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Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective

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ABSTRACT

The indirect immunofluorescence assay (IIFA) on HEp-2 cells is widely used for detection of antinuclear antibodies (ANA). The dichotomous outcome, negative or positive, is integrated in diagnostic and classification criteria for several systemic autoimmune diseases. However, the HEp-2 IIFA test has much more to offer: besides the titre or fluorescence intensity, it also provides fluorescence pattern(s). The latter include the nucleus and the cytoplasm of interphase cells as well as patterns associated with mitotic cells. The International Consensus on ANA Patterns (ICAP) initiative has previously reached consensus on the nomenclature and definitions of HEp-2 IIFA patterns. In the current paper, the ICAP consensus is presented on the clinical relevance of the 29 distinct HEp-2 IIFA patterns. This clinical relevance is primarily defined within the context of the suspected disease and includes recommendations for follow-up testing. The discussion includes how this information may benefit the clinicians in daily practice and how the knowledge can be used to further improve diagnostic and classification criteria.

INTRODUCTION

Autoantibodies, as detected by the indirect immunofluorescence assay (IIFA) on HEp-2 cells (IIFA HEp-2), are recognised as important diagnostic markers in a plethora of autoimmune diseases, in particular the systemic autoimmune rheumatic diseases (SARD).¹ Although somewhat dated by today's standards, members of the American College of Rheumatology (ACR) prepared an evidence-based guideline for the usefulness of the HEp-2 IIFA results for diagnostic and prognostic purposes and also for meeting diagnostic criteria.² That guideline was based on reactivity with nuclear antigens as detected by IIFA on rodent tissue or HEp-2 cells. More recently, the IIFA on HEp-2 cells was reinforced as the gold standard for autoantibody screening in SARD.³

Interestingly, the HEp-2 IIFA test reveals much more information than the mere absence or presence of autoantibodies, that is, the level of antibody as well as the HEp-2 IIFA pattern. Based on titration or appropriate evaluation of the fluorescence intensity, the antibody level can be determined and this information has general concordance with the

clinical relevance of the test result. Indeed, higher antibody levels are better associated with SARD and have an increased likelihood to identify the autoantigen in follow-up testing.^{4–6} The importance of the level of autoantibodies is also recognised in the ACR guideline as well as by the recommendations issued by the European Autoimmunity Standardization Initiative (EASI) and the International Union of Immunologic Societies (IUIS) Autoantibody Standardization Subcommittee.^{2,7}

The HEp-2 IIFA pattern may also reveal clinically relevant information. This information is not restricted to giving direction to follow-up testing for antigen-specificity, but, for instance, the centromere pattern is included in the classification criteria for systemic sclerosis,⁸ while the nuclear dense fine speckled pattern is reported to be more prevalent in apparently healthy individuals as compared with patients with SARD.⁹ To harmonise the names and descriptions of the distinct HEp-2 IIFA patterns, an ordered classification taxonomy was proposed.¹⁰ This proposal was subsequently elaborated on by the International Consensus on ANA Patterns (ICAP), initiated in parallel to the 12th International Workshop on Autoantibodies and Autoimmunity (2014) held in Sao Paulo, Brazil. During this workshop, a consensus was reached on the nomenclature and definitions of 28 HEp-2 IIFA patterns. Each HEp-2 IIFA pattern was ascribed an alphanumeric code from AC-1 to AC-28.¹¹ The consensus nomenclature for each pattern and representative images were also made available online at the ICAP website (<http://www.ANAPatterns.org>).

In addition to the nuclear patterns, important cytoplasmic and mitotic patterns may also be observed in HEp-2 IIFA analysis. Although reporting non-nuclear patterns is considered clinically relevant,⁷ for various jurisdictional reasons there is no clear-cut consensus viewpoint on reporting non-nuclear patterns as a negative or positive test.¹² With the understanding that the term 'Antinuclear antibody (ANA) test' may be inappropriate to designate a test that also addresses autoantibodies to antigens in the cytoplasm and mitotic apparatus, an alternative name, anticellular antibodies, was suggested in the EASI/IUIS recommendations.⁷ Recent publications from ICAP have preferred the term HEp-2 IIFA as it covers the



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whole spectrum of patterns that can be observed when using the HEp-2 cells as substrate.^{13 14}

Originally, the HEp-2 IIFA patterns were associated with diseases, but it was anticipated that many of these associations are only valid if the antigen-specificity was confirmed by follow-up testing. In subsequent ICAP workshops, it was agreed that the disease associations should be replaced by clinical relevance. In this current paper, we present the consensus on the clinical relevance of the distinct HEp-2 IIFA patterns as achieved by consecutive workshops and discussions among the executive ICAP members.

MATERIALS AND METHODS

For discussion about the structure of clinical relevance templates were prepared for AC-2 (LECA), AC-3 (JD) and AC-5 (MS). This formed the basis of a guideline for description of each AC pattern (EC). Of highest importance, it was agreed that the information should be objective and helpful for the clinician, the pattern-antigen associations should be put in the right clinical context and information should be evidence-based.

In preparation for the third ICAP workshop in Kyoto (2016), composition of the clinical relevance documents was started for the nuclear patterns (JD, LECA, MS), cytoplasmic patterns (CAvM, EKLC) and mitotic patterns (MH, TM). As far as already available, the documents were commented on by the ICAP executive board and, after appropriate adjustment, discussed with the workshop participants. The feedback from participants mainly focused on the structure of the information provided, on the required level of detail and the format of recommended follow-up testing.

In anticipation of the fourth ICAP workshop in Dresden (2017), the set of clinical relevance documents was completed for all patterns. Further comments from the ICAP executive board were included. The resulting documents were individually discussed with the workshop participants for nuclear (JD), cytoplasmic (CAvM) and mitotic (MH) patterns. Besides several substantive comments, there was general agreement that the information should be provided in tabular format at two distinct levels. The first level should contain information on relevant follow-up testing in the respective clinical context, the recommended follow-up tests should be commercially available and detailed test characteristics should not be given because of potential geographic and jurisdictional differences. Information based on case reports or small patient cohorts, as well as information on possible follow-up testing that is only available in specialised research laboratories, should only be provided in the second level information.

Tables for nuclear, cytoplasmic and mitotic patterns were prepared for first and second level information (JD). These tables were commented by the ICAP executive board and finalised by JD. Of note, since the starting point of the tables on clinical relevance is the HEp-2 IIFA pattern and not the clinically suspected disease, the tables do not list all autoantibodies related to the respective disease.

RESULTS

Nuclear HEp-2 IIFA patterns

To date, a total of 15 nuclear HEp-2 IIFA patterns have been described, that is, AC-1–AC-14 and AC-29. Table 1 summarises the clinical relevance of these patterns.^{8 9 14–79} Since AC-29 was only recently described,¹⁴ the advice for follow-up testing for autoantibodies to topoisomerase I (Scl-70) in case of clinical suspicion of systemic sclerosis is also added as a note to the

clinical relevance of AC-1. In particular, disease-specific immunoassays, like autoimmune liver disease profile, inflammatory myopathy profile, systemic sclerosis profile, are often only available in specialty clinical laboratories.

For six nuclear HEp-2 IIFA patterns (AC-3, 5, 7, 8, 12 and 13), additional information about clinical relevance is summarised in online supplementary table S1. Although some assays for anti-CENP-A antibodies are commercially available, these antibodies are included in online supplementary table S1 because the majority of sera revealing the AC-3 pattern are also reactive with CENP-B. In contrast to CENP-A, CENP-B is included in many routine extractable nuclear antigens profiles.

Cytoplasmic HEp-2 IIFA patterns

Table 2 summarises the clinical relevance of the nine cytoplasmic HEp-2 IIFA patterns, that is, AC-15–AC-23.^{26 33 80–101} It is recognised that the distinction between AC-19 (dense fine speckled) and AC-20 (fine speckled) can be challenging. Moreover, within the spectrum of anti-tRNA synthetase antibodies, not all produce an HEp-2 IIFA pattern and only some anti-Jo-1 antibodies are considered to give the AC-20 pattern, while the other anti-tRNA synthetase antibodies (EJ, KS, OJ, PL-7 and PL-12) are more likely to reveal the AC-19 pattern. Solid information on the pattern of two additional anti-tRNA synthetase antibodies (Ha and Zo) is lacking. Overall, the relation between these two cytoplasmic HEp-2 IIFA patterns and the distinct anti-tRNA synthetase antibodies is subject to further discussion. In clinical practice, the complete spectrum of the anti-tRNA synthetase antibodies should be determined irrespective of the subtype of cytoplasmic speckled pattern, that is, AC-19 or AC-20.

For seven cytoplasmic HEp-2 IIFA patterns (AC-15–AC-19, AC-22 and AC-23), more detailed information is provided in online supplementary table S2. In particular, for AC-16–AC-18, the clinical associations are quite diverse, depending on the antigen recognised. Overall, the clinical associations provided are primarily based on antigen-specific immunoassays and not on the HEp-2 IIFA pattern as such.

Mitotic HEp-2 IIFA patterns

The clinical relevance of the five mitotic patterns is summarised in table 3,^{102–122} with more detailed information in online supplementary table S3. As for the cytoplasmic patterns, clinical associations for the mitotic patterns are primarily based on antigen-specific immunoassays and not on the HEp-2 IIFA pattern as such.

DISCUSSION

In the current paper, we present the ICAP consensus on the clinical relevance of 29 HEp-2 IIFA patterns defined by ICAP.^{11 14} The consensus on clinical relevance is defined in the clinical context of the patient, that is, suspected disease, and includes recommended follow-up testing within the spectrum of antigen-specificities that are commercially available. Obviously, if follow-up testing identifies the antigen, the clinical relevance can be further refined.¹²³

Defining the clinical relevance of HEp-2 IIFA patterns in the context of disease manifestations is meant to be an important tool for the clinician in the diagnostic work-up of patients suspected of SARD. Unfortunately, good data on the association between HEp-2 IIFA patterns and the distinct diseases are lacking, probably due to reasons summarised below. There are several reasons for not finding a perfect association between HEp-2 IIFA patterns and diseases. First, pattern assignment in

Table 1 Nuclear HEp-2 IIFA patterns

Code	AC pattern—clinical relevance	Refs
AC-1	<p>HOMOGENEOUS</p> <ul style="list-style-type: none"> ▶ Found in patients with SLE, chronic autoimmune hepatitis or juvenile idiopathic arthritis ▶ If SLE is clinically suspected, it is recommended to perform a follow-up test for anti-dsDNA antibodies, alone or in combination with dsDNA/histone complexes (nucleosomes/chromatin); anti-dsDNA antibodies are included in the classification criteria for SLE ▶ If chronic autoimmune hepatitis or juvenile idiopathic arthritis is suspected, follow-up testing is not recommended because the respective autoantigens revealing the AC-1 pattern are not completely defined <p>Notes: Although autoantibodies to Topoisomerase I (formerly Scl-70) may be reported as nuclear homogeneous, they typically reveal a composite AC-29 HEp-2 IIFA pattern; as such, clinical suspicion of SSc may warrant follow-up testing for reactivity to this antigen.</p> <p>Although AC-1 is the most prevalent pattern in chronic autoimmune hepatitis, other HEp-2 IIFA patterns may occur, but also for these patterns the autoantigens are not completely defined.</p>	<p>15, 16</p> <p>17</p> <p>14, 18</p> <p>19</p>
AC-2	<p>DENSE FINE SPECKLED</p> <ul style="list-style-type: none"> ▶ Commonly found as high titer HEp-2 IIFA-positive in apparently healthy individuals or in patients who do not have a systemic autoimmune rheumatic disease (SARD) ▶ The negative association with SARD is only valid if the autoreactivity is confirmed as being directed to DFS70 (also known as LEDGF/p75) and if no other common ENA is recognized ▶ Both in apparently healthy individuals as well as patients who do not have a SARD the AC-2 pattern may be caused by autoantibodies to other antigens than DFS70 <p>Note: Confirmatory assays for anti-DFS70 antibodies may be available only in specialty clinical laboratories.</p>	<p>9</p> <p>20, 21</p> <p>22</p>
AC-3	<p>CENTROMERE (see online supplementary table S1 for further details)</p> <ul style="list-style-type: none"> ▶ Commonly found in patients with limited cutaneous SSc, and as such included in the classification criteria for SSc ▶ In combination with Raynaud phenomenon, the AC-3 pattern is prognostic for onset of limited cutaneous SSc ▶ Strongly associated with antibodies to CENP-B; especially in case of low titers, confirmation by an antigen-specific immunoassay is recommended to support the association with limited cutaneous SSc; the CENP-B antigen is included in many routine ENA profiles ▶ The AC-3 pattern is also apparent in a subset of patients with PBC; these patients often have both SSc as well as PBC 	<p>8, 15, 23</p> <p>15, 23</p> <p>15</p> <p>15</p>
AC-4	<p>FINE SPECKLED</p> <ul style="list-style-type: none"> ▶ Present to a varying degree in distinct SARD, in particular SjS, SLE, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, congenital heart block, DM, SSc, and SSc-AIM overlap syndrome ▶ If SjS, SLE, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, or congenital heart block is clinically suspected, it is recommended to perform follow-up tests for anti-SS-A/Ro (Ro60) and anti-SS-B/La antibodies; in most laboratories these antigens are included in the routine ENA profile ▶ Autoantibodies to SS-A/Ro are part of the classification criteria for SjS (the criteria do not distinguish between Ro60 and Ro52/TRIM21) ▶ If SSc, AIM, or to a lesser extent SLE, is clinically suspected, it is recommended to perform follow-up tests for detecting autoantibodies to Mi-2, TIF1γ, and Ku; these antigens are typically included in disease specific immunoassays (i.e., inflammatory myopathy profile*) ▶ Autoantibodies to Mi-2 and TIF1γ are associated with DM; autoantibodies to TIF1γ in patients with DM, although rare in the overall AC-4 pattern, is strongly associated with malignancy in old patients ▶ Autoantibodies to Ku are associated with SSc-AIM and SLE-SSc-AIM overlap syndromes <p>Notes: Anti-SS-A/Ro (Ro60) and AIM-specific autoantibodies may be undetected in HEp-2 IIFA-screening.</p>	<p>15</p> <p>15</p> <p>25</p> <p>26</p> <p>26, 27</p> <p>26</p> <p>28</p>
AC-5	<p>LARGE/COARSE SPECKLED (see online supplementary table S1 for further details)</p> <ul style="list-style-type: none"> ▶ Present to a varying degree in distinct SARD, in particular SLE, SSc, MCTD, SSc-AIM overlap syndrome, and UCTD (i.e. patients with rheumatic symptoms without a definite SARD diagnosis) ▶ If SLE is clinically suspected, it is recommended to perform follow-up tests for anti-Sm and anti-U1RNP antibodies; these antigens are commonly included in the routine ENA profile; anti-Sm antibodies are included in the classification criteria for SLE ▶ If SSc is clinically suspected, it is recommended to perform a follow-up test for anti-RNApol III antibodies (e.g., SSc profile*); the anti-RNApol III antibodies are included in the classification criteria for SSc ▶ If MCTD is clinically suspected, it is recommended to perform a follow-up test for anti-U1RNP antibodies; the antigen is commonly included in the routine ENA profile; anti-U1RNP antibodies are included in the diagnostic criteria for MCTD ▶ If the SSc-AIM overlap syndrome is clinically suspected, it is recommended to perform follow-up tests for anti-U1RNP and anti-Ku antibodies; these antigens are included in the routine ENA profile (U1RNP), or in disease specific immunoassays (Ku, i.e., inflammatory myopathy profile* and SSc profile*) ▶ In non-SARD individuals in the general population, the presence of the AC-5 pattern is not associated with the autoantigens mentioned above and most often concerns low antibody titers 	<p>29</p> <p>16, 30, 31</p> <p>8</p> <p>32</p> <p>26, 33</p>

Continued

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Table 1 Continued

Code	AC pattern—clinical relevance	Refs
AC-6	<p>MULTIPLE NUCLEAR DOTS</p> <ul style="list-style-type: none"> ▶ Found in a broad spectrum of autoimmune diseases, including PBC, AIM (DM), as well as other inflammatory conditions 34 ▶ If PBC is clinically suspected, it is recommended to perform follow-up tests for anti-Sp100 (and PML/Sp140) antibodies; in particular anti-Sp100 antibodies have the best clinical association with PBC and have added value, especially when associated with AMA; the Sp100 (and PML-Sp140) antigen is included in disease specific immunoassays (ie, liver profile*) 17, 35, 36 ▶ If DM is clinically suspected, it is recommended to perform a follow-up test for anti-MJ/NXP-2 antibodies; these anti-MJ/NXP-2 antibodies are highly specific for AIM, are found in up to one third of patients with juvenile DM, and have been reported to be associated with malignancies in adult AIM patients; the antigen is included in disease specific immunoassays (i.e., inflammatory myopathy profile*) 37–39 	
AC-7	<p>FEW NUCLEAR DOTS (see online supplementary table S1 for further details)</p> <ul style="list-style-type: none"> ▶ The AC-7 pattern has low positive predictive value for any disease 40, 41 ▶ Antigens primarily localized in the dots include p80-coilin and SMN complex; specific immunoassays for these autoantibodies are currently not commercially available 42, 43 	
AC-8	<p>HOMOGENEOUS NUCLEOLAR (see online supplementary table S1 for further details)</p> <ul style="list-style-type: none"> ▶ Found in patients with SSc, SSc-AIM overlap syndrome, and patients with clinical manifestations of other SARD 44–46 ▶ If limited cutaneous SSc is clinically suspected, it is recommended to perform a follow-up test for anti-Th/To antibodies; the antigen is included in disease specific immunoassays (ie, SSc profile*) 44, 45 ▶ If SSc-AIM overlap syndrome is clinically suspected, it is recommended to perform a follow-up test for anti-PM/Scl antibody reactivity; the antigen may be included in the routine ENA profile and is included in disease specific immunoassays (i.e., inflammatory myopathy profile* and the SSc profile*); in general, anti-PM/Scl antibodies yield a diffuse nuclear fine speckled staining in addition to the AC-8 pattern 46 ▶ Other antigens recognized include B23/nucleophosmin, No55/SC65, and C23/nucleolin, but the clinical significance of these autoantibodies is not well established; specific immunoassays for these autoantibodies are currently not commercially available <p>Notes: Although some anti-Th/To antibody immunoassays are commercially available, technical issues relating to the limited sensitivity of these immunoassays should be taken into consideration. 44, 47</p>	
AC-9	<p>CLUMPY NUCLEOLAR</p> <ul style="list-style-type: none"> ▶ Found in patients with SSc 48 ▶ If SSc is clinically suspected, it is recommended to perform a follow-up test for anti-U3RNP/fibrillarin antibodies; the antigen is included in disease specific immunoassays (i.e, SSc profile*) 48 ▶ If confirmed as anti-U3RNP/fibrillarin reactivity by immunoassay, the clinical association is with diffuse SSc, increased incidence of pulmonary arterial hypertension, skeletal muscle disease, severe cardiac involvement, and gastrointestinal dysmotility 23, 48–50 ▶ Among SSc patients, anti-U3RNP/fibrillarin antibodies are most commonly found in African American and Latin American patients 48, 49, 51 <p>Notes: Although some anti-U3RNP/fibrillarin immunoassays are commercially available, technical issues relating to the limited sensitivity of these immunoassays should be taken into consideration. 24</p>	
AC-10	<p>PUNCTATE NUCLEOLAR</p> <ul style="list-style-type: none"> ▶ The AC-10 pattern can be seen in various conditions, including SSc, Raynaud's phenomenon, SjS, and cancer 52–56 ▶ If the AC-10 pattern is observed in the serum of patients with conditions mentioned above, follow-up testing for anti-NOR90(hUBF) antibodies is to be considered; the antigen is included in disease specific immunoassays (i.e. SSc profile*) 54, 55 ▶ While AC-10 is associated with anti-RNAPol I antibodies, these antibodies almost always coexist with anti-RNAPol III antibodies which reveal the AC-5 pattern; therefore, if SSc is clinically suspected, it is recommended to perform a follow-up test for anti-RNAPol III antibodies (See also AC-5); specific immunoassays for anti-RNAPol I antibodies are currently not commercially available 52, 53, 57 	
AC-11	<p>SMOOTH NUCLEAR ENVELOPE</p> <ul style="list-style-type: none"> ▶ The AC-11 pattern is infrequently found in routine autoantibody testing and has been described in autoimmune-cytopenias, autoimmune liver diseases, linear scleroderma, APS, and SARD; current information on clinical associations is based mainly on case reports and small cohorts 58–60 ▶ Antigens recognized include lamins (A, B, C) and LAP-2; specific immunoassays for these autoantibodies are currently not commercially available 58–60 	
AC-12	<p>PUNCTATE NUCLEAR ENVELOPE (see online supplementary table S1 for further details)</p> <ul style="list-style-type: none"> ▶ Found in patients with PBC, as well as patients with other autoimmune liver diseases and SARD 61 ▶ If PBC is clinically suspected, it is recommended to perform a follow-up test for anti-gp210 antibodies; the antigen is included in disease specific immunoassays (ie, extended liver profile*) 62–64 ▶ Other antigens recognized include p62 nucleoporin, LBR, and Tpr; specific immunoassays for these autoantibodies are currently not commercially available 65–68 	

Continued

Table 1 Continued

Code	AC pattern—clinical relevance	Refs
AC-13	PCNA-like (see online supplementary table S1 for further details) <ul style="list-style-type: none"> ▶ The AC-13 pattern has formerly been considered highly specific for SLE, but this specificity is debated ▶ If SLE is clinically suspected, it is recommended to perform a follow-up test for anti-PCNA antibodies; the antigen is included in several routine ENA profiles ▶ Recent studies with antigen-specific immunoassays show clinical associations also with SSc, AIM, RA, HCV and other conditions 	69, 70 69 70–73
AC-14	CENP-F-like <ul style="list-style-type: none"> ▶ The majority of sera exhibiting the AC-14 pattern are from patients with a diversity of neoplastic conditions (breast, lung, colon, lymphoma, ovary, brain); paradoxically, the frequency of the AC-14 pattern in patient cohorts with these malignancies is low ▶ The AC-14 pattern is also seen in inflammatory conditions (Crohn’s disease, autoimmune liver disease, SjS, graft-versus-host disease); current information on clinical associations is based mainly on case reports and series of cases ▶ Possible associations only hold if the reactivity to CENP-F is confirmed in an antigen-specific immunoassay; current information on clinical associations is based mainly on case reports and series of cases; specific immunoassays for this autoantibody are currently not commercially available 	74–78
AC-29	TOPOI-like <ul style="list-style-type: none"> ▶ The AC-29 pattern is highly specific for SSc, in particular with diffuse cutaneous SSc and more aggressive forms of SSc ▶ If SSc is clinically suspected, it is recommended to perform a follow-up test for anti-Topoisomerase I (formerly Scl-70) antibodies; the anti-Topoisomerase I antibodies are included in the classification criteria for SSc and the antigen is included in routine ENA profiles 	14, 18, 23 8, 23, 79

*Availability of the inflammatory myopathy profile, the SSc profile and the (extended) liver profile may be limited to specialty clinical laboratories. AIM, autoimmune myopathy; AMA, antimitochondrial antibodies; APS, antiphospholipid syndrome; CENP, centromere-associated protein; DFS, dense fine speckled; DM, dermatomyositis; ENA, extractable nuclear antigens; HCV, hepatitis C virus; IIFA, indirect immunofluorescence assay; LAP, lamin-associated polypeptide; LBR, lamin B receptor; LEDGF, lens epithelial derived growth factor; NOR, nucleolus organiser region; NXP, nuclear matrix protein; PBC, primary biliary cholangitis; PCNA, proliferating cell nuclear antigen; PML, promyelocytic leukaemia; PM/Scl, polymyositis-scleroderma; RA, rheumatoid arthritis; RNAPol, RNA polymerase; RNP, ribonucleoprotein; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SMN, survival of motor neuron; SSc, systemic sclerosis; SjS, Sjögren’s syndrome; TIF, transcription intermediary factor; TRIM, tripartite motif; Tpr, translocated promoter region; UCTD, undifferentiated connective tissue disease; dsDNA, double stranded DNA; hUBF, human upstream binding factor.

clinical laboratories is rather inconsistent as shown by external quality assessments.^{14 124 125} This is exactly the reason why ICAP was initiated: the consensus on nomenclature and definitions of HEp-2 IIFA patterns allows to align pattern description across laboratories. Also, the integration of computer-aided immunofluorescence microscopy (CAIFM) may further improve the consistency in pattern assignments.^{126–131} As such, it is promising that several companies involved in CAIFM have declared their intention to accommodate to the ICAP classification. Second, even apparently healthy individuals may have autoantibodies as detected by the HEp-2 IIFA. Such autoantibodies, being either innocent bystander antibodies or predictive antibodies, may still be present on development of SARD and interfere with the SARD-related pattern. Interestingly, the pattern best associated with apparently healthy individuals is the nuclear dense fine speckled pattern (AC-2), but this association only holds if the specificity is confirmed as monospecific for DFS70.^{20 21 132} Third, the HEp-2 IIFA patterns may slightly differ depending on the cellular substrate used. For this reason, the ICAP website contains for each pattern multiple pictures taken from different brands of HEp-2 slides. Fourth, diseases like systemic lupus erythematosus and autoimmune inflammatory myopathies may be associated with distinct autoantibodies, each associated with a distinct HEp-2 IIFA pattern. If the autoantigens are ill defined, as is the case, for instance, in autoimmune hepatitis, only the most prevalent patterns are included. Altogether, it is evident that, with the exception of the centromere pattern (AC-3), all patterns are to be confirmed by antigen-specific immunoassay for a solid association with the respective autoimmune diseases.

While consensus statements have been generated for all 29 HEp-2 IIFA patterns, and it is highly recommended to report

patterns,^{7 11} it is anticipated that laboratories may restrict their reports to the so-called ‘competent level’ patterns (<http://www.ANAPatterns.org>).¹³³ Although, for instance, the nucleolar patterns may not be reported as distinct entities (AC-8, AC-9 and AC-10), all three subtypes represent autoantibodies reactive with antigens associated with systemic sclerosis, either alone or in combination with autoimmune inflammatory myopathies. Follow-up testing, therefore, anyhow involves the systemic sclerosis multiparameter assay including all the relevant autoantibodies. Traditionally, only nuclear HEp-2 IIFA patterns have been considered as a true positive HEp-2 IIFA test, and this is most likely related to the time-honoured terminology ‘Antinuclear Antibody Test’,¹² but it is evident from this report that even for nuclear HEp-2 IIFA patterns, the clinical associations are quite diverse. In particular, the nuclear dense fine speckled pattern (AC-2) seems to have an inverse association with SARD.^{9 134} On the other hand, the cytoplasmic HEp-2 IIFA patterns, and to a lesser extent the mitotic patterns, are also clinically relevant and may demand dedicated follow-up testing in daily clinical practice. Therefore, the ICAP executive board advocates that information on HEp-2 IIFA patterns should be reported to the clinician and should also be incorporated in diagnostic and classification criteria instead of the simple assignment ‘ANA-positive’.¹³⁵

Although the HEp-2 IIFA has been considered the gold standard for autoantibody detection in SARD,³ the limitations of this assay are understood.^{136–138} Indeed, up to 35% of healthy controls may be positive if a screening dilution of 1/40 is used.¹³⁹ Therefore, in the EASI/IUIS recommendations, it is advocated that each laboratory verifies that the screening dilution is defined by a cut-off set at the 95th percentile.⁷ However, by

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Table 2 Cytoplasmic HEp-2 IIFA patterns

Code	AC pattern—clinical relevance	Refs
AC-15	<p>FIBRILLAR LINEAR (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> ▶ Found in patients with AIH type 1, chronic HCV infection, and celiac disease (IgA isotype); rare in SARD 17 ▶ If AIH type 1 is clinically suspected, it is recommended to confirm reactivity with smooth muscle antibodies (IgG isotype), typically detected by IIFA on rodent tissue (liver, stomach, kidney); anti-smooth muscle antibodies are included in the international criteria for AIH type 1 17,80 ▶ F-actin is the main target antigen of anti-smooth muscle antibodies in AIH type 1; autoantibodies to F-actin are of more clinical importance than antibodies to G-actin 81–83 <p>Notes: Although anti-F-actin immunoassays are commercially available, technical issues relating to the sensitivity of these immunoassays should be taken into consideration.</p>	
AC-16	<p>FIBRILLAR FILAMENTOUS (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> ▶ Found in various diseases, but AC-16 is not typically found in SARD ▶ Antigens recognized include cytokeratins 8, 18, & 19, tubulin, and vimentin; specific immunoassays for these autoantibodies are currently not commercially available 	
AC-17	<p>FIBRILLAR SEGMENTAL (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> ▶ Found very infrequently in a routine serology diagnostic setting ▶ Antigens recognized include α-Actinin and Vinculin; specific immunoassays for these autoantibodies are currently not commercially available 	
AC-18	<p>DISCRETE DOTS (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> ▶ Autoantibodies revealing the AC-18 pattern have been reported in distinct SARD and in a variety of other diseases; their prevalence in unselected or specified disease cohorts has not been thoroughly studied 84 ▶ Antigens recognized include GW-body (Processing or P body) antigens (Ge-1/Hedls, GW182, and Su/Ago2) and endosomal antigens (EEA1, CLIP-170, GRASP-1, and LBPA); specific immunoassays for these autoantibodies are currently not commercially available <p>Notes: Autoantibodies to GW-bodies and endosomes may yield slightly different HEp-2 IIFA patterns. 84, 85</p>	
AC-19	<p>DENSE FINE SPECKLED (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> ▶ Found in patients with SLE and the anti-synthetase syndrome (a subset of AIM), interstitial lung disease, polyarthritis, Raynaud's phenomenon, and mechanic's hands; these features may occur in various combinations or as an isolated manifestation, especially interstitial lung disease 33, 86, 87 ▶ If SLE is clinically suspected, follow-up tests for antibodies to ribosomal P phosphoproteins (P0, P1, P2, C22 RibP peptide) are recommended; these antigens may be included in the routine ENA profile ▶ Anti-RibP antibodies have been associated in some studies with neuropsychiatric lupus, and in childhood-onset SLE with autoimmune hemolytic anemia 86, 88, 89 ▶ If AIM, in particular the anti-synthetase syndrome, is suspected, it is recommended to perform follow-up tests for antibodies to tRNA synthetases; antigens are included in disease specific immunoassays (ie, inflammatory myopathy profile*) 26, 33 ▶ If AIM, in particular necrotizing myopathy, is suspected, it is recommended to perform follow-up tests for anti-SRP antibodies; the antigen is included in disease specific immunoassays (ie, inflammatory myopathy profile*) 26 <p>Notes: The fine distinction between AC-19 and -20 may depend on HEp-2 substrates and/or antibody concentration; antibodies to both RibP as well as tRNA synthetases may be undetected in HEp-2 IIFA-screening.</p>	
AC-20	<p>FINE SPECKLED</p> <ul style="list-style-type: none"> ▶ Found in patients with the anti-synthetase syndrome (a subset of AIM), interstitial lung disease, polyarthritis, Raynaud's phenomenon, and mechanic's hands; these features may occur in various combinations or as an isolated manifestation, especially interstitial lung disease 33, 90 ▶ Autoantibodies associated with the AC-20 pattern are primarily reported for the anti-Jo-1 antibody, which recognizes histidyl-tRNA synthetase; since AC-20 is not specific for Jo-1, it is recommended to perform a follow-up test for anti-Jo-1 antibodies; the antigen is included in the routine ENA profile, as well as in disease specific immunoassays (ie, inflammatory myopathy profile*); the anti-Jo-1 antibodies are included in the classification criteria for AIM 91, 92 <p>Notes: The fine distinction between AC-19 and -20 may depend on HEp-2 substrates and/or antibody concentration; antibodies to Jo-1 may be undetected in HEp-2 IIFA-screening.</p>	

Continued

Table 2 Continued

Code	AC pattern—clinical relevance	Refs
AC-21	<p>RETICULAR/AMA</p> <ul style="list-style-type: none"> Commonly found in PBC, but also detected in SSc, including PBC-SSc overlap syndrome and PBC-SjS overlap syndrome If PBC is clinically suspected it is recommended to perform a follow-up test for AMA, historically detected by IIFA on rodent tissue (liver, stomach, kidney); these autoantibodies are primarily directed to the PDH complex, and in particular the E2-subunit (PDH-E2); the antigen is included in disease specific immunoassays (i.e., liver profile*) as well as in some routine ENA profiles Additional antigens recognized include the E1α and E1β subunits of PDH, the E3-binding protein of PDH, and the 2-OGDC; these antigens are only included in extended disease specific immunoassays (i.e., extended liver profile*) 	<p>93–97</p> <p>93, 94</p> <p>93, 94</p>
AC-22	<p>POLAR/GOLGI-like (see online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> Found in small numbers of patients with a variety of conditions Antigens recognized include giantin/macrogolgin and distinct golgin molecules; specific immunoassays to detect autoantibodies directed to specific Golgi antigens are currently not commercially available 	85
AC-23	<p>RODS and RINGS (see (online supplementary table S2 for further details)</p> <ul style="list-style-type: none"> Most commonly found in HCV patients who have been treated with pegylated interferon-α/ribavirin combination therapy, but autoantibodies revealing the AC-23 patterns were undetected prior to treatment; as the use of interferon-α/ribavirin in HCV treatment is decreasing, the frequency and clinical associations of the AC-23 pattern may change Specific immunoassays to detect autoantibodies directed to specific Rods and Rings antigens, for instance IMPDH2, are not commercially available <p>Note: Presence of the AC-23 pattern depends on the HEP-2 cell substrate.</p>	98–101

*Availability of the inflammatory myopathy profile, the SSc profile and the (extended) liver profile may be limited to specialty clinical laboratories. AIH, autoimmune hepatitis; AIM, autoimmune myopathy; AMA, anti-mitochondrial antibodies; APS, antiphospholipid syndrome; Ago, argonaute protein; CENP, centromere-associated protein; CLIP, class II-associated invariant chain peptide; DFS, dense fine speckled; DM, dermatomyositis; EEA, early endosome antigen; ENA, extractable nuclear antigens; HCV, hepatitis C virus; IFA, indirect immunofluorescence assay; LAP, lamin-associated polypeptide; LBR, lamin B receptor; LEDGF, lens epithelial derived growth factor; NOR, nucleolus organizer region; NXP, nuclear matrix protein; PBC, primary biliary cholangitis; PCNA, proliferating cell nuclear antigen; PML, promyelocytic leukaemia; PM/ScI, polymyositis-scleroderma; RA, rheumatoid arthritis; RNApol, ribonucleic acid polymerase; RNP, ribonucleoprotein; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SMN, survival of motor neuron; SRP, signal recognition protein; SSc, systemic sclerosis; SjS, Sjögren's syndrome; TIF, transcription intermediary factor; TRIM, tripartite motif; Tpr, translocated promoter region; dsDNA, double stranded deoxyribonucleic acid; HUBF, human upstream binding factor; tRNA, transfer ribonucleic acid.

taking into account that the HEP-2 IIFA nowadays is ordered by a wide spectrum of clinical disciplines,¹ the number of clinically unexpected positive results, that is, positive test results with no clinical evidence of an associated autoimmune disease, is ever increasing and may even equal the likelihood of a clinically true-positive result.^{140 141} A study performed in a community setting concluded that many patients with a positive ANA test are incorrectly given a diagnosis of systemic lupus erythematosus and sometimes even treated with toxic medications.¹⁴² These arguments are used to introduce a gating strategy in order to restrict test-ordering to those cases that have a sufficiently high pretest probability for having a SARD. However, it can also be argued that patients with a low pretest probability should be tested using the HEP-2 IIFA in order to prevent true cases, especially those with very early disease manifestations, from being missed. This is a paradigm shift to disease prediction and prevention.¹⁴³ In this strategy, the HEP-2 IIFA could be integrated in multianalyte 'omic' profiles for case finding and establishing an early diagnosis and preventing severe complications.^{143 144} Obviously, it is anticipated that the added value of the HEP-2 IIFA in this approach can be increased by incorporating information on both patterns as well as titres in combination with well-directed advices on follow-up testing.

Although the current consensus on the clinical relevance of HEP-2 IIFA patterns has come across after extensive discussion and debate within the ICAP executive board as well as with the workshop participants, the information provided is not based on a systematic review or meta-analysis of the existing literature. Because of the short history of ICAP, being founded in 2014, inclusion of older literature might have been hampered

by potential differences in pattern nomenclature and definitions. For instance, the nuclear dense fine speckled (AC-2) and topo I-like (AC-29) patterns were previously often considered homogeneous, speckled or even mixed patterns. The centromere pattern (AC-3) or the cytoplasmic reticular/AMA (AC-21) patterns, on the other hand, are examples that probably have been less prone to change in pattern definition over time. The universal use of the ICAP nomenclature and pattern definitions, both in daily clinical practice as well as in the scientific literature, may enable systematic reviews in the future, and may well fine-tune current consensus based on expert opinions only.

In conclusion, the consensus statements on clinical relevance should be readily available to clinicians and this will enable further harmonisation of test-result interpretation with respect to HEP-2 IIFA patterns. Obviously, clinicians should be aware of the clinical suspicion for the respective patient, and therefore should order specific tests accordingly, also taking into account the anticipation of prevalence of HEP-2 IIFA negative (AC-0)¹³ results in SARD. The information on clinical relevance of HEP-2 IIFA patterns is intended to support the decision strategy of the clinician. Information presented in the online supplementary tables 1–3 is primarily intended to be used for complex cases in the consultation of the laboratory specialist by the clinician. Depending on various jurisdictional regulations, follow-up testing can be automated in predefined algorithms which eventually will shorten the diagnostic delay. Eventually, appropriate integration of HEP-2 IIFA pattern information may help to better define disease criteria and even enable a paradigm shift in the pretest probability paradox.

Recommendation

Table 3 Mitotic HEp-2 IIFA patterns

Code	AC pattern—clinical relevance	Refs
AC-24	<p>CENTROSOME (see online supplementary table 3 for further details)</p> <ul style="list-style-type: none"> ▶ The AC-24 pattern has low positive predictive value for any disease ▶ Within the spectrum of the SARD, the AC-24 pattern is found in patients with Raynaud's phenomenon, localized scleroderma, SSc, SLE and RA, either alone or in combination with other SSc-associated antibodies; ▶ Antigens recognized include α-enolase, γ-enolase, ninein, Cep-250, Mob1, PCM-1/2, pericentrin; specific immunoassays for these autoantibodies are currently not commercially available 	102–105 104, 106–108
AC-25	<p>SPINDLE FIBERS (see online supplementary table 3 for further details)</p> <ul style="list-style-type: none"> ▶ The AC-25 pattern has low positive predictive value for any disease ▶ Found very infrequently in a routine serology diagnostic setting ▶ Antigen recognized includes HsEg5; specific immunoassays for this autoantibody, or other spindle fiber targets, are currently not commercially available 	109 109 110, 111
AC-26	<p>NuMA-like</p> <ul style="list-style-type: none"> ▶ Approximately one-half of the patients with the AC-26 pattern have clinical features of a SARD (SJS, SLE, UCTD, limited SSc, or RA); the AC-26 pattern is also observed in patients with organ-specific autoimmune diseases and less frequently in non-autoimmune conditions, especially when in low titer ▶ Found very infrequently in a routine serology diagnostic setting ▶ Antigens recognized include NuMA, centrophilin, SP-H antigen and NMP-22; specific immunoassays for these autoantibodies are currently not commercially available 	109, 111–114 109 115
AC-27	<p>INTERCELLULAR BRIDGE (see online supplementary table 3 for further details)</p> <ul style="list-style-type: none"> ▶ The AC-27 pattern has low positive predictive value for any disease ▶ Found very infrequently in a routine serology diagnostic setting ▶ Antigens recognized include, among other, CENP-E, CENP-F, TD60, MSA36, KIF-14, MKLP-1, MPP1/KIF20B, and INCENP; specific immunoassays for these autoantibodies are currently not commercially available 	116 117 116, 118, 119
AC-28	<p>MITOTIC CHROMOSOMAL (see online supplementary table 3 for further details)</p> <ul style="list-style-type: none"> ▶ The AC-28 pattern has low positive predictive value for any disease ▶ Found very infrequently in a routine serology diagnostic setting ▶ Antigens recognized include DCA, MCA1, and MCA5; specific immunoassays for these autoantibodies are currently not commercially available 	120 120–122

CENP, centromere-associated protein; Cep, centrosomal protein; DCA, dividing cell antigen; IIFA, indirect immunofluorescence assay; INCENP, inner centromere protein; KIF, kinesin family; MCA, mitotic chromosomal antigen; MKLP, mitotic kinesin-like protein; MPP, M-phase phosphoprotein; MSA, mitotic spindle apparatus; NMP, nuclear matrix protein; NuMA, nuclear mitotic apparatus; PCM, pericentriolar material; RA, rheumatoid arthritis; SARD, systemic autoimmune rheumatic diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SJS, Sjögren's syndrome; UCTD, undifferentiated connective tissue disease.

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