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Assessment of antinuclear antibodies by indirect immunofluorescence assay: report from a survey by the American Association of Medical Laboratory Immunologists

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Abstract

Background: The indirect immunofluorescence assay (IFA) using HEp-2 cell substrates is the preferred method by some for detecting antinuclear antibodies (ANA) as it demonstrates a number of characteristic staining patterns that reflect the cellular components bound as well as semi-quantitative results. Lack of harmonized nomenclature for HEp-2 IFA patterns, subjectivity in interpretation and variability in the number of patterns reported by different laboratories pose significant harmonization challenges. The main objectives of this study were to assess current practice in laboratory assessment of HEp-2 IFA, identify gaps and define strategies to improve reading, interpretation and reporting.

Methods: We developed and administered a 24-item survey based on four domains: educational and professional background of participants, current practice of HEp-2 IFA testing and training, gap assessment and the perceived value of International Consensus on Antinuclear Antibody Patterns (ICAP) and other factors in HEp-2 IFA assessment. The Association of Medical Laboratory Immunologists (AMLI) and American Society for Clinical Pathology administered the survey from April 1 to June 30, 2018, to members involved in ANA testing. This report summarizes the survey results and discussion from a dry workshop held during the 2019 AMLI annual meeting.

Results: One hundred and seventy-nine (n=179) responses were obtained where a significant number were

clinical laboratory scientists (46%), laboratory directors (24%), supervisors (13%) or others (17%). A majority of respondents agreed on the need to standardize nomenclature and reporting of HEp-2 IFA results. About 55% were aware of the ICAP initiative; however, among those aware, a significant majority thought its guidance on HEp-2 IFA nomenclature and reporting is of value to clinical laboratories. To improve ICAP awareness and further enhance HEp-2 IFA assessment, increased collaboration between ICAP and the clinical laboratory community was suggested with emphasis on education and availability of reference materials.

Conclusions: Based on these suggestions, future efforts to optimize HEp-2 IFA reading, interpretation and reporting would benefit from more hands-on training of laboratory personnel as well as continuous collaboration between professional organizations, in vitro diagnostic manufacturers and clinical laboratories.

Keywords: antinuclear antibodies; interpretation; performance; reporting; survey.

Introduction

The first International Consensus on antinuclear antibody (ANA) patterns (ICAP) published in 2015 [1] addressed antibody patterns detected by indirect immunofluorescence assay (IFA) on HEp-2 cell substrates. The goal of the inaugural and subsequent publications was to optimize usage of HEp-2 IFA patterns in patient care, by promoting standardization, harmonization and understanding of autoantibody test nomenclature, and providing guidelines for antinuclear antibody (ANA) test interpretation and reporting [1–5].

To-date, 30 HEp-2 IFA nuclear, cytoplasmic and mitotic patterns have been elucidated by ICAP and presented in a classification tree (www.anapatterns.org). The utility of this nomenclature classification tree is that these patterns and corresponding descriptions should be included in research analytics and clinical laboratory test reports. The ICAP recommendations are generally in accord with the earlier 2014 study and Delphi exercise of experts in ANA testing [6]. The ICAP guidelines also indicate that ‘expert-level’ laboratories would report all

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the HEp-2 IFA patterns, whereas those self-designated as ‘competent-level’ laboratories would report six nuclear and five cytoplasmic HEp-2 IFA patterns. More recent ICAP publications have started to address the clinical relevance associated with each IFA pattern [3, 4]. Several limitations to the nomenclature have been identified, and these include consensus about reporting HEp-2 IFA cytoplasmic or mitotic patterns when an ‘ANA’ test is ordered [7]. Although many laboratories around the world would like to adopt the ICAP nomenclature and embrace the recommendations provided in these consensus guidelines, some challenges may persist for laboratories because variability in technologist training and experience, equipment, reflex testing algorithms, protocols or test offerings, and specific reagents utilized may create difficulties in reliably identifying, reporting and harmonizing certain HEp-2 IFA patterns [8, 9].

With increasing awareness of the ICAP initiatives and recognizing the need for clinical laboratories to optimize ANA reading, interpretation and reporting, we sought to investigate the current practice of ANA testing. To address these objectives, the authors developed a survey which was administered through two professional laboratory organizations, which included current practice domains in ANA reporting and interpretation by laboratory professionals, current state of ANA training in professional curricula including gap assessment, perception of the need for the harmonization of HEp-2 IFA nomenclature, and the role of ICAP, other professional societies and in vitro diagnostic (IVD) manufacturers or distributors.

Materials and methods

Questionnaire

The 24-item questionnaire based on four main domains aimed to identify current practices and gaps in the assessment, interpretation and reporting of the HEp-2 IFA was developed by the authors.

1. Domain 1: focused on the educational and professional background of participants.
2. Domain 2: documented the current practice of ANA testing and training.
3. Domain 3: examined gaps in the use of nomenclature and the diagnostic relevance of HEp-2 IFA patterns and titers.
4. Domain 4: explored the role of ICAP, other professional societies and IVD manufacturers or distributors in the harmonization of the HEp-2 IFA nomenclature and reporting.

The questionnaire, primarily a multiple-choice format, relied on self-reported knowledge about nomenclature and diagnostic relevance of HEp-2 IFA patterns and titers. Participants were asked to select either

a single answer or all responses that apply, with one open-ended question regarding the value of the ICAP HEp-2 IFA nomenclature and interpretation. In addition, many of the multiple-choice questions contained an ‘Other’ response with the option of entering text as an alternative response.

Administration of survey and collection of data

An official email was sent out with the questionnaire to the members of the Association of Medical Laboratory Immunologists (AMLI) as well as a subset of members of the American Society for Clinical Pathology (ASCP). Responses from the participants were collected via the link https://aruplab.qualtrics.com/jfe/form/SV_9EvxSPuxIFIMn9b. The deadline for the survey was June 30, 2019. Survey responses referring to specific entities that could not be validated or traced were not included in this report.

Ethics statement

This study involved an anonymous survey on the practice of antinuclear antibody testing. Individual participation in the survey was voluntary.

Results

Survey respondents

One hundred and seventy-nine ($n=179$) responses were received with 96% of the respondents residing in the United States of America. Other respondents included individuals from Canada ($n=2$), Turkey ($n=2$), Brazil ($n=1$), Portugal ($n=1$) and Qatar ($n=1$).

Domain 1: Educational and professional background of respondents

Almost half of the 179 respondents were medical technologists (MT), medical laboratory scientists (MLS) or medical laboratory technologists (MLT); 24% were laboratory directors, and 13% were laboratory supervisors. The ‘other’ write-in responses included a phlebotomist, a technical consultant, trainees (fellows and residents), a laboratory manager and a laboratory assistant. Approximately two thirds of respondents worked in hospital laboratories, while 12% were employed by private reference laboratories, 6% by university or academic laboratories and 4% by IVD manufacturers or distributors. More than half of respondents (57%) had worked in their current roles for over 10 years;

approximately one quarter (26%) for 1 to 5 years; 12% for 6 to 10 years and 5% for less than 1 year (Table 1).

Domain 2: Current practice of ANA testing

Eighty-three percent (83%) of the respondents indicated that they were personally involved in ANA testing. Of those, three quarters reported utilizing microscopic (IFA or indirect immunoperoxidase) detection assays. Over one half of the respondents were involved in reviewing HEp-2 IFA results for quality control and quality assurance purposes or for supervising or training of staff performing ANA by microscopy. Approximately one third of the respondents were involved in the selection of ANA methods/kits, consulting with providers about ANA results and/or writing ANA interpretive results or comments. Relatively few respondents were involved in ordering tests as a clinician or involved in IVD manufacturing (Table 1). Most of the respondents' institutions screen for ANA by IFA using HEp-2 or HEp-2000 substrates. Approximately one third of

these laboratories used an automated HEp-2 IFA platform for this testing [10]. Other methods used for ANA screening include multi-analyte arrays (18%), enzyme-linked immunosorbent assays (ELISA; 17%) and indirect immunoperoxidase assays using HEp-2 or HEp-2000 substrates (8%). With respect to formal training on HEp-2 IFA testing, approximately 65% of respondents indicated they received training on the job, while a minority received training in formal training programs (Medical Laboratory Science [MLS]: <30%, MD, MD/PhD or PhD fellowship: <20%). Approximately 10% of the respondents reported that they did not receive formal training. The 'other' write-in responses specified that other respondents received training in workshops and meetings and by colleagues. Some respondents questioned the terminology used, indicating that they were not sure that they would regard their training as 'formal'. When asked about external resources used by their laboratories to train on HEp-2 IFA assessment, approximately 45% indicated they used ICAP, 28% used an IVD manufacturer, 17% did not know if external resources were used and 15% reported that their laboratories had not used external resources to train on HEp-2 IFA assessment. The 'other' write-in responses included the following: CAP surveys/survey samples; workshops; vendor websites, promotional material and training documents (charts, booklets, online ANA training modules, etc.); training books and CDs; medical training website, University of Washington; Birmingham Online ANA Quiz; IfQ-Lübeck; reference laboratory and MLS student instruction only. Regarding competency for HEp-2 IFA testing, the majority (~90%) of the respondents reported using external proficiency testing such as the College America Pathologists (CAP) program, while 30–40% of individuals indicated they used internal proficiency and training modules. Less than 10% of the responders reported using programs provided by IVD manufacturers or specimen exchange (Figure 1).

Domain 3: Gap assessment

The vast majority of respondents indicated that their laboratories reported homogenous (anti-cell [AC]-1; 98%), speckled (AC-2, 4, 5, 29; 97%), centromere (AC-3; 96%) and nucleolar (AC-8, 9, 10; 95%) nuclear HEp-2 IFA patterns. Fewer labs reported other nuclear patterns such as discrete nuclear dots (AC-6, 7; 41%), dense fine speckled (AC-2; 32%), pleomorphic (PCNA-like) (AC-13, 14; 28%) and the nuclear envelope (AC-11, 12; 27%) patterns (Figure 2). Overall, fewer respondents indicated that their laboratories reported both cytoplasmic and mitotic patterns. Of the cytoplasmic patterns, respondents indicated that

Table 1: Characteristics of 179 survey respondents.

Features	Percentage, %
Professional role	
Clinical laboratory scientist (MT, MLS or MLT)	46
Laboratory director	24
Laboratory supervisor	13
Other	17
Place of employment	
Hospital clinical laboratory	68
Private reference laboratory	12
University/academic laboratory	6
IVD manufacturer	4
Other	10
Length of time in current role	
> 10 years	57
6–10 years	12
1–5 years	26
< 1 year	5
Role in ANA testing	
Performing ANA test by microscopy	73
Review of test performance (QA/QC)	53
Supervising or training ANA test performers	46
Selection of ANA methods/kits	40
Consulting with providers regarding ANA results	31
Writing ANA interpretive reports and comments	28
Order ANA tests as clinician	3
IVD manufacturer	3
Other	4

MT, medical technologist; MLS, medical laboratory scientist; MLT, medical laboratory technologist; IVD, in vitro diagnostic; ANA, antinuclear antibodies; QA/QC, quality assurance/quality control.

Figure 1: Resources used to evaluate competency for ANA testing at respondents' institutions. CAP, College of American Pathologists; IVD, in vitro diagnostic.

Figure 2: HEP-2 indirect immunofluorescence assay (IFA) patterns.

Comparison between the nuclear HEP-2 IFA patterns reported by respondents' laboratories, those that respondents indicated must be reported and those that respondents indicated that they are uncomfortable reporting.

their laboratories report speckled (AC-18, 19, 20; 43%), reticular/anti-mitochondrial antibody (AMA, AC-21; 36%), polar/Golgi (AC-22; 30%), rods and rings (AC-23; 17%) and fibrillar (AC-15, 16, 17; 17%) cytoplasmic patterns (Figure 3). Regarding the mitotic patterns, respondents indicated their laboratories reported spindle fibers (NuMA-like, AC-25, 26; 39%), centrosome (AC-24; 32%), mitotic chromosomal (AC-28; 21%) and intercellular bridge (AC-27; 11%) patterns (Figure 4).

When asked which HEP-2 IFA patterns should be reported by clinical laboratories, for nuclear HEP-2 IFA patterns the responses tended to mirror the previous responses of those patterns reported by their laboratories. The majority of respondents indicated the homogenous (AC-1; 95%), speckled (AC-2, 4, 5, 29; 95%), centromere (AC-3; 92%) and nucleolar (AC-8, 9, 10; 90%) nuclear ANA patterns must be reported. Fewer respondents answered pleomorphic

(PCNA-like) (AC-13, 14; 37%), discrete nuclear dots (AC-6, 7; 36%), dense fine speckled (AC-2; 34%) and the nuclear envelope (AC-11, 12; 33%) patterns must be reported (Figure 2). Overall, fewer respondents indicated cytoplasmic and mitotic ANA patterns must be reported. Of the cytoplasmic ANA patterns, respondents thought the reticular/anti-mitochondrial antibody (AMA, AC-21; 42%), speckled (AC-18, 19, 20; 41%), polar/Golgi (AC-22; 28%), rods and rings (AC-23; 22%) and fibrillar (AC-15, 16, 17; 17%) cytoplasmic ANA patterns must be reported (Figure 3). For the mitotic ANA patterns, respondents indicated the spindle fibers (NuMA-like AC-25, 26; 31%), centrosome (AC-24; 29%), mitotic chromosomal (AC-28; 23%) and intercellular bridge (AC-27; 14%) patterns must be reported (Figure 4).

We asked respondents to indicate the settings in which additional training would be most useful. Eighty percent of respondents indicated that additional training

Figure 3: Cytoplasmic HEP-2 IFA patterns.

Comparison between the cytoplasmic HEP-2 IFA patterns reported by respondents' laboratories, those that respondents indicated must be reported and those that respondents indicated that they are uncomfortable reporting.

Figure 4: Mitotic HEP-2 IFA patterns.

Comparison between the mitotic HEP-2 IFA patterns reported by respondents' laboratories, those that respondents indicated must be reported and those that respondents indicated that they are uncomfortable reporting.

requirements for HEP-2 IFA assessment would be useful in the clinical laboratories performing the testing. Three quarters of respondents thought that additional training requirements would be useful in medical laboratory scientist or technologist training programs. Just over one half of the respondents (51%) answered additional training would be useful in physician/healthcare provider continuing education programs, and 42% indicated that additional training requirements for HEP-2 IFA assessment would be useful during postgraduate training programs for laboratory professionals. Four percent of respondents did not know where additional training requirements for HEP-2 IFA assessment would be most useful. One respondent indicated that they would be useful in continuing education programs for laboratory professionals, and one respondent did not think that any further training initiatives were necessary.

Nearly all (95%) respondents indicated that their laboratories reported HEP-2 IFA results as negative or

positive along with titers and reference ranges (qualitative and semi-quantitative). However, 2% indicated their laboratory reported HEP-2 IFA results qualitatively as positive or negative (and/or indeterminate). Three percent reported that they did not know whether their laboratory reported titers and reference ranges for HEP-2 IFA. Almost half (48%) of respondents indicated that HEP-2 IFA or solid phase assay reports from their laboratory provide a statement about the clinical significance of the results and 38% provided recommendations for additional testing. In contrast, 33% of the respondents reported that their laboratories do not provide either recommendations for additional testing or information about the clinical significance of HEP-2 IFA or solid phase assays. About 10% of the respondents did not know whether HEP-2 IFA or solid phase reports include additional information about clinical relevance or follow-up testing.

Domain 4: Role of ICAP and other organizations in providing guidance on ANA testing

Fifty-five percent (55%) of respondents answered that they were aware of the ICAP initiatives. Even more (67%) thought ICAP guidance on ANA nomenclature and interpretation provides value to the clinical laboratory, whereas 31% answered that they did not know and only 3% answered no. Respondents were asked to provide their own answer for the value provided by the ICAP guidance: 61% answered that it provides value in promoting standardization and/or consistency, 24% answered that it provides value as a reference and/or educational tool, 10% indicated it increases clinical correlation and fills a gap and 5% did not know.

Since the ICAP HEP-2 IFA classification tree (www.anapatterns.org) includes nuclear, cytoplasmic and mitotic patterns, with designations of competent level or expert level, survey respondents were asked to indicate which of the patterns listed in the survey they were not comfortable identifying. Respondents were most comfortable identifying the homogeneous (AC-1), speckled (AC-2, 4, 5, 29), nucleolar (AC-8, 9, 10) and centromere (AC-3) nuclear HEP-2 IFA patterns. These patterns are considered competent level by ICAP (Figure 2). Approximately 20% of respondents were not comfortable identifying the discrete nuclear dot pattern (AC-6, 7), and nearly half of respondents were not comfortable identifying the dense fine speckled pattern (AC-2), both considered competent level by ICAP. Of the expert-level ICAP nuclear ANA patterns, 40% and 55% of respondents were not comfortable identifying the nuclear envelope (AC-11, 12) and pleomorphic (PCNA-like, AC-13, 14) patterns, respectively. Overall, respondents were less comfortable identifying cytoplasmic and mitotic HEP-2 IFA patterns than they were identifying nuclear patterns. Of the cytoplasmic patterns queried, respondents were least comfortable identifying the fibrillar (AC-15, 16, 17; 61%) and rods and rings (AC-23; 57%) patterns, while

for polar/Golgi (AC-22), speckled (AC-18, 19, 20) and reticular/anti-mitochondrial antibody (AMA, AC-21) patterns the number of respondents not comfortable identifying the patterns ranged from 34% to 39% (Figure 3). Of the expert-level mitotic patterns, nearly three quarters of respondents were not comfortable identifying the intracellular bridge pattern (AC-27), 60% were not comfortable identifying the mitotic chromosomal pattern (AC-28), and 37% and 39% of respondents were not comfortable identifying the spindle fibers (NuMA-like, AC-25, 26) and centrosome (AC-24) patterns, respectively (Figure 4).

Responses to specific competencies for optimal microscopic analysis of HEP-2 IFA by the respondents indicated areas where guidance would be beneficial (Figure 5). More than 80% of participants indicated a need for guidance in classification of HEP-2 IFA staining and patterns with other suggestions for guidance on cytoplasmic and mitotic patterns, minimum requirements for performing ANA by IFA testing, troubleshooting discrepant ANA results and suggesting confirmatory testing based on the HEP-2 IFA pattern.

Only 8% of respondents were aware of other initiatives to improve HEP-2 IFA assessment. When respondents were asked to provide their own answers for other initiative(s) for improving HEP-2 IFA assessment they were aware of, responses included the CAP Diagnostic Immunology and Flow Cytometry Committee, the American Association of Clinical Chemistry, the University of Washington (Seattle) training module on ANA (<https://www.medtraining.org>) and the European Autoimmunity Standardization Initiative.

Discussion

This report follows the administration of a survey on the performance and utilization of the HEP-2 IFA test and reflects the essence of the discussions that followed when

Figure 5: Responses on specific areas where additional guidance on HEP-2 IFA analysis would be beneficial to laboratories.

the results were presented at the most recent Association of Medical Laboratory Immunologists 2019 meeting in Cleveland, OH. Similar surveys were led by professionals in Brazil [11, 12] and more recently by the European Autoimmunity Standardization Initiative (EASI) [13], ICAP (personal communication) and the Korean Society of Laboratory Medicine (presented at the 60th Annual Meeting in Busan, Korea, September 26–28, 2019). Despite its shortcomings, the HEp-2 IFA test is regarded by some as the ‘gold standard’ method for detecting ANA in the evaluation of systemic autoimmune rheumatic diseases [14–17]. Subjectivity in the identification and interpretation of HEp-2 IFA and variability in the number and nomenclature of patterns reported, however, poses significant challenges in its utilization. Furthermore, an increasing number of test orders coming from physicians other than rheumatologists [18] along with the possibility that repeat and/or confirmatory testing may be performed at a different laboratory highlights the importance of harmonization of testing and interpretation of results.

Our survey reflected the current state-of-the-art in HEp-2 IFA testing, primarily in laboratories in the USA. Although a limitation of our study is that the 179 respondents are only a small proportion of those involved in ANA testing, it nevertheless documented an appreciation for the value of standardized international nomenclature (i.e. ICAP) and reporting, as well as areas of inconsistent practice. Survey results indicated that the majority of respondents’ laboratories perform HEp-2 IFA testing, report the results as negative or positive with titers and report at least four of the six designated competent-level nuclear HEp-2 IFA patterns. The majority of respondents also indicated that they are comfortable interpreting and think that there is clinical value in reporting at least four of the six ICAP competent-level nuclear HEp-2 IFA patterns. In addition, the majority of respondents reported they primarily received training on HEp-2 IFA testing on the job and that ICAP guidance on HEp-2 IFA nomenclature and interpretation provides value to the clinical laboratory.

Gaps and differences identified by the survey include a lack of widespread awareness of ICAP and other initiatives for increasing standardization in the interpretation and reporting of HEp-2 IFA results. The importance of standardized reporting, currently being addressed by ICAP [4], is highlighted by respondents’ answers. Thus demonstrating variability in the reporting of test results, the comfort level of identifying HEp-2 IFA patterns and in particular the clinical relevance of the dense fine speckled (AC-2) and discrete nuclear dot (AC-6,7). It also highlights the wide spectrum of identification and reporting competent-level and expert-level cytoplasmic and mitotic

HEp-2 IFA patterns. Differences were also observed in whether respondents’ laboratories provide suggestions for confirmatory testing. Last, respondents indicated that additional guidance is needed for the classification and identification of HEp-2 IFA staining patterns and also for the minimum requirements for establishing reference ranges and troubleshooting discrepant HEp-2 IFA results.

Remarkably, nearly one third of reporting laboratories indicated that they use automated HEp-2 IFA digital reading platforms [reviewed in 10], a trend that is in keeping with other areas worldwide [4]. Since only a handful of the HEp-2 IFA patterns are currently suggested by automated instruments, efforts to increase the ‘bandwidth’ of automated IFA readers to make them more robust have potential [9]. Incorporation of recent guidance on HEp-2 IFA classification and nomenclature will facilitate this process. In addition, availability of validated index reagents, standardization of HEp-2 cell substrates and standardization of techniques to capture and classify images (e.g. by artificial intelligence and deep learning algorithms) may lead to better inter-assay concordance and inter-laboratory commutability.

In the meantime, laboratories and partner agencies can focus on training personnel using guidance for assessing HEp-2 IFA to improve laboratory consistency and accuracy. Our survey indicates some of the strengths as well as the opportunities for improvement in performing the HEp-2 IFA test. Hands-on and wet bench workshops designed to train clinical laboratory scientists and address the lack of standardization among IVD manufacturers [9, 19] and improvements in proficiency programs would also be helpful in optimizing the detection and measurement of HEp-2 IFA results and reports [19, 20].

As mentioned above, similar studies have been conducted in other areas of the world [11–13, unpublished data]. However, the design of these studies (except the Korean study referenced above which has not yet been published) are quite different, and hence it is difficult to draw comparisons. For example, we are not aware of any other study that has surveyed participants based on ICAP nomenclature. A consensus from these surveys is that variability exists in the interpretation and reporting of HEp-2 IFA results which highlights the importance of addressing this issue through standardization and harmonization. The implementation of the ICAP nomenclature is widely thought to be a logical first step toward this common goal.

Respondents’ interest in additional guidance and more ‘hands on’, wet bench training indicates an opportunity for AMLI, ICAP, CAP, other professional organizations and IVD manufacturers to create and disseminate such guidance and provide additional training. In addition,

there is a need for control/reference materials for training and competency assessment for patterns that respondents are not comfortable reporting. Reference materials for some of these patterns are currently available through the Autoantibody Standardization Committee (www.AutoAb.org), with continued efforts to generate and validate additional patterns [21–23].

Take home messages

1. Survey respondents agree on the need for standardization in the nomenclature and reporting of ANA results.
2. ICAP guidance on HEp-2 IFA nomenclature and reporting is of value to the clinical lab, but gaps exist in its implementation.
3. Challenges in implementation of ICAP nomenclature and reporting include increasing the awareness of the guidance, training personnel and the availability of reference materials for more of the ICAP competent- and expert-level patterns.
4. Future efforts should focus on collaboration between professional organizations, IVD manufacturers and clinical laboratories, with the common goal of achieving standardization and harmonization in the nomenclature and reporting of ANA results.

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