reported using SPA as the screening methodology (rather than IIF on HEp-2 cells), the use of SPA for ANA determination continues to grow, despite the fact that the equivalence of these two testing methodologies has not yet been fully established [2]. In this regard, it has been reported that SPA without HEp-2 extracts have a low sensitivity and high specificity, particularly in non-selected populations [16]. Consequently, in cases with a positive SPA screening test, the finding should be confirmed by IIF on HEp-2 cells, which is exactly the approach taken by the laboratories in this survey.

We found that the ANA pattern conditioned follow-up testing for specific antigen-related antibodies in most laboratories. Follow-up testing is essential for specific patterns (e.g., nucleolar, cytoplasmic, centromere, or liver disease associated patterns) that have a strong association with specific pathologies. Moreover, although both pattern and titer are directly associated with the likelihood of disease [13, 17], a substantial proportion of the laboratories (20%) do not titrate samples with these specific patterns, but instead directly perform the associated antigenic determination. Our data show that the methods used to detect these specificities varied widely among the participating laboratories. In any case, suspected antigen-related specificities should be characterized with individualized antigenic tests.

The two most commonly reported screening dilution ratios in this survey were 1:160 (52% of laboratories) and 1:80 (38%). By contrast, two recent surveys, one conducted by the ICAP [14, 18] and the other by the European Autoimmune Standardization Initiative (EASI) [14, 18], found that most laboratories use the 1:80 screening dilution ratio (80% and 60.5%, respectively). We found that laboratory practices related to diluting, estimating, and serializing positive sera to obtain the final titer were highly heterogeneous. Similarly, the frequency of repeat testing for ANA and related antigen specificities was also highly variable. This clear lack of consensus on these items is probably due to the absence of specific recommendations in current guidelines. The appropriate time interval for test repetition should be established by the consensus between the requesting physician and the testing laboratory, based on the pathogenicity of the antibodies and their importance for the diagnosis and/or follow-up of the specific disease.

Our survey showed that slightly less than half (48%) of the laboratories use the ICAP nomenclature. Given the importance of harmonizing data reporting, this finding is highly relevant. In this regard, we hope that the results of the present survey will encourage all laboratories in Spain to incorporate this nomenclature into their routine practice. Curiously, the proportion of laboratories using this nomenclature in our survey is substantially higher than other surveys, such as the one conducted by the ICAP, where only 27% of laboratories either incorporate AC pattern descriptions or are in the process of modifying their reporting practices to include AC codes in the final ANA report [19]. Unfortunately, the survey conducted by the EASI did not report this percentage, but the authors did mention that the use of the ICAP nomenclature would help to improve the standardization of methodologies, tests, and interpretation of results [18].

We found wide variability in the techniques used to detect specificities for ANA-related antigens. The participating laboratories applied the same procedures for only a few items in this area, such as testing for anti-dsDNA antibodies, with most respondents using a confirmatory method (mainly CLIFT). In addition, the most common option for cytoplasmic and nucleolar specificities was line blot. These findings further underscore the need for clear recommendations to harmonize testing procedures.

Table 2 Conclusions and recommendations

- The technique of choice for ANA detection is IIF on HEp-2 cells (or variants):
  - Results should be reported as negative or positive with titer and pattern
  - Use of the ICAP nomenclature is strongly recommended
  - Screening dilution should be at least 1:80. Estimated or alternate dilutions can be used to obtain the final titer
- The ANA pattern and titer should be used to determine the efficient search for autoantibodies:
  - If the ANA test result shows a pattern known to be associated with a specific antigen and this pattern does not vary with the course of the disease, then the test does not need to be repeated
  - In cases with a less specific ANA pattern, screening can be done with techniques that use antigenic mixtures. If those screening tests are positive, then antigen-related specificities should be characterized by performing individualized antigenic tests
- When SPA is used for ANA screening, these assays must include HEp-2 cell extracts, which may also be enriched with antigen mixtures
  - When the test result is positive, another technique should be performed to identify the individual antigenic specificities. In these cases, HEp-2 IIF is strongly recommended to confirm that the ANA pattern is consistent with the result obtained
  - When the result is negative, it is not appropriate to carry out determinations of antibodies against specificities clearly located in the nucleus. However, if cytoplasmic antigens are suspected, then specific antigen profiles should be obtained
- Quantitative methods are preferred to determine the presence of anti-dsDNA antibodies. When positive, the findings should be corroborated by other techniques such as CLIFT or radioimmunoassay
- ANA determination and testing for antigenic specificities (with the exception of dsDNA) should not be repeated until at least 3 months have passed
- Continuous communication with clinicians is important to ensure adequate assessment of the results and to determine the need for further testing based on the patient's clinical condition

Among the limitations of our study, it should be noted that the presence of low-titer ANAs, which can be present in up to 40% of healthy individuals and act as a confounding factor, was not considered in the survey.

Finally, although we did not assess clinician-laboratory communication in the present survey, we strongly believe that close communication between the laboratory and the requesting clinician is essential to ensure that the appropriate tests and to enable proper interpretation of the results. In this regard, the patient's clinical condition, together with the results of ANA testing, should determine the need to test for antigen-related specificities.

Conclusions

The results of this survey provide a real-world picture of how detection of antinuclear antibodies and related antigens is performed in autoimmunity laboratories in Spain. In most of the laboratories surveyed, the recommendations of experts and international guidelines are followed, but there is still some variability, probably due to the lack of clear recommendations for certain procedures.

Based on the findings of this survey, the GEAI committee held a series of meetings to reach consensus on best practices and to draw up specific conclusions and recommendations (Table 2).

Overall, this survey shows that most autoimmune laboratories in Spain use a similar approach to ANA testing. However, the survey also reveals a clear need to further standardize testing and reporting protocols. In this regard, surveys such as this can provide valuable data to promote greater harmonization among laboratories. We believe that this survey should be repeated approximately 3 years from now to ascertain whether the recommendations developed by the GEAI committee have been implemented. Greater harmonization of testing procedures would provide many benefits, including optimization of the resource use. More importantly, this would provide better quality and more reliable results, which would ultimately benefit patients.

ANA, antinuclear antibodies; *IIF*, indirect immunofluorescence; *ELISA*, enzyme-linked immunosorbent assay; *FEIA*, fluoroenzyme immunoassay; *CLIA*, chemiluminescence; *SPA*, solid phase assays; *dsDNA*, double-strand DNA; *ENA*, extractable nuclear antigen antibodies; *CLIFT*, IIF in *Crithidia luciliae* cells; *RIA*, radioimmunoassay; *AC*, anti-cell.

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Declarations

Conflict of interest The authors declare no competing interests.

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