The International Consensus on ANA Patterns (ICAP) in 2021—The 6th Workshop and Current Perspectives

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Abstract: The establishment of the International Consensus on ANA Patterns (ICAP) in 2014–2015 was welcomed by members of the medical community as a significant improvement in guiding harmonization of ANA test interpretation and reporting. In the subsequent years, several itinerant meetings and continuous interaction with the community contributed to disseminate the ICAP harmonization on the immunofluorescence patterns observed in the indirect immunofluorescence assay on HEp-2 cells (HEp-2 IFA) and to promote progressive improvement in the classification of HEp-2 IFA patterns. The 6th ICAP Workshop was held in person on September 6, 2021 as a satellite meeting of the 15th Dresden Symposium on Autoantibodies. This article summarizes the major discussions at the meeting as well as outlining the current plans for the ICAP committee.

Autoantibodies to intracellular antigens, historically known as antinuclear antibodies (ANA), are serological biomarkers that have a central role in the diagnosis and classification of systemic autoimmune rheumatic diseases (1). There is a continuing need for harmonization of the methods for autoantibody determination and reporting, both in the research setting for the identification of novel autoantibodies and in the clinical laboratory setting, where numerous assay methods and platforms have become available over the past decades. In 2009, the American College of Rheumatology ANA Task Force position statement recommended the indirect immunofluorescence

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assay (IFA) using HEp-2 substrate as the "gold standard" for primary ANA detection. However, some clinical laboratories use solid-phase immunoassays, in some cases as a reflex test to supplement HEp-2 IFA screening test, or even replace HEp-2 IFA testing. Nevertheless, most clinical laboratories worldwide depend heavily on HEp-2 IFA as the primary screening method.

The Committee for the Standardization of Autoantibodies in Rheumatic and Related Diseases was established in the early 1980s as the "Committee on ANA Serology" based on the recognized need for reference human autoimmune sera that were critical for academic and clinical laboratories, as well as in vitro diagnostic industries. In the past 15 years, the committee has used the truncated name "Autoantibody Standardization Committee (ASC)" and established the affiliated website www.AutoAb.org. To date, the ASC serves as a subcommittee of the Quality Assessment and Standardization Committee of the International Union of Immunological Societies. A primary objective of the ASC is to uphold the highest standards of patient care by promoting accuracy in autoantibody testing. To date, the ASC has identified 23 reference reagents that are available free of charge to research laboratories, diagnostic laboratories, and commercial organizations developing autoantibody diagnostic kits via the ASC website (www.AutoAb.org under the Reference Materials tab) and are distributed by the Plasma Services Group (www.plasmaservicesgroup.com) on a not-for-profit basis.

Another achievement of the ASC is the establishment of the International Consensus on ANA Patterns (ICAP) initiative during the 12th International Workshop on Autoantibodies and Autoimmunity held in São Paulo, Brazil in 2014 (2). The goal of ICAP is to promote harmonization and understanding of HEp-2 IFA staining pattern nomenclature, as well as optimizing usage in patient care by providing interpretation guidelines for HEp-2 IFA test results (3). Because this methodological platform allows the identification of autoantibodies targeted to antigens localized not only in the nucleus, but also in the cytoplasm and mitotic cells, many specialists consider that the term "antinuclear antibody test" is no longer appropriate (4, 5). Accordingly, ICAP prefers the term HEp-2 IFA that harmonizes with the broader scope of the assay. The set of autoantibodies detected in the HEp-2 IFA test can be also more correctly described as autoantibodies to cellular antigens (4) or more recently as recommended by ICAP, anticell antibodies (6).

To date, there have been 6 ICAP workshops. The first and third ICAP workshops were held as satellite meetings immediately before the International Workshop on Autoantibodies and Autoimmunity in São Paulo, Brazil, in 2014 and in Kyoto, Japan, in 2016, respectively. The second and fourth to sixth ICAP workshops were all held in Dresden, Germany, 1 day before the series of Dresden Symposium on Autoantibodies. The sixth and most recent ICAP Workshop took place on September 6, 2021, with 80 registered participants. The program was organized with a 2-hour session in the morning and another 2-hour session in the afternoon. A presentation was provided for updating the ICAP classification chart, with several improvements based on feedback and recommendations from the user community to the ICAP committee.

Since the first ICAP Workshop, 30 different HEp-2 IFA patterns have been categorized into 4 major groups: negative ($n = 1$), nuclear ($n = 15$), cytoplasmic ($n = 9$), and mitotic patterns ($n = 5$) (2, 3, 5, 7, 8). The conceptual basis of the ICAP algorithm was elaborated by a team of international experts in the field using the template of the Brazilian Consensus on HEp-2 ANA Patterns started in 2000 (9). The resulting consensus nomenclature was arranged into a classification tree that is displayed on the ICAP website (www.ANApatterns.org). Each pattern is assigned an alphanumeric AC code (anticell). For example, the nuclear homogeneous pattern is

designated AC-1 and the cytoplasmic reticular pattern is AC-21. In addition to representative images for each pattern, the website presents other important information, such as the recommended (and historical) designation of each pattern, a consensus description of the main features of the patterns and, most importantly, possible associated autoantibody target specificities and the clinical relevance of each HEp-2 IFA pattern (3). The clinical relevance of each pattern is primarily defined within the context of the suspected diseases and includes recommendations for follow-up or confirmatory testing of disease-associated autoantibodies when appropriate. The website provides didactic material with the classification tree and free-of-charge downloadable associated images. In addition to English, the content of the website is available in 12 other languages including Portuguese, Spanish, Italian, German, Chinese, French, Turkish, Russian, Greek, Hungarian, Bosnian, and Korean. Other translations, including Japanese and Thai, are in final stages of implementation. The translation process typically involves a team of specialists in the respective countries and this team is encouraged to discuss and disseminate ICAP concepts among the specialist community in the respective country or among different countries. For example, the translation team for Spanish is coordinated by Ignacio García-De La Torre in Mexico, with 3 colleagues: Orlando Gabriel Carballo in Argentina, Aresio Plaza López in Spain, and Carlos Casiano in the USA. The newest translation is Korean, posted in February 2021 and is coordinated by Kyeong-Hee Kim, together with 4 other colleagues in different academic institutions in South Korea and endorsed by the Korean Society of Diagnostic Immunology. Thus, the translation into several languages has promoted a productive discussion on HEp-2 IFA terms among participating members during the process.

From the very beginning, ICAP agreed to ground its commentary, concepts, and advances with full public discussion involving all participants in the successive ICAP workshops, which take place in international congresses with participation of all potentially interested parties. In addition, there is opportunity of interaction and participation of the world community through the ICAP website. Anyone may submit candidate images relevant to the ICAP patterns, and these images are subjected to a vetting process that selects the representative images to be displayed online at the ICAP website. Therefore, the current panel of images has contributions from academic and industry specialists from several parts of the world that depict IFA staining nuances obtained with different HEp-2 slide brands. In addition, a "frequently asked questions" (FAQ) section on the website offers opportunity for interaction between the world community and a panel of ICAP specialists. For example, it is feasible for registered ICAP users to ask questions and to submit unique or apparently unclassifiable IFA images through the FAQ portal and get clarification and advice. This activity is coordinated among the ICAP committee members and answers are typically returned to users usually within a couple of days. Questions that are of common interest are written up and edited by ICAP members for posting in the FAQ section on the ICAP website (https://ANApatterns.org/addFaq.php). A number of users have commented positively on the usefulness of the FAQ section.

ICAP develops educational projects that are available free of charge on the website. The training module, available after registration, can be accessed via the Training Tab at www.ANApatterns.org. The first module introduces the ICAP concept, the classification tree, and other technical aspects necessary for the best experience in navigating through the website. This training also presents technical recommendations on how to perform the HEp-2 IFA. The crucial points in the technical recommendations will ensure test quality, accuracy, and reproducibility. Participants are asked to take an initial survey to provide some feedback on the background of the user. After

taking the training module, participants can take the final assessment and receive a certificate stating their successful completion of the course. This first module was released in July 2020 and so far 949 individuals have assessed the educational modules and 45.4% completed the final assessment and obtained the certificate of training. Professionals from 76 countries have participated. Translation of the training module to Chinese has been completed and more than 300 users have already participated. Spanish translation for the training module is currently in progress. Additional training modules are being developed to focus on basic (competent) and advanced (expert) patterns.

There are several key points in the revision of the classification tree that came as a result of the most recent 2021 ICAP meeting in Dresden, Germany. The chart (Fig. 1A, B) was revised to provide a better visual separation between nuclear and cytoplasmic patterns, as well as a clear separation between competent- and expert-level patterns. To achieve these, several changes were implemented. First, the overarching nuclear envelope (AC-11,12) and pleomorphic (AC-13,14) patterns, not the individual patterns, are changed to the "competent-level." The suggestion to change the nuclear envelope to competent-level followed early user feedback when it was suggested that these nuclear envelope patterns should be readily recognized because they bear significant clinical relevance (3). The pleomorphic patterns are also considered competent-level because HEp-2 IFA pattern readers, even though they may not have sufficient experience to distinguish each one of them readily, are encouraged to recognize the cell cycle-dependent patterns. Second, the nuclear dense fine-speckled (AC-2) and Topo I-like (AC-29) patterns are realigned closer to the nuclear homogeneous pattern (AC-1) to highlight their similarities in staining of both interphase nuclei and mitotic condensed chromatin. This change results in the overarching nuclear speckled pattern (AC-4,5), classified at the competent-level to represent only the fine-speckled (AC-4) and large speckled (AC-5) patterns, both of which do not stain the mitotic condensed chromatin; this arrangement is more practical as it is consistent with the understanding that many laboratories do not distinguish between AC-4 and AC-5, but do distinguish AC-2 and AC-29. Third and last, the cytoplasmic discrete dots pattern (AC-18) is separated from cytoplasmic dense fine-speckled (AC-19) and cytoplasmic fine-speckled (AC-20) patterns based on the obvious difference between AC-18 and the 2 more closely related AC-19 and AC-20. The AC-18 pattern remains an expert-level pattern.

Several discussions with respect to adjustment of the classification tree have not resulted in a consensus. For instance, suggestions to include N (nucleus), C (cytoplasmic), and M (mitotic) in the AC codes were not incorporated, because the ICAP committee originally included these codes to allow for easy and objective access and reference to the web-based consensus patterns available on the ICAP website. The codes are to be considered analogous to the cluster differentiation-nomenclature of cell-specific membrane surface molecules, predominantly relevant to leukocyte antigens. Therefore, use of AC codes in the HEp-2 IFA report is recommended in terms of harmonization (6). Finally, it became apparent in several discussions that the extension "-like" is often overlooked because this extension is not included in the pattern names in the classification tree due to insufficient space. However, the extension is intentionally added to several patterns, for instance PCNA-like (AC-13), NuMA-like (AC-26), or Topo I-like (AC-29), to emphasize that the pattern requires confirmation by antigen-specific immunoassays. For 2 other patterns, i.e., the nuclear dense fine-speckled pattern (AC-2) and the cytoplasmic reticular/AMA pattern (AC-21), the -like extension is not added, but also in these cases confirmation by antigen-specific immunoassays is mandatory for appropriate clinical interpretation. However, in all

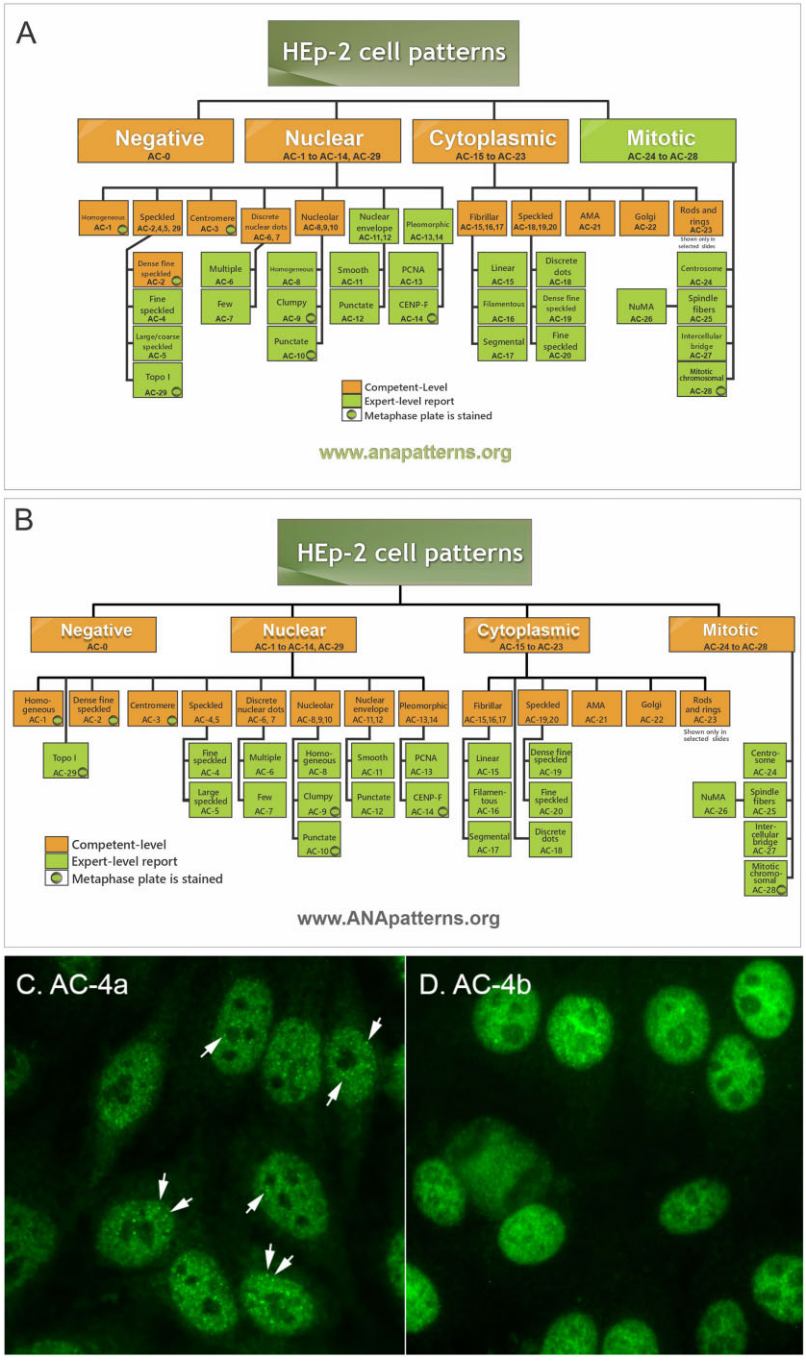

Fig. 1. Change in ICAP classification chart and refined definition of nuclear fine-speckled (AC-4). (A) ICAP classification chart in use from 2015 to 2021. (B) The revised ICAP chart in use since September 2021. (C and D) Indirect immunofluorescence of HEp-2 cells showing the proposed AC-4a and AC-4b patterns. AC-4a (C) exhibits the characteristic myriad discrete nuclear speckles that are essentially absent in AC-4b (D). See text for discussion.

these cases, assigning the pattern is independent from the result of the confirmation assay, as the pattern classification should be strictly under morphological criteria. Distinction of "subpatterns" based on the result of antigen-specific immunoas says by the prefix "pseudo-" is, therefore, currently not encouraged.

Throughout successive ICAP workshops, it became evident that the panel of ICAP patterns may be occasionally enriched with the addition of novel patterns. In fact, an active self-reevaluation process while exercising the ICAP classification system has indicated multiple opportunities for improvement. In addition, the participation of the international community has contributed new inputs. A new ICAP initiative entitled Clinical and Immunological Characterization of HEp-2 IFA patterns (HEp-2 CIC) is coordinated by Luis Andrade, São Paulo, Brazil. The HEp-2 CIC project has 3 branches: (*a*) determination of the prevalence of ICAP patterns worldwide—preliminary data was presented by Trischna Martins, Berlin, Germany at the 6th ICAP Workshop; (*b*) establishment of clinical associations of selected ICAP patterns; and (*c*) characterization of the antigenic specificity of selected ICAP patterns. Differently from branch 1, which comprises a single project/study and has been coordinated by Luis Andrade, branches 2 and 3 should comprise several projects/studies (each chosen pattern can represent one subproject) to be coordinated by interested participants. For example, a colleague interested in coordinating the study of the clinical associations in patients with AC-22 pattern would align with branch 2 and this colleague will be the coordinator of the study. Similarly, another colleague may want to coordinate the study of the immunologic characterization (branch 3) of a novel IFA pattern and this colleague can be the coordinator of this study. Branches 2 and 3 can serve to explore the collaborative potential of several experts in multicenter collaboration. It is encouraged that for collecting clinical data (not only diagnosis, but in particular clinical manifestations) a standard format will be designed for the whole study. These projects are highly collaborative with laboratories worldwide to understand better all these associations in diverse geographic and ethnical contexts.

For the 6th ICAP Workshop, there were several other very interesting presentations and discussions. Presentations from Lieve van Hoovels and Martine Vercammen in Belgium discussed results based on surveys including ICAP users on current laboratory and clinical practices in reporting and interpreting HEp-2 IFA patterns (10) and on the methodological aspects of HEp-2 IFA testing. Manfred Herold, Innsbruck, Austria, described the experience with implementing ICAP recommendations in his country demonstrating increased interactions between local and regional laboratories. Amira Cerimagic from Sarajevo, Bosnia-Herzegovina, the coordinator of the local translation team, described their experience on how to decide on the translation and implementation of ICAP in a region with remarkable language heterogeneity. Maria Infantino, Florence, Italy, presented their team work on improving decision trees for routine diagnostics and research based on pilot immunoblot panels (11, 12). Manfred Herold also gave a presentation to provide examples how to report rare and multiple patterns using ICAP terminology. Luis Andrade presented their experience in the heterogeneity in results from using substrates from different HEp-2 brands. Rico Hiemann, Senftenberg, Germany, provided an interesting discussion on how artificial intelligence can be applied for pattern recognition in automatic IFA systems according to ICAP terminology.

Last, Ed Chan discussed several questions raised from ICAP users along the past few years. For example, a frequently asked question is whether there is an ICAP AC pattern for monospecific anti-Ro52. The consensus is that anti-Ro52 antibodies do not produce a distinctive staining pattern on commercially prepared HEp-2 cells, which is consistent with published studies (13, 14).

Anti-Ro52 autoantibodies also do not show reactivity in double immunodiffusion or classical radioimmunoprecipitation assays. If an anti-Ro52 positive sample shows any reactivity in HEp-2 IFA, it probably contains additional autoantibodies against antigens other than anti-Ro52. Another participant asked, "What is the point of classifying an IFA pattern as NuMA-like (AC-26) or CENP-F-like (AC-14) when there is no commercially available assay for validation?" The reply was that ICAP encourages partners of the diagnostic industry to work on these important antigen-specific assays to facilitate their identification and further studies on their clinical relevance. Another, rather negative comment posed the question of identifying few nuclear dots (AC-7) or NuMA-like (AC-26) when there is no known relevant clinical association. The answer to this is, again, that the "no relevant clinical association" status may change as more research is conducted. Also important to note is that when a HEp-2 IFA pattern has been demonstrated to have no clinical relevance, this can be useful information for clinicians as well, as is broadly recognized for the AC-2 pattern. Overall, the 6th ICAP Workshop in Dresden was a successful venue to promote interactions that were missed so much during the COVID-19 pandemic.

A couple of new publications from the ICAP group are worthy of mention. There was a consensus that HEp-2 IFA results should be communicated to clinicians in a standardized way, adding value to laboratory findings and helping with critical clinical decisions. A Test Report template, based on the practices informed by 118 laboratories in 68 countries, with ICAP recommendations has been accepted for publication (6). The major focus is placed on the report format containing endpoint titers, immunofluorescence patterns together with AC nomenclature, possible autoantibody associations, and remarks on follow-up or reflex testing. Special situations addressed include serum screening dilutions and endpoint titers, relevance of immunofluorescence patterns with special attention to cytoplasmic patterns, mixed and compound patterns, and how to report different titers corresponding to multiple patterns or autoantibodies in the same sample. This ICAP pro forma report represents a further step in harmonizing the way relevant clinical information could be provided by laboratories (6).

Another study by several ICAP members on AC-4 patterns has recently been accepted for publication (15). AC-4 (fine-speckled nuclear pattern) is associated with anti-SS-A/Ro, anti-SS-B/La, and other autoantibodies. Anti-SS-A/Ro sera may contain antibodies to Ro60 and Ro52. A variation of AC-4 (preliminarily designated AC-4a), characterized by myriad of discrete nuclear speckles (Fig. 1C), was reported in 2013 by the Andrade laboratory to be associated with anti-SS-A/Ro60 (16). The plain fine-speckled pattern (herein designated AC-4b, Fig. 1D) is seldom associated with anti-SS-A/Ro. This new study reports the experience of 4 expert laboratories on AC-4a and AC-4b patterns (15). The association of the AC-4a pattern and anti-SS-A/Ro60 is confirmed in contrast to the AC-4b pattern. Results support the worldwide applicability of these AC-4 pattern variants and their incorporation into ICAP classification under codes AC-4a and AC-4b. The AC-4 pattern should be maintained as an umbrella pattern for cases in which one cannot discriminate AC-4a and AC-4b patterns. The ability to recognize the AC-4a pattern and its strong association to anti-SS-A/Ro60 should add value to HEp-2 IFA interpretation. The separation of AC-4 into AC-4a and AC-4b will be discussed at the next ICAP meeting to formally acknowledge this addition.

ICAP established a collegial community focus on an accurate reading, interpretation, and reporting of HEp-2 IFA images for national and international audiences. Committee members have strong practical experience and are genuinely interested in HEp-2 IFA images. In addition, ICAP

continues to solicit comments from the community and recruit younger colleagues to participate in the discussion. The 6th ICAP Workshop has rejuvenated many of us to renew our efforts and put the COVID-19 pandemic in the rearview mirror the sooner the better.

Nonstandard Abbreviations: ICAP, International Consensus on ANA Patterns; ANA, antinuclear antibodies; IFA, immunofluorescence assay, ASC, Autoantibody Standardization Committee; AC, anticell; FAQ, frequently asked questions.

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