


POSITION ARTICLE AND GUIDELINES

Open Access

V Brazilian consensus guidelines for detection of anti-cell autoantibodies on hep-2 cells



Wilson de Melo Cruvinel^{1*} , Luis Eduardo Coelho Andrade², Carlos Alberto von Mühlen³, Alessandra Dellavance⁴, Antônio Carlos Ximenes⁵, Carlos David Bichara⁶, Cleonice Bueno⁷, Cristóvão Luis Pitangueira Mangueira⁸, Eloísa Bonfá⁹, Fabiano de Almeida Brito¹⁰, Fernanda Bull Flumian¹¹, Glaucielen Gomes da Silva¹², Jozelia Rêgo¹³, Lisiane Maria Ericoni dos Anjos¹⁴, Natasha Shhessarenko¹⁵, Sandra Gofinet Pasoto¹⁶, Suzane Pretti Figueiredo Neves¹⁷, Valéria Valim¹⁸, Wilton Silva dos Santos¹⁹ and Paulo Luiz Carvalho Francescantonio^{1*}

Abstract

Background: The V Brazilian Consensus for determination of autoantibodies against cellular constituents on HEp-2 cells, held in Brasília (DF, Brazil) on August 27, 2016, discussed the harmonization between the Brazilian Consensus on ANA (BCA) guidelines and the International Consensus on ANA Patterns (ICAP) recommendations (www.anapatterns.org). Initial guidelines were formulated by the group of Brazilian experts with the purpose of guiding and enabling Brazilian clinical laboratories to adopt recommendations and to provide a common standard for national and international consensuses.

Mainbody: Twenty Brazilian researchers and experts from universities and clinical laboratories representing the various geographical regions of the country participated in the meeting. Three main topics were discussed, namely the harmonization between the BCA guidelines and latest recommendations of the ICAP initiative, the adjustment of the terminology and report on HEp-2 patterns, and a reassessment of quality assurance parameters. For the three topics, our aim was to establish specific guidelines. All recommendations were based on consensus among participants. There was concrete progress in the adjustment of the BCA guidelines to match the ICAP guidelines. To a certain extent, this derives from the fact that ICAP recommendations were largely based on the algorithm and recommendations of the IV Brazilian ANA Consensus, as consistently recognized in the ICAP publications and presentations. However, although there is great overlap between the two Consensuses, there are some point divergences. These specific items were individually and extensively discussed, and it was acknowledged that in several points ICAP improved recommendations previously issued by the Brazilian ANA Consensus and these changes were readily implemented. Regarding some specific topics, the BCA panel of experts felt that the previously issued recommendations remained relevant and possibly will require further discussion with ICAP. The term anti-cell antibodies was adopted as the recommended designation, recognizing that the assay addresses antibodies against antigens in the nucleus and in other cell compartments. However, the acronym ANA HEp-2 was maintained due to historical and regulatory reasons. It was also signaled that the latest trend in ICAP is to adopt the term Indirect Immunofluorescent Assay on HEp-2 cell substrate (HEp-2 IIFA). In addition, the quality assurance strategies previously presented were ratified and emphasized.

(Continued on next page)

* Correspondence: melocruvinel@gmail.com; paulo_luiz1@hotmail.com

Organizing committee: Wilson de Melo Cruvinel, Luis Eduardo Coelho Andrade, Wilton Silva dos Santos and Paulo Luiz Carvalho Francescantonio

Coordinator: Paulo Luiz Carvalho Francescantonio

¹Pontifícia Universidade Católica de Goiás (PUC Goiás), Escola de Ciências Médicas, Farmacêuticas e Biomédicas, Avenida Universitária 1.440, Setor Universitário, Goiânia, GO, CEP 74605-010, Brazil

Full list of author information is available at the end of the article



(Continued from previous page)

Conclusion: The V BCA edition was successful in establishing an overall harmonization with the ICAP recommendations for interpretation of the HEp-2 IIFA test, pinpointing the perspectives in filling the remaining gaps between both initiatives.

Keywords: ANA, Autoantibodies, HEp-2 cells, Antinuclear antibodies, Indirect immunofluorescence, Standardization

Background

Initiatives for the standardization of the Antinuclear Antibody assay on HEp-2 cells in Brazil began in 2000, with the First Brazilian Consensus for Antinuclear Antibody (ANA) Determination (BCA HEp-2) in Goiânia [1]. This initiative was successful in disseminate as standardized classification of ANA patterns in the country. In addition, BCA HEp-2 initiative inspired the launching of the International Consensus on ANA Patterns initiative in 2014. The present paper reports on the V BCA HEp-2 dedicated to the harmonization of the BCA HEp-2 with the ICAP guidelines. Aiming to keep coherence with the current international literature, the present report will use the recently consensed ICAP designation for the ANA test, i.e., indirect immunofluorescence assay on HEp-2 cells (HEp-2 IIFA), despite the fact that the designation recommended by the BCA HEp-2 is ANA-HEp-2 (see below).

The First BCA HEp-2 focused mainly on the definition of criteria for interpreting and reporting the test, elaboration of a classification algorithm for HEp-2 IIFA patterns based on topographic groups (nuclear, nucleolar, cytoplasmic and mitotic apparatus), and on the definition of general guidelines for the test procedure. At the occasion, it was established as conceptual framework that the pattern recognition and classification should be fundamentally based on morphological features [1].

The II BCA HEp-2, carried out in 2002 in Goiânia, defined the guidelines for issuing the descriptive report of the test. It was established that definite staining of any cell compartment, including the cytoplasmic, would be granted a positive test report. It was recognized that the coexistence of more than one autoantibody specificity in the sample can yield overlapping patterns and, therefore, the II BCA HEp-2 defined the group of mixed patterns. In addition, the II BCA HEp-2 defined preliminary clinical relevance and associations related to several HEp-2 IIFA patterns [2].

In 2007, the III BCA HEp-2 focused efforts on encouraging Brazilian laboratories to adopt quality control programs for the HEp-2 IIFA test, as well as educational programs. The relevance of conjugate titration was highlighted as a crucial step to adjust the reading system and harmonize the titer of results among different laboratories [3, 4].

In 2012, at the IV BCA HEp-2, in Vitória, three new patterns were included in the BCA HEp-2 classification

algorithm: the cytoplasmic rods and rings pattern, the nuclear quasi-homogeneous pattern and the nuclear CENPF pattern. Among the guidelines, the screening dilution was recommended at 1/80 and further serum dilution to at least 1/640. Recommendations were made regarding the pattern reproducibility in different commercial HEp-2 slide preparations and pertaining to the use of alternative methodologies for autoantibody screening, as well as for detection of specific autoantibodies [5].

The International Consensus on ANA Patterns (ICAP) was launched in São Paulo, in 2014, during the 12th International Workshop on Autoantibodies and Autoimmunity (IWAA). The purpose of the initiative is to discuss and promote consensus regarding patterns observed in HEp-2 IIFA, extending the concept and guidelines of the Brazilian ANA Consensus to specialists around the world [5], but always considering also the standardization initiatives from other countries [6–9].

The aim of ICAP was to establish global guidelines for the nomenclature and classification of HEp-2 IIFA patterns [10]. A group of 13 specialists from academic institutions in different countries were selected, with individual groups being responsible for studying the relevant literature and presenting a proposal to classify the HEp-2 IIFA patterns in each cell compartment. The session was attended by international specialists from academic, clinical laboratory and industry settings, who participated actively in the discussion. The underlying conceptual criteria were mainly based on the morphological details, but also considered in some cases the target autoantigens or target organelles. The ICAP classification algorithm mirrors that of the BCA HEp-2, but contains some peculiar aspects [10]. One innovation of ICAP is that each HEp-2 IIFA pattern was ascribed an alphanumeric code (Anti-Cell pattern) from AC-1 to AC-29. These codes allow for fast access and reference to all patterns classified under the ICAP algorithm. The ICAP recommendations have been published in successive papers [10, 11] and in the official website (www.anapatterns.org). This page has high penetration worldwide, currently presenting versions in ten languages, including Portuguese. The website www.anapatterns.org has been visited by more than 108,000 users in 171 countries, since its creation on May 2015. Recently, the ICAP team has published on detailed information on the clinical relevance of each of the 29 distinct HEp-2 IIFA patterns [12]. ICAP has

broad international acceptance and its recommendations have been adopted around the world by academic institutions, clinical laboratories and the industry of kit manufacture. Currently, the insert of several HEp-2 cell slide kits contains the ICAP classification algorithm.

In view of the worldwide acceptance of ICAP, nowadays adopted in most countries, the specialists of the BCA HEp-2 group understood that it would be appropriate to seek harmonization between both initiatives. To this end, the V BCA HEp-2 was organized with the purpose of reviewing the recommendations and algorithm of the BCA HEp-2 from the perspective of the ICAP guidelines. This paper presents the recommendations of the V BCA HEp-2, enabling Brazilian clinical laboratories to update the recommendations as well as to provide an update for clinicians who order and interpret the test. This report is also available online at the Brazilian Consensus website (www.hep-2.com.br).

Methods

On August 27, 2016, during the XXXIII Brazilian Rheumatology Congress held in Brasília, 20 experts on the HEp-2 IIFA test participated in a full-day workshop dedicated to undertaking an analytical comparison between the BCA HEp-2 and the ICAP algorithms and recommendations. Participants included HEp-2 IIFA experts from academic and clinical laboratories across the country. Objectives of the meeting were: 1) to identify potentially diverging and similar points, devising strategies for harmonization between both initiatives; 2) to determine adequacy of the current and possible alternatives for a correct denomination of the ANA HEp-2 test; and 3) to ratify and update previous recommendations from BCA HEp-2. Each topic was introduced to participants, followed by extensive discussion until agreement, as in previous sessions of the BCA HEp-2. The standard strategy adopted in the former sessions of the Brazilian Consensus on ANA Patterns is based on an initial period of free and thorough discussion of each item on the agenda. In most cases, this discussion naturally leads to consensus on each item. In very few cases, in which no natural consensus is attained, a further discussion session is promoted. If any divergence persists, we generally postpone the decision to the next meeting of the group, recommending full discussion of the subject in the meantime. Very rarely, we had to promote a voting process for final decision. This procedure has been clarified in the previous publications of the group. In the present version of the meeting, a natural consensus was attained for all points in the agenda.

Recommendations

Harmonization of BCA HEp-2 with ICAP recommendations

The V BCA HEp-2 recommends the harmonization with the ICAP pattern classification algorithm available at

www.anapatterns.org and summarized on Fig. 1. Accordingly, all BCA-HEp-2 algorithms were adapted to match as much as possible the ICAP algorithm. In addition, the appropriate ICAP alphanumeric code (Anti-cell indications - AC) was added to each pattern, which contributes for consistency of the scientific information at an international level. ICAP has so far classified 30 patterns (Fig. 1) of autoantibodies on HEp-2 cells, which were ascribed the respective alpha-numeric AC codes, starting at AC-0, which corresponds to the negative pattern, ending in AC-29, which corresponds to the compound pattern of anti-topoisomerase I antibodies [10, 12, 13].

By means of minor adjustments, the ICAP algorithm was harmonized into the BCA HEp-2 original algorithm. The incorporation of AC codes corresponding to all HEp-2 IIFA patterns recognized by ICAP (Fig. 1) in the V BCA HEp-2 classification tree is depicted in Fig. 2. Based on the ICAP classification, the nucleolar patterns are allocated within the nuclear group. As the conceptual framework for the pattern classification in BCA HEp-2 is based on the analysis of fluorescence across four cell regions (nucleus, nucleolus, cytoplasm and mitotic apparatus), the V BCA HEp-2 chose to keep the nucleolar patterns as a distinct group, with the understanding that this arrangement does not contradict the ICAP conceptual framework and contributes for harmonization of both initiatives (Fig. 1).

The group of nuclear speckled patterns in the ICAP algorithm is not split into the subgroups with stained and non-stained mitotic chromatin, respectively. Instead, ICAP algorithm signals with an icon the patterns with stained plate. The V BCA HEp-2 maintains the original subdivision by understanding that this favors laboratory technicians in pattern analysis, facilitating the classification and improving the learning process. This division is also useful for the interpretation of possible autoantibodies associated with each pattern. As an example, the nuclear centromeric pattern in the ICAP classification tree is connected directly to the nuclear patterns box, and it is not classified within the nuclear speckled group. The V BCA HEp-2 maintains the centromeric pattern (AC-3) linked to the nuclear speckled branch. In fact, the ICAP classification algorithm has several HEp-2 IIFA patterns directly linked to the root box (Fig. 1). However, the V BCA HEp-2 decided to maintain the classification guideline, in which individual HEp-2 patterns are linked to subgroups that derive from the root boxes (Fig. 2). Such tiered hierarchic algorithm contributes to the learning process and day-to-day operation by assisting in the comprehension and memorization of the several patterns.

The V BCA HEp-2 maintained the nuclear discrete dots pattern within the subgroup of speckled patterns with non-stained metaphase plate. This contrasts with

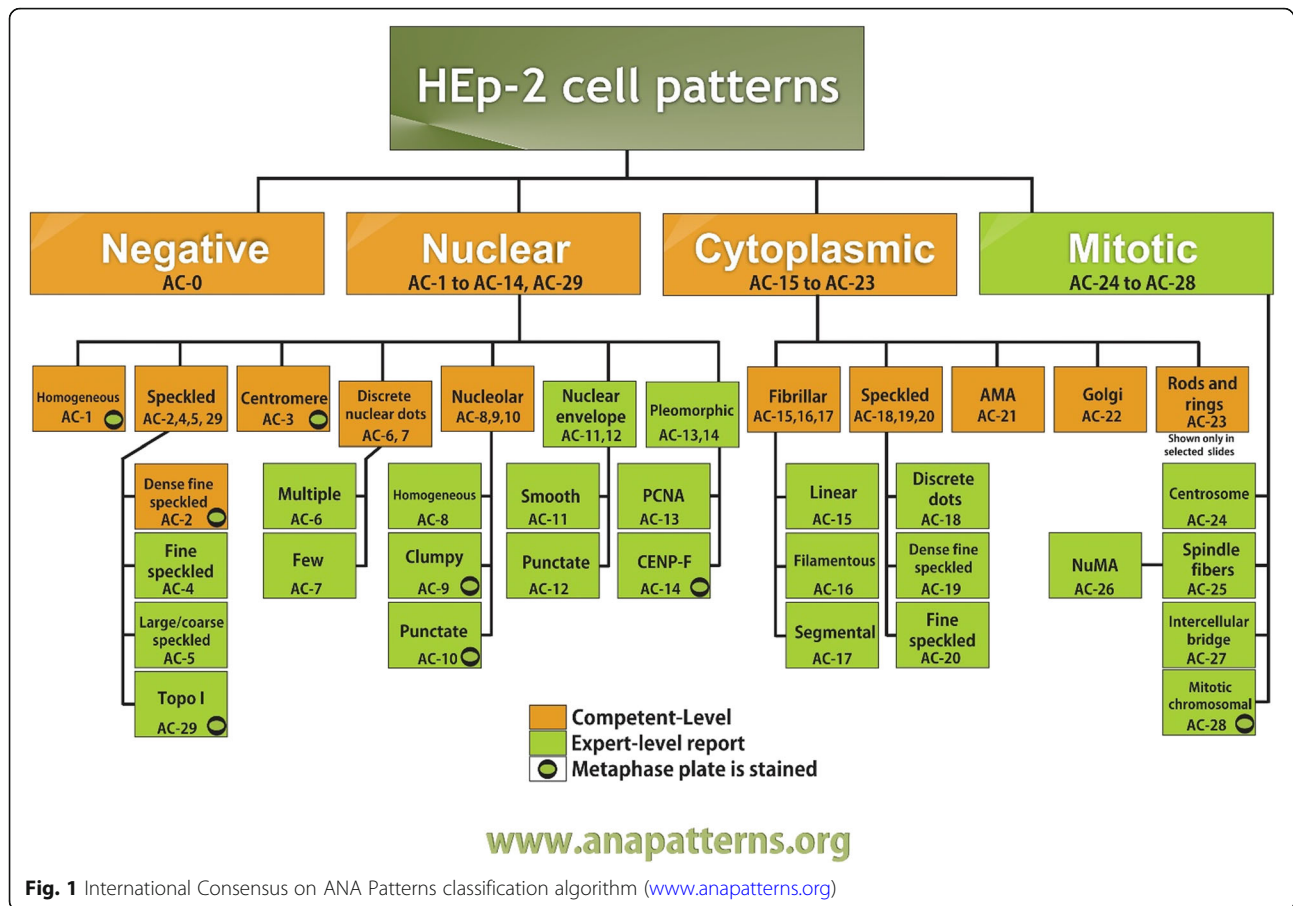


Fig. 1 International Consensus on ANA Patterns classification algorithm (www.anapatterns.org)

the ICAP algorithm, which links this pattern directly to the root box of nuclear patterns. In addition, ICAP discriminates nuclear discrete dots patterns into two distinct patterns: multiple discrete nuclear dots (AC-6) and few discrete nuclear dots (AC-7) [10]. The AC-7 pattern has a low positive predictive value for systemic autoimmune diseases and is frequently reported in healthy individuals [14]. It is characterized by the decoration of 1 to 6 discrete nuclear dots resulting from the recognition of p80 coilin autoantigen [14] or SMN (survival of motor neuron) protein [15]. The AC-6 pattern (multiple discrete nuclear dots) is characterized by the recognition of 6 to 15 discrete nuclear dots of heterogeneous size and fluorescence in interphase cells [16, 17]. This pattern is associated with reactivity against the autoantigens Sp100 [15] or MJ/NXP-2 [18] and is observed in different autoimmune diseases such as Primary Biliary Cholangitis, dermatomyositis and other systemic rheumatic diseases [19].

The accumulated experience of the successive editions of the BCA HEP-2 has identified significant difficulty for analysts in the counting of the discrete dots and discrimination of few versus multiple discrete nuclear dots. This is especially true because the number and size of nuclear

bodies stained by anti-p80 coilin and anti-sp 100 antibodies may vary, depending on the cellular substrate in use. Thus, although an experienced observer can safely suggest the most likely pattern in most cases, the number of points per nucleus does not seem to be an absolute criterion in distinguishing both patterns [3]. Therefore, since the third BCA HEP-2, the recommendation is that the rare discrete nuclear dots pattern (AC-7) and multiple discrete nuclear dots (AC-6) pattern are equally reported as “discrete nuclear pattern (AC6/AC7)”.

The pleomorphic speckled nuclear pattern (PCNA) (AC-13) remains in the group of nuclear speckled patterns with negative metaphase plate. In ICAP, this pattern is linked directly to the root nuclear box, and classified as expert level, which corresponds to the BCA HEP-2 optional report level. This is in contrast with the BCA HEP-2 recommendation that assigns the PCNA as a mandatory report pattern. This recommendation was maintained by the V BCA HEP-2, due to its significance in diagnosing systemic lupus erythematosus. An additional divergence refers to the CENP-F pattern, which is classified as a nuclear pattern in the ICAP algorithm and as a mixed pattern in the BCA HEP-2.

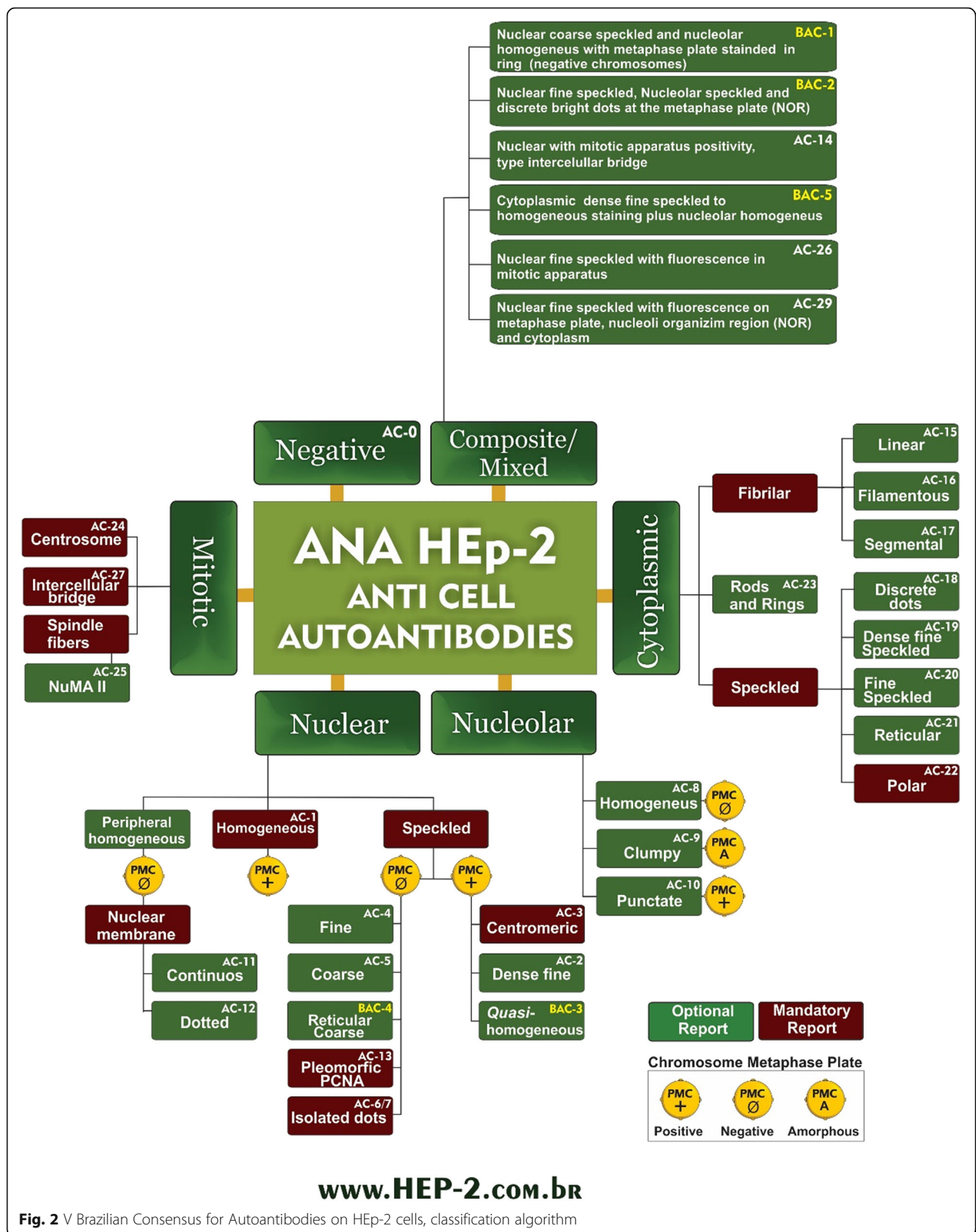


Fig. 2 V Brazilian Consensus for Autoantibodies on HEp-2 cells, classification algorithm

It is important to note that the above related divergent points between ICAP and BCA HEp-2 concern the hierarchical strategy for the pattern classification across the algorithms, but do not affect fundamental aspects, such as the definition and description of the patterns, as well as their associations with specific autoantibodies and clinical correlations. In that sense, laboratory analysts and clinicians will find a harmonical set of information in both algorithms, but at this point the BCA HEp-2 experts feel that the BCA HEp-2 algorithm provides further support in pattern identification and classification. In fact, the particularities of the BCA HEp-2 algorithm provide further support in pattern identification and classification.

Regarding cytoplasmic patterns, ICAP also recognizes the fibrillar and speckled subgroups, in the same way as BCA. However, mitochondrial and polar patterns are directly linked to the cytoplasmic patterns root box, whereas BCA HEp-2 classifies them within the secondary box of speckled cytoplasmic patterns (Fig. 2).

In the mitotic pattern group, ICAP includes the NuMA-like pattern (AC-26), characterized by nuclear fine speckled pattern plus spindle fibers decoration directly linked to the mitotic patterns root box. In fact, ICAP does not have now a classification group exclusively dedicated to mixed or compound patterns. In contrast, the BCA HEp-2 classifies the NuMA pattern (AC-26) as a compound pattern. The concept of compound patterns refers to patterns composed of staining of more than one cellular compartment and caused by a single autoantibody system targeting a single autoantigen, as is the case for NuMA-like pattern in which anti-NuMA antibodies react with the nucleoplasm and with the mitotic apparatus. ICAP and BCA HEp-2 fit the spindle fiber pattern (AC-25) within the mitotic patterns root box, side by side with other mitotic patterns such as the centrosome (AC-24) and the intercellular bridge (AC-27) patterns. However, BCA HEp-2 discriminates two closely related patterns, the NuMA 2 pattern (with extensive staining of the spindle fibers, anaphase intercellular bridge and the midbody) and the spindle fiber pattern (exclusive staining of the spindle fibers). The V BCA HEp-2 acknowledges and follows ICAP [10] by replacing the term “centriole pattern” with the term centrosome pattern (AC-24), considering the possible recognition not only of centrioles but also of other integrated components at this cellular domain [20].

Still in the mitotic apparatus group, ICAP adds a new pattern, the mitotic chromosomal envelope pattern, the only pattern classified by ICAP (AC-28) not yet recognized by BCA. This pattern is described as rare in discoid lupus erythematosus, chronic lymphocytic leukemia, Sjögren’s syndrome and polymyalgia rheumatica [21, 22]. The V BCA HEp-2 decided that participants should evaluate this novel pattern for further discussion in the future.

ICAP does not include some of the patterns classified by the BCA HEp-2, such as the reticulated coarse speckled nuclear pattern and the quasi-homogeneous speckled nuclear pattern. In addition, ICAP does not include the concept of compound patterns and, thus, does not include the compound patterns associated with anti-Ku (nuclear coarse speckled plus nucleolar homogeneous with a peripheral staining of the metaphase plate) and the P-ribosomal-associated pattern (dense fine speckled cytoplasmic plus weak homogeneous nucleolar patterns) [5, 10, 11]. The V BCA HEp-2 understood that these patterns should be kept in the Brazilian classification tree, since they have been useful for the interpretation of findings, for the establishment of possible associations and indicating further testing for specific autoantibodies. For a better inter-laboratory communication, in analogy with ICAP AC codes, the BCA created a transient alpha-numeric coding system (BAC: Brazilian anti-cell autoantibodies) to classify these patterns not yet recognized by ICAP. The main items that are peculiar to each of the two Consensuses are detailed in Table 1.

The compound pattern comprising nuclear fine speckled with similar staining on the metaphase plate, nucleolar organizing region (NOR) and cytoplasmic fine speckled staining, was recently classified by the ICAP as AC-29, so called Topo I-like pattern, which is directly linked to the nuclear pattern root box [23]. This pattern, originally described by DELLAVANCE et al. [24] and not present in the former version of the BCA HEp-2 algorithm, is now incorporated in its upgraded algorithm, in the category of compound patterns. Table 2 shows six compound patterns recognized by the BCA HEp-2, three of which are already classified by ICAP, although not classified as compound patterns. Nevertheless, the V BCA HEp-2 ascribes the same AC code used in ICAP for these three patterns.

As mentioned above, the V BCA HEp-2 confirms the concept of compound patterns as those in which a single autoantibody specificity yields distinct fluorescence patterns, be it in different cell compartments (nucleus, nucleolus, cytoplasm or mitotic apparatus) or different fluorescence patterns observed in the same compartment [24].

The V BCA HEp-2 sought to harmonize the Brazilian Consensus and the ICAP algorithms, maintaining the most valued peculiarities of the Brazilian classification algorithm and incorporating major advances from ICAP, with synergistic advantages for the interpretation of the patterns and autoantibodies associations.

Recommendations for naming the ANA test

Participants of the V BCA recommend the terminology “anti-cell antibody test”, but keeping the acronym ANA

Table 1 Main differences between the Brazilian Consensus for Autoantibodies on HEp-2 cells (BCA) and the International Consensus on Ana Patterns (ICAP)

| | BCA | ICAP |
|------------------------------------|---|--|
| Total patterns | 33 | 30 |
| Classification groups | (5) nuclear, nucleolar, mitotic cytoplasmic, mixed/compound | (3) nuclear, mitotic, cytoplasmic |
| Patterns not recognized | AC-28 mitotic chromosome | BAC-1, BAC-2, BAC-3, BAC-4 and BAC-5# |
| Nuclear dense fine speckled (AC-2) | optional report | competent-level |
| AC-6 and AC-7 | reported together | reported isolated |
| AC-9 | metaphase plate classified as amorphous | metaphase plate classified as positive |
| AC-13 | designated as mandatory report | designated as expert-level report |
| AC-14 | mixed/compound | nuclear |
| AC-21 | designated as optional report | designated as competent-level |
| AC-23 | designated as optional report | designated as competent-level |
| AC-24 | designated as mandatory report | designated as expert-level report |
| AC-25 | designated as mandatory report | designated as expert-level report |
| AC-27 | designated as mandatory report | designated as expert-level report |

BCA Brazilian Consensus on Autoantibodies, ICAP International Consensus on Ana Patterns. #BAC-1 nuclear coarse speckled plus nucleolar homogeneous with peripheral staining of metaphase plate, BAC-2 nuclear fine speckled and nucleolar speckled plus discrete bright dots at the metaphase plate (NOR), BAC-3 nuclear quasi-homogeneous, BAC-4 nuclear reticular coarse speckled

(in Portuguese, FAN, for fator antinúcleo). This trend had been predicted at the III BCA, when six options were recommended to designate the HEp-2 IIFA test, with the intention of gradually migrating to a single denomination. In the opinion of the BCA HEp-2 experts, ANA is the most used and most recognized term, as per rheumatologists and other specialists who commonly order this test. In addition, this is a recognized terminology in the official Clinical Pathology institutional agencies portfolio of tests in Brazil, which is of relevance for reimbursement purposes. In a recent study carried out by Silva and collaborators (2018), with participation of 53 Brazilian clinical laboratories, it was verified that most laboratories are using five of the six terms recommended by III BCA HEp-2. The term “ANA” was present in most of these denominations [25]. Recognizing the ICAP recommendation for naming the test as indirect immunofluorescence assay on HEp-2 cells (HEp-2 IIFA), used in the most recent publications [13, 23] and aiming to harmonize this report with the international current nomenclature, we will adopt in the term IIFA HEp-2 in this manuscript.

Choice of commercial brands and reproducibility of patterns

Recommendations of the IV BCA HEp-2 [5] regarding the distinct capacity of several commercial slides to express the various patterns was reiterated. The alert is emphasized due to a range of variation among different commercial substrates available in Brazil, which may affect the characterization and recognition of HEp-2 IIFA patterns. Variations can also be related to different

lots of the same slide brand, something inherent to the manufacturing process.

Therefore, it is suggested that laboratories consider the possibility of using more than one brand for samples generating unexpected results, considering the limitation of some substrates in the expression of certain patterns [26]. Another recommendation is that, for each new lot or new brand, representative samples of different patterns should be tested, preferentially contemplating different cell compartments. The use of a control panel for validation of HEp-2 cell lots and brands is a measure that ensures greater reliability and safety of results.

It is the laboratory responsibility to address occasional problems with substrates that do not adequately express certain patterns, such as the fine speckled nuclear pattern associated with the presence of anti-SS-A/Ro antibodies (AC-4), the coarse speckled nuclear pattern associated with antibodies against RNP/Sm (AC-5), the pleomorphic nuclear pattern suggestive of antibodies anti-PCNA (AC-13), the compound pattern suggestive of the presence of anti-CENP-F antibodies (AC-14), the cytoplasmic fine speckled pattern suggestive of anti-Jo-1 (AC-20) antibodies, the cytoplasmic rods and rings pattern (AC-23), the anti-NuMA-1-like pattern (AC-26) and the anti-NuMA-2 pattern (AC-25) [10, 26].

Solid-phase methods for screening autoantibodies

BCA HEp-2 does not recommended the use of enzyme immunoassays and chemiluminescence assays in the autoantibody screening as a replacement for the HEp-2 IIFA method. This recommendation is due to the fact that the HEp-2 IIFA method allows for the

Table 2 Compound/mixed patterns of the Brazilian consensus for autoantibodies on HEp-2 cells

| ICAP code | Pattern | Morphological description | Associated autoantibodies |
|-----------|--|--|---------------------------------------|
| BAC-1 | Nuclear fine speckled, nucleolar speckled plus discrete bright dots at the metaphase plate (NOR) | Cells in interphase with fine speckled nuclear and nucleolar decoration, highlighted with speckled pattern (individual points). Cytoplasm not stained. Metaphase plate with five to 10 dots, corresponding to the nucleolar organizing regions (NOR) | Anti-RNA polymerase I and II |
| BAC-2 | Nuclear coarse speckled plus nucleolar homogeneous with peripheral staining of the metaphase plate (non-stained chromosomes) | Cells in interphase with nuclear fine speckled staining, nucleolar homogeneous staining, metaphase cells with fluorescent decoration around the metaphase plate. | Anti-Ku |
| AC-14 | Variable-intensity nuclear speckled with mitotic centromere and intercellular bridge | Nuclear speckled pattern with variability in fluorescence intensity, greater in G2 phase and negative/moderate in G1 phase. Centromeres stained only in prometaphase and metaphase. Cells during pro-metaphase show delicate decoration of the nuclear envelope. Cytoplasm in mitotic cells diffusely stained | Anti-CENP-F |
| BAC-5 | Cytoplasmic dense fine speckled to homogeneous staining plus nucleolar homogeneous | Nucleoli with weak homogeneous fluorescence or negative. The cytoplasm expresses intense fine dense (tending to homogeneous) speckled staining. Negative nuclei and mitotic cells. | Anti-rRNP (ribosomal anti-protein P). |
| AC-26 | Nuclear fine speckled with fluorescence in mitotic apparatus | Interphase cells show nucleus stained as fine speckled, usually at high titre. Mitotic cells in metaphase and anaphase show a well-defined and delicate staining of the pericentrosome region, also of the proximal parts of the mitotic spindle. At telophase, there is speckled staining of the newly formed nuclei and no staining of the intercellular bridge. | Anti-NuMA1. |
| AC-29 | Nuclear fine speckled with fluorescence at the metaphase plate, nucleolar organizing region (NOR) and cytoplasm | Prominent nuclear fine speckled staining in interphase cells, fine speckled staining of condensed chromatin in mitotic cells with strong staining of nucleolar organizing region (NOR). Weak cytoplasmic staining in interphase (and mitotic) cells and variable nucleolar staining that can appear as a punctate nucleolar or perinucleolar staining. | Anti-DNA topoisomerase I (Sci-70) |

BAC Brazilian AC code. Preliminary designation that may be changed according to future ICAP determinations. AC Anti-cell code (designation according to ICAP)

detection of a large array of autoantibodies, including so far uncharacterized antibodies and some known autoantibodies not addressed in commercial multiplex reagents. Although the industry has promoted a virtuous progress in the improvement of several diagnostic methods, the use of solid phase-based methodologies has not yet surpassed the screening for autoantibodies by indirect immunofluorescence on HEp-2 cells in which experienced analysts are able to identify the different patterns indicating a large array of autoantibodies [5]. Careful morphological analysis of the fluorescence patterns directs the specific investigation of the most plausible autoantibodies present in the patient's serum, conferring to the indirect immunofluorescence method on HEp-2 cells a unique and non-comparable dimension that currently surpasses other autoantibody testing modalities.

Screening dilution

An important issue with the HEp-2 IIFA method is the occurrence of positive results in an expressive proportion of healthy individuals (~ 13% in the Brazilian population at 1/80 dilution) [27]. In general, healthy subjects tend to have low titer (1/80 and 1/160), whereas most autoimmune patients show moderate (1/160 and 1/320) to high titers ($\geq 1/640$) [27, 28]. Thus, the V BCA HEp-2 recommends maintenance of the screening dilution at 1/80, as suggested in the previous Brazilian Consensus [5].

Titration of the conjugate

From a technical point of view, the determination of autoantibodies on HEp-2 cells depends on several factors. From those, microscope bulbs are of the utmost importance. According to a research across clinical laboratories in Brazil, 62% use 100-W bulbs, but the observed range

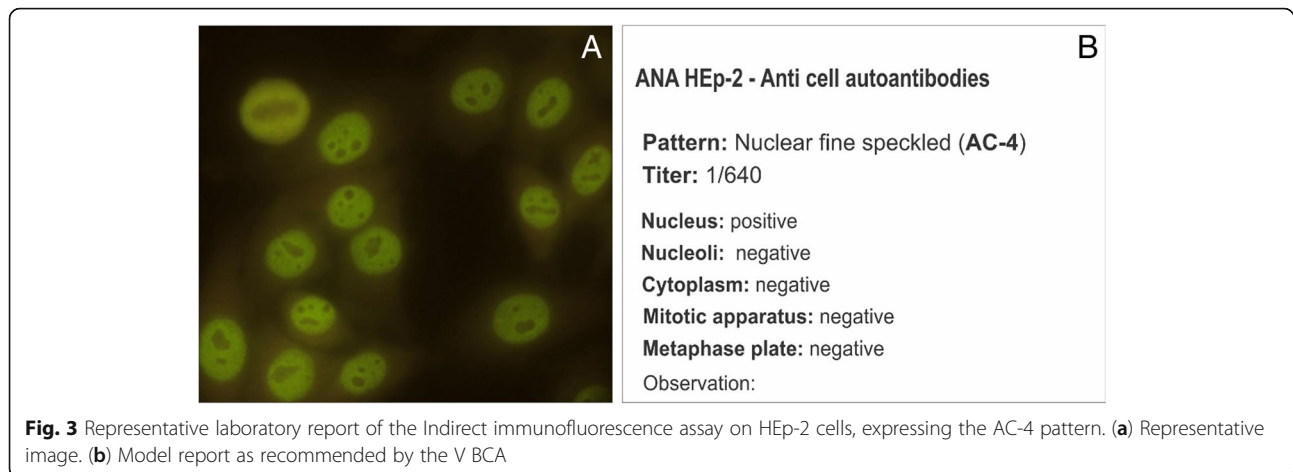


Fig. 3 Representative laboratory report of the Indirect immunofluorescence assay on HEp-2 cells, expressing the AC-4 pattern. (a) Representative image. (b) Model report as recommended by the V BCA

encompasses 20, 30, 50, 120 and 200-W lamps [25]. This may represent an important factor of pattern recognition and end-titer disparity among laboratories. Other important aspects contributing to the heterogeneity of results are the protein/fluorescein ratio in conjugates, use of different control sera for system calibration (or its absence), lack of training and subjectivity inherent to the method. The conjugate titration is a step of great importance, allowing the adjustment of the system, ensuring recognition of the nominal titre of the control sera and greatly increasing the objectivity and accuracy of the test [3–5]. It is recommended that this procedure be performed for each new brand and lot of slide kit, thus, assuring consistency of titer in kits of different lots and even if a new slide brand is used. Low titer control serum samples represent an invaluable tool in this process [4]. The V BCA HEp-2 reinforces the relevance of this measure to improve the quality of the test, especially regarding the semiquantitative dimension that the test offers and adds value in clinical practice.

Assay quality control

The V BCA HEp-2 maintains the recommendation of need for rigorous quality control, already disclosed at the III and IV BCA HEp-2 [3–5]. Such a measure is fundamental in order to restrict the occurrence of false-positive reactions in individuals without autoimmune disease and as a measure to standardize results among different laboratories [25]. It is recommended that laboratories seek to participate in institutional quality programs and educational programs. Another parameter that integrates the quality control efforts is the accomplishment of the titration of the conjugate, as previously detailed.

The test report

The V BCA HEp-2 maintains the structured report recommended by the IV BCA HEp-2. There is one additional recommendation, that the ICAP code, whenever available, be added to the name of the pattern. A

thoughtful description for each cellular compartment, which is a useful procedure for technical professionals performing and recording test results, favors appropriate pattern classification and clinical interpretation. Figure 3 shows an example of the HEp-2 IIFA report, classified by the V BCA and ICAP as AC-4 [10].

Conclusion

The recommendations of the V BCA HEp-2 are summarized in Table 3. The fifth edition represents another relevant initiative for the Brazilian process of standardization the HEp-2 IIFA test, initiated in the year 2000. Successful harmonization between recommendations by BCA HEp-2 and ICAP was achieved. The remaining divergent points between both initiatives were clearly pointed out and

Table 3 Fifth Brazilian Consensus for Autoantibodies on HEp-2 cells: summary of recommendations

- 1 - Harmonization to the international guidelines for the anti-cell autoantibodies on HEp-2 cells and adoption of the corresponding ICAP AC code.
- 2 - Creation of preliminary Brazilian AC codes (BAC) for the patterns recognized by the BCA and not addressed in the ICAP algorithm at this time.
- 3 - Maintenance of the original BCA conceptual arrangement of groups and subgroups of patterns in the classification tree.
- 4 - Adequacy of the test designation: anti-cell autoantibodies (ANA HEp-2)
- 5 - Attention to the reproducibility of different patterns, depending on the commercial HEp-2 cell substrate used.
- 6 - Alert that solid phase-based tests should not replace Indirect Immunofluorescence on HEp-2 cells as a screening test for anti-cell antibodies.
- 7 - Dilution of screening at 1/80.
- 8 - Titration of the conjugate for each new brand and lot of the same brand.
- 9 - Use of quality control strategies.
- 10 - Adequacy of the report to express the name of the pattern, ICAP code, titer, fluorescence pattern in each cellular compartment and classification of the chromosome metaphase plate

perspectives for future harmonization were pinpointed. The Brazilian Consensus on ANA remains a key element allowing immunology laboratories to correctly perform and interpret the HEp-2 cell test in Brazil. In addition to harmonizing and incorporating several improvements from ICAP, the V BCA HEp-2 emphasized essential actions discussed in previous editions regarding quality control, strongly recommending that quality assessment actions must be implemented in the laboratory practice so that the HEp-2 IIFA test reaches the aimed high standard of quality and efficiency.

Abbreviations

AC: Anti-cell; ANA: Antinuclear antibody; ASC: Autoantibody Standardization Committee; BCA: Brazilian Consensus on ANA; CENP-F: Centromere-associated protein F; DFS: Dense fine speckled; HEp-2: Human epithelial type 2 cells; ICAP: International Consensus on ANA Patterns; IIFA: Indirect Immunofluorescent Assay; IUIS: International Union of Immunology Societies; IWA: International Workshop on Autoantibodies and Autoimmunity; NOR: Nucleolar organizer regions; NuMA: Nuclear Mitotic Apparatus; PCNA: Proliferating cell nuclear antigen; RNP: Ribonucleoprotein; SMN: Survival of motor neuron

Acknowledgements

The authors would like to thank the Sociedade Brasileira de Reumatologia (SBR) and the Sociedade Brasileira de Patologia Clínica e Medicina Laboratorial (SBPC/ML). The SBR for support providing the infra-structure and a slot in the timeframe of the XXX Brazilian Congress of Rheumatology for the development of the activities of the V Brazilian Consensus on ANA. The two Societies for the aid in the dissemination of the initiative among Rheumatologists and Clinical Pathologists.

- Sociedade Brasileira de Reumatologia (SBR).
- Sociedade Brasileira de Patologia Clínica e Medicina Laboratorial (SBPC/ML).

Authors' contributions

WMC, LEA, PCF are design of the meeting. WMC, LEA, PCF, CAM, AD, ACX, CDB, CB, CLM, EB, FAB, FBF, GGS, JR, LMA, NS, SGP, SPN, VV, WSS. PCF are participation in overall discussions during the meeting. WMC Elaboration of the first Draft in Portuguese. WMC, LEA, GGS are translation into English. LEA, PCF, CAM, AD, ACX, CDB, CB, CLM, EB, FAB, FBF, GGS, JR, LMA, NS, SGP, SPN, VV, WSS, PLCF are review of the manuscript. WMC Elaboration of figures. All authors read and approved the final manuscript.

Funding

There is no funding to be declared, except for the support of the Brazilian Society of Rheumatology in providing the infra-structure and a slot in the timeframe of the XXX Brazilian Congress of Rheumatology for the development of the activities of the V Brazilian Consensus on ANA.

Availability of data and materials

This manuscript refers to the proceedings of a meeting with a panel of experts and, therefore, there is available research data or materials.

Ethics approval and consent to participate

This manuscript refers to the proceedings of a meeting with a panel of experts and, therefore, there is no pertinent research ethical involvement.

Consent for publication

All authors comply with the content of the manuscript.

Competing interests

The authors of the manuscript declare that they have no competing interests.

Author details

¹Pontifícia Universidade Católica de Goiás (PUC Goiás), Escola de Ciências Médicas, Farmacêuticas e Biomédicas, Avenida Universitária 1.440, Setor Universitário, Goiânia, GO, CEP 74605-010, Brazil. ²Disciplina de Reumatologia,

Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), Divisão de Imunologia, Fleury Medicina e Saúde, São Paulo, SP, Brazil. ³Sociedade Brasileira de Autoimunidade, Porto Alegre, RS, Brazil. ⁴Divisão de Pesquisa, Inovação e Desenvolvimento, Fleury Medicina e Saúde, São Paulo, SP, Brazil. ⁵Diretor Clínico do Hospital Geral de Goiânia Alberto Rassi, Goiânia, GO, Brazil. ⁶Faculdade de Medicina Famaz, Amaral Costa Medicina Diagnóstica, Belém, PA, Brazil. ⁷Laboratórios de Investigação Médica, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo (FM-USP), São Paulo, SP, Brazil. ⁸Departamento de Patologia Clínica e Anatomia Patológica, Hospital Israelita Albert Einstein, São Paulo, SP, Brazil. ⁹Hospital das Clínicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil. ¹⁰Instituto Hermes Pardini, Belo Horizonte, MG, Brazil. ¹¹Grupo DASA, São Paulo, SP, Brazil. ¹²Faculdade Carajás, Marabá, PA, Brazil. ¹³Faculdade de Medicina, Universidade Federal de Goiás (UFG), Goiânia, GO, Brazil. ¹⁴Universidade do Sul de Santa Catarina (UNISUL) e Universidade do Vale do Itajaí (UNIVALI), Florianópolis, SC, Brazil. ¹⁵Universidade Federal de Mato Grosso (UFMT) e Grupo DASA Cuiabá, Cuiabá, MT, Brazil. ¹⁶Serviço de Reumatologia e Laboratório de Autoimunidade da Divisão de Laboratório Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (USP), São Paulo, SP, Brazil. ¹⁷Departamento de Propedêutica Complementar da Faculdade de Medicina, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. ¹⁸Universidade Federal do Espírito Santo (UFES), Vitória, ES, Brazil. ¹⁹Escola Superior de Ciências da Saúde do Distrito Federal e Laboratório Sabin, Brasília, DF, Brazil.

Received: 26 February 2019 Accepted: 20 June 2019

Published online: 03 July 2019

References

- Dellavance A, Júnior AG, Cintra AFU, Ximenes AC, Nuccitelli B, Mühlen CA, et al. I Consenso Nacional para Padronização dos Laudos de FAN HEp-2. *J Bras Patol Med Lab.* 2002;38(3):201–16.
- Dellavance A, Júnior AG, Cintra AFU, Ximenes AC, Nuccitelli B, Taliberti BH, et al. II Consenso Brasileiro de Fator Antinuclear em Células HEp-2. *Rev Bras Reumatol.* 2003;43(3):129–40.
- Dellavance A, Júnior AG, Nuccitelli B, Taliberti BH, Mühler CA, Bichara CDA, et al. 3o Consenso Brasileiro para pesquisa de autoanticorpos em células HEp-2 (FAN). Recomendações para padronização do ensaio de pesquisa de autoanticorpos em células HEp-2, controle de qualidade e associações clínicas. *Rev Bras Reumatol.* 2009;49(2):89–109.
- Franciscantonio PLC, Andrade LEC, Cruvinel WM, Araújo FI, Dellavance A, Júnior AG, et al. III Consenso Brasileiro para Pesquisa de Autoanticorpos em Células HEp-2: perspectiva histórica, controle de qualidade e associações clínicas. *J Bras Patol Med Lab.* 2009;45(3):185–99.
- Franciscantonio PLC, Cruvinel WM, Dellavance A, Andrade LEC, Taliberti BH, Mühler CA, et al. IV Brazilian guidelines for autoantibodies on HEp-2 cells. *Rev Bras Reumatol.* 2014;54(1):44–50.
- Pham B-N, Albaredo S, Guyard A, Burg E, Maisonneuve P. Impact of external quality assessment on antinuclear antibody detection performance. *Lupus.* 2005;14:113–9.
- Sack U, Conrad K, Csernok E, Frank I, Hiepe F, Krieger T, et al. Autoantibody detection using indirect immunofluorescence on HEp-2 cells. *Ann N Y Acad Sci.* 2009;1173:166–73.
- Wiik AS, Hoier-Madsen M, Forslid J, Charles P, Meyrowitsch J. Antinuclear antibodies: a contemporary nomenclature using HEp-2 cells. *J Autoimmun.* 2010;35:276–90.
- Carballo OG, Ingenito FB, Ginaca AA, Carabajal P, Costa MA, Balbaryski J. First argentine consensus for standardization of antinuclear antibodies by indirect immunofluorescence-HEp-2. *Acta Bioquím Clín Latinoam.* 2012;46:3–13.
- Chan EKL, Damoiseaux J, Carballo OG, Conrad K, Cruvinel WM, Franciscantonio PLC, et al. Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014–2015. *Front Immunol.* 2015;6:412.
- Chan EKL, Damoiseaux J, Cruvinel WM, Carballo OG, Conrad K, Franciscantonio PLC, et al. Report on the second international consensus on ANA pattern (ICAP) workshop in Dresden 2015. *Lupus.* 2016;25(8):797–804.
- Damoiseaux J, Andrade LEC, Carballo OG, Karsten C, Franciscantonio PLC, Fritzer MJ et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective.

- Ann Rheum Dis 2019; 12 March 2019. doi: <https://doi.org/10.1136/annrheumdis-2018-214436>.
13. Herold M, Klotz W, Andrade LEC, Conrad K, Cruvinel WM, Damoiseaux J, et al. International consensus on antinuclear antibody patterns: defining negative results and reporting unidentified patterns. *Clin Chem Lab Med*. 2018;56(10):1799–802.
 14. Andrade LE, Chan EK, Raska I, Peebles CL, Roos G, Tan EM. Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. *J Exp Med*. 1991;173(6):1407–19.
 15. Satoh M, Chan JY, Ross SJ, Ceribelli A, Cavazzana I, Franceschini F, et al. Autoantibodies to survival of motor neuron complex in patients with polymyositis: immunoprecipitation of D, E, F, and G proteins without other components of small nuclear ribonucleoproteins. *Arthritis Rheum*. 2011;63(7):1972–8.
 16. Sternsdorf T, Guldner HH, Szostecki C, Grötzinger T, Will H. Two nuclear dot-associated proteins, PML and Sp100, are often co-autoimmunogenic in patients with primary biliary cirrhosis. *Scand J Immunol*. 1995;42(2):257–68.
 17. Stinton LM, Swain M, Myers RP, Shaheen AA, Fritzier MJ. Autoantibodies to GW bodies and other autoantigens in primary biliary cirrhosis. *Clin Exp Immunol*. 2011;163(2):147–56.
 18. Ceribelli A, Fredi M, Taraborelli M, Cavazzana I, Franceschini F, Quinzanini M, et al. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. *Arthritis Res Ther*. 2012;14(2):R97.
 19. Cozzani E, Drosera M, Riva S, Parodi A. Analysis of a multiple nuclear dots pattern in a large cohort of dermatological patients. *Clin Lab*. 2012;58(3–4):329–32.
 20. Mack GJ, Rees J, Sandblom O, Balczon R, Fritzier MJ, Rattner JB. Autoantibodies to a group of centrosomal proteins in human autoimmune sera reactive with the centrosome. *Arthritis Rheum*. 1998;41(3):551–8.
 21. Blaschek M, Muller S, Youinou P. Anti-“dividing cell antigen” autoantibody: a novel antinuclear antibody pattern related to histones in systemic lupus erythematosus. *J Clin Immunol*. 1993;13(5):329–38.
 22. Gitlits VM, Macaulay SL, Toh BH, SENTRY JW. Novel human autoantibodies to phosphoepitopes on mitotic chromosomal autoantigens (MCAs). *J Investig Med*. 2000;48(3):172–82.
 23. Andrade LEC, Klotz W, Herold M, Conrad K, Rönnelid J, Fritzier MJ, et al. Executive committee of ICAP. International consensus on antinuclear antibody patterns: definition of the AC-29 pattern associated with antibodies to DNA topoisomerase I. *Clin Chem Lab Med*. 2018;56(10):1783–8.
 24. Dellavance A, Gallindo C, Soares MG, da Silva NP, Mortara RA, Andrade LE. Redefining the Scl-70 indirect immunofluorescence pattern: autoantibodies to DNA topoisomerase I yield a specific compound immunofluorescence pattern. *Rheumatology (Oxford)*. 2009;48(6):632–7.
 25. Silva GG, Gomes CM, Dellavance A, Francescantonio PLC, Andrade LEC, Cruvinel WM. Implementation of the ANA HEp-2 consensus guidelines in Brazilian clinical laboratories. *J Bras Patol Med Lab*. 2017;53(6):368–76.
 26. Dellavance A, Cruvinel WM, Francescantonio PLC, Manguiera CLP, Drugowick IC, Rodrigues SH, et al. Variability in the recognition of distinctive immunofluorescence patterns in different brands of HEp-2 cell slides. *J Bras Patol Med Lab*. 2013;49(3):182–90.
 27. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE. Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum*. 2011;63(1):191–200.
 28. Tan EM, Feltkamp TE, Smolen JS, Butcher B, Dawkins R, Fritzier MJ, et al. Range of antinuclear antibodies in “healthy” individuals. *Arthritis Rheum*. 1997;40(9):1601–11.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

