Recommendations for Improved Standardization of Immunohistochemistry

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Abstract: Immunohistochemistry (IHC) continues to suffer from variable consistency, poor reproducibility, quality assurance disparities, and the lack of standardization resulting in poor concordance, validation, and verification. This document lists the recommendations made by the Ad-Hoc Committee on Immunohistochemistry Standardization to address these deficiencies. Contributing factors were established to be underfixation and irregular fixation, use of nonformalin fixatives and ancillary fixation procedures divested from a deep and full understanding of the IHC assay parameters, minimal or absent IHC assay optimization and validation procedures, and lack of a standard system of interpretation and reporting. Definitions and detailed guidelines pertaining to these areas are provided.

Key Words: immunohistochemistry, pathology, assay, oncology, standardization, procedure, tissue, fixation

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Immunohistochemistry (IHC) is widely used in surgical pathology and serves as a diagnostic, prognostic, and predictive tool. It was recognized over a decade ago that IHC assay standardization was vital for reproducible and reliable results. Agencies, including the Biologic Stain Commission, CLSI (previously NACCLS), FDA, and the manufacturing sector established guidelines, standards, and recommendations for reagents and package inserts. These efforts have resulted in consistent, high-quality assay components and instruments on which contemporary IHC is performed.[1–4] It has also allowed the development and use of so-called black box IHC stainers in which IHC assays have preset parameters set by the manufacturer.[5]

Despite the improvements of reagents and automation, authors over the years have consistently noted the inconsistent quality of IHC assays.[6–11] Unlike previous IHC-epochs, most of the causative responsibility rests with the individual laboratory performing the IHC and specifically, the lack of standardization and attention to quality assurance programs.[12,13] Prior consensus conferences identified the likely causative factors (Table 1).[14] Recent studies strongly suggest that these problems are widespread and are not insignificant.[15–17] A 2-day *ad-hoc* consensus conference was convened in August 2006, charged with the goal of making practical and feasible recommendations directed at standardizing these laboratory and pathologist factors. The recommendations listed below are the product of this meeting that included input from the directors of IHC laboratories from a broad range of clinical practices and representing the governmental, private, and academic sectors. These guidelines do not diverge from general practice recommendations and are supported by scientific data. Unfortunately, laboratories often do not appreciate the negative impact on their specimens and the validity of IHC performed on them created by diverging away from these recommendations.

PREANALYTIC FACTORS

Fixation

Recommendation 1

Tissue should be fixed in 10% neutral-pH, phosphate-buffered formalin for a minimum of 8 hours. If formalin or formalin-alcohol mixture is a component solution on the tissue processor instrument, tissue should be fixed in formalin for 6 to 12 hours before being loaded

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TABLE 1. IHC Assay Total Test Concept

Preanalytic
Test selection
Specimen type, acquisition, transport time
Fixation: type and time
Tissue processing, type, and temperature
Analytic
AR procedure
Protocol, control selection
Regent validation
Technician training/certification
Laboratory certification
Postanalytic
Control evaluation
Results interpretation
Results reporting
Pathologist, experience and CME

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onto the tissue processor. Non–formalin-based fixatives and or alternative fixation methodologies are strongly discouraged in regard to IHC, in large part because performance data are limited and extrapolation from formalin-fixed data is unreliable.

Comments

Formalin is aqueous, completely dissolved formaldehyde. The diffusion coefficient of formaldehyde is 0.79, meaning it permeates tissue at approximately 1 mm/h.[18,19] However, permeation is not fixation. Formaldehyde becomes available after disassociating from methylene glycol in solution at a rate that is measured in hours, referred to as a "clock" reaction.[19–21] Although complete tissue fixation requires 24 hours, a minimum of 6 preprocessor hours of formalin fixation is required for consistent IHC assay results.[19,22,23]

Nonformalin fixatives and or alternative fixation methodologies are strongly discouraged for several reasons. First, tissue fixed in formalin for less than 8 hours is countered by relatively greater alcohol dehydration fixation. Although tissues fixed in this manner can produce hematoxylin and eosin-stained slides that are similar in appearance to formalin-fixed tissues, the 2 specimens have discrete differences in both histomorphology and staining characteristics by IHC. Many alcohol-fixed tissue antigens do not require antigen retrieval (AR) to be sufficiently immunoreactive and in general, the less formalin fixation, the less AR required.[24] Tissues fixed in formalin for over 8 hours requires standard amounts of AR. Five to eight hours of formalin fixation is the transition-zone between no-AR and complete-AR, and varies depending on the antigen, antibody, and tissue. The majority of suboptimal and inconsistent IHC stains result from the mixing of specimens with different AR requirements. Some antigens, especially small secreted peptides are difficult to stain and localize in alcohol or alcohol-phase–fixed tissue, as a result of a lack of cross linking and diffusion throughout the tissue.

Second, a collective body of knowledge of expected or characteristic immunoreactivity of neoplasms and lesions has accrued over time consisting of the accumulated weight of numerous published studies and decades of pathologists' personal experience. It functions as the vital foundation on which pathologists make interpretative decisions and plays a significant role in the validation of an antibody. This knowledge base is almost entirely based on IHC assays performed on antigen-retrieved sections of formalin-fixed paraffin-embedded tissue. Tissues fixed in nonformalin fixatives and or alternative fixation methodologies can produce high-quality appearing IHC slides.[25,26] However, a similar appearance is not similar antigen expression. Neoplastic and lesional tissues fixed in an alternative nonformalin method may not produce the same patterns of antigen immunoreactivity patterns as formalin-fixed tissues.[27] Some microwave processors require using the manufacturer's proprietary (and undisclosed) reagents.[28] Although similar results have been obtained in tissue microarrays, there have been no direct comparisons of antibody panel results of formalin and non–formalin-fixed standard tissue blocks, needle core, or endoscopic biopsy tissue specimens.[29] Microwave fixation induces inhomogeneous tissue fixation reactions that varies with the size specimen and composition of tissue types.[30–33] It is a flawed assumption to think that the patterns and shades of intensities in neoplasms are the same in formalin and non–formalin-fixed tissues. Demonstration of differences in the clinical significance between alternative-fixed and formalin-fixed tissues may not be readily apparent, however side by side comparisons of statistically significant numbers of specimens, usually requiring at least 30 specimens per variable are required to demonstrate equivalency. Given the increasing role IHC assays play as predictive markers of oncologic chemotherapeutic agent response, faster turnaround times and laboratory cost-containment should not be prioritized as goals ahead of the laboratory procedures required for reliable, accurate, and reproducible IHC assays.[34–37]

Third, most antibodies used by laboratories in IHC assays are classified as analyte specific reagents (ASR) and regulated as a class 1 in vitro diagnostic (IVD) device by the FDA.[38–40] The Clinical Laboratory Improvement Amendments Act stipulates that the laboratory that uses the ASR in its in-house IHC assay takes responsibility to understand, *verify*, and *validate* its performance characteristics in lesions fixed and processed in their laboratory. It seems obvious to state that the Act means laboratories are required to validate and verify that lesions fixed in their laboratory express a characteristic immunophenotype using IHC assays developed in their laboratory. Validation means that the assay performs as designed to detect the specified antigen. Verification means the assay detects the antigen, as the assay was design-based on the specimen being analyzed. For many markers, verification requires a small number of samples processed in a consistent and identical manner to the test sample to demonstrate true positive and negative staining patterns.

However, as the complexity of the IHC assay increases, especially to prognostic and predictive markers, verification of the IHC assay requires a significantly larger number of samples.

Fourth, class 2 or 3 "kits or systems" for in IVD use requires the laboratory that uses the kit or system to perform the assay in the precise and exact manner designated by the manufacturer. Examples of such kits include the HER2 HercepTest (Dako Co, Carpenteria, CA,) and Pathway (Ventana Co, Tucson, AZ). Class 2 or 3 kits or systems IVDs stipulate the permitted fixatives on which the assay can be applied, which is almost always formalin. Results of these kits or system IVDs are invalid and deemed inaccurate and potentially unreliable when they are applied to tissues fixed in method or solution not stipulated as allowable by the manufacturer.

Recommendation 2

The time and type of preprocessor fixative and the time, type, and component solutions of the tissue processor should be recorded for every IHC tissue specimen. Delineation of neutral-buffered formalin alone is inadequate; rather, specification of the type of buffer and its molar concentration is required. The same is true for reagents on the tissue processing instrument, with special attention to xylene alternatives and type of embedding paraffin. This information should accompany all tissue that is sent to an outside laboratory where the IHC staining is performed. When IHC studies are performed in a reference laboratory, the originating laboratory is responsible for recording this information for each case. The reference laboratory that performs the IHC staining should record this information for each case. This information should be permanently maintained as part of the daily work run logs of the IHC laboratory. This information should be checked against the quality of the IHC stains and be incorporated into the IHC stain quality review log performed the laboratory director or their designate.

Comment

These recommendations are in step with the NCCLS (CLSI) guidelines for HER2 IHC assays that the type of fixative and length of fixation be included in the pathology report.[41,42] Insufficient, inconsistent, and different types of fixation and tissue processing are the key factors behind suboptimal and inconsistent IHC stains on automated IHC staining instruments. In general, underfixation is a substantially larger problem than overfixation.[43,44] This recommendation plays a key role in standardizing fixation parameters for IHC assays. Ideally, the time tissue is placed into fixative would be known, in reality, this information would be difficult to obtain for most laboratories. An acceptable surrogate of fixation-start time is the time that the specimen was accessioned into the laboratory computer system or when logged into the department, providing the specimens are immersed in formalin at or before this time point. The start time of the tissue processor instrument on which the tissue block is loaded is an acceptable end-fixation time point. If tissues are fixed in formalin or formalin-alcohol solutions for an additional time period on the tissue processor, this additional length of time should be added to the total fixation time of the specimen. It is widely assumed that tissue processing is a black box process and that differences or variations in this process have little impact on the specimen. This is not true. Current evidence suggests that *any* changes in the processing system have the capacity to alter the chemical makeup of the tissue specimen. Alternative processing systems, especially those that include alterative reagents and microwaves have not been adequately validated. IHC can be performed on tissue from these systems; however, there are no well-validated studies to demonstrate equivalency.

AR

Recommendation 3

AR is presumed to "restore" the antigenicity after the formalin fixation.[45–50] The parameters of an AR protocol must be balanced to match the unique length and type of tissue fixation of the individual laboratory and the characteristics of the individual antibody.[51–53] For tissues fixed in formalin for at least 6 hours before being loaded onto a tissue processor, one AR protocol is usually adequate. Different types of AR, such as low pH buffers, high pH buffers, various types of heating devices, enzyme digestion, etc, should be available for each AR protocol, depending on the optimization parameters of individual antibodies (see Optimization and Validation section below). Although enzyme digestion is not generally considered as a component of AR, it functions as an alternative method for practical purposes. It may be the preferred method of tissue pretreatment for some antibodies.

Comment

For most contemporary oncologic antigens, a minimum threshold level amount of energy must be applied via AR procedure to all types of tissue specimens, regardless of the length of time above 6 hours the tissue specimen has been fixed.[54,55] The method of applying the energy (pressure cooker, microwave, waterbath) is substantially less important than the amount of energy applied to the slides by the AR system.[56] AR energy above this threshold generally does not improve antigen detection.[57] Poor or inconsistently optimized AR relative to tissue fixation is responsible for most suboptimal contemporary automated IHC assay results. This creates a problem of inadequate (either over or under) AR. Over-AR relative to the amount of formalin fixation produces high-background staining, section fall-off, tissue section holes and rents, indistinct nuclear detail, muddy chromatin, pseudo-nucleolar staining, and overly strong edge staining of small biopsy specimens. This type of imbalance most commonly occurs when an AR protocol intended for tissues fixed in formalin for at least 8 hours is applied to tissues fixed in formalin less than 6 hours.

Under-AR relative to the amount of formalin fixation produces false negative immunoreactivity. This type of imbalance most commonly occurs when an AR protocol intended for tissues fixed in formalin for 4 to 8 hours is applied to tissues fixed for more than 16 hours (overnight fixation) (unpublished data).

It is very difficult to optimize AR on poorly fixed and processed tissues. Adequate fixation is key to achieving reproducible and optimal AR. It is not uncommon for laboratories to encounter a mixture of under and overfixed tissues, resulting in a large spectrum of problems, which combine together to produce result in inconsistent and unreliable IHC assays. AR is a part of clinical IHC, and the best solution is to standardize fixation in an effort to obtain consistent IHC.

Analytic Factors

Proper analysis of IHC assays is critical for correct tumor identification and prognostic/predictive assessment. Pathologists who have documented expertise in this field should interpret IHC assays. Expertise is attained by documenting experience with literature use and proficiency testing. IHC is an adjunct to pathologic interpretation, and all IHC assays should always be interpreted within the context of morphology.

DEVELOPING NEW ASR ANTIBODIES INTO IHC ASSAYS

Developing new ASR antibodies into clinical IHC assays is a 2-step process of optimization and validation. Optimization is the process in which the laboratory serially tests and modifies component procedures with the end point of producing a consistent high-quality assay. Antibody optimization must be completed before moving on to the validation step. Antibody validation is the process whereby the parameters of the IHC assay, including its accuracy, reliability, and reproducibility are established.

Antibody Optimization

Recommendation 4

Reagent package inserts should be read completely and in detail before beginning the optimization process. The laboratory director should sign the package insert and it should be maintained in an easily accessible laboratory manual as a reference source during the active life of the antibody or reagent. The manufacturer recommendations listed in the package insert should be followed (Table 2).

Comments

Work by the Biologic Stain Commission, FDA regulations, and AR pretreatment procedures have led to a generally high level of consistency in commercial antibodies and reagent products from manufacturers.[2–4,39] Satisfactory antibody staining is usually achieved by strictly following the manufacturer's package insert instructions.

TABLE 2. List of Factors That Could be Adjusted During the Antibody Optimization

Parameter	Description
No pretreatment	Some antibodies still perform best without any type of pretreatment
Enzyme digestion	Few antibodies perform best only when enzyme digestion was used without the need for heat induced epitope retrieval
Retrieval buffer	The combination of the type of buffer (ie, citration, ethylenediaminetetraacetic acid, trishydroxymethylaminomethane), and pH level can result in dramatically different signal intensity and signal-to-noise ratio
Heating device	That is, pressure cooker, electronic water bath, microwave, steamer, hot plate
Primary antibody incubation time	This varies depending on the affinity of the antibody to its antigen target, the primary antibody concentration, incubation temperature, and antigen levels in target tissue
Detection system	Polymer detection systems may allow to further dilute the antibody titer, given their generally higher sensitivity than avidin-biotin systems. Tyramine amplification systems are the most sensitive, but also most cumbersome
Chromogen	Prolonging the application of chromogen often lead to more intense signal, but could also compromise the signal-to-noise ratio

Recommendation 5

The test tissue used to optimize the IHC assay should contain the target antigen. Tissue specimens used for antibody optimization should be selected on the basis of the intended use of the IHC clinical assay and the level of target antigen expression in the respective tissue specimen.

Comments

The expression levels of some antigens can vary between neoplasms from different patients, between benign and malignant tissues, and between different types of neoplasms.[53]

Three examples of this issue are provided:

Example 1: the level of CD117 (C-Kit) expressed in gastrointestinal stromal tumors (GISTs) from different patients can range from none to high. Mast cells within GISTs and the adjacent bowel wall strongly express CD117, often at a substantially higher level than the adjacent neoplastic cells. The sensitivity of the CD117 IHC assay should be set such that weak-CD117 expressing GISTs are immunoreactive. If a strongly CD117 expressing GIST or mast cells are used as the test tissue against which the CD117 antibody is optimized, weakly CD117 expressing GISTs may fall below the threshold of immunoreactivity and be interpreted as CD117-negative.

Example 2: some mantle cell lymphomas have low levels of nuclear BCL1 (cyclin D1) antigen, whereas other mantle cell lymphomas and benign tissues such as breast acini have high levels of BCL1. If one of the latter tissues

is used to optimize the BCL1 antibody, mantle cell lymphomas with low-level BCL1 antigen expression can fall below this immunoreactivity cut-point and appear BCL1-negative.

Example 3: CD10 expression in follicular lymphomas and reactive germinal centers is usually substantially lower than in some carcinomas. If CD10 IHC assay is optimized against a follicular lymphoma or tissue abundant in reactive germinal centers, the same assay could be overly intense and possibly uninterpretable when being used as a marker of renal cell carcinoma or to identify bile canaliculi in hepatocellular adenocarcinoma.

The simplest method of establishing multiple IHC assays of a single antibody across a range of clinically different types of tissue after the IHC assay has been optimized in one type of tissue specimen (see Recommendation 7) is to vary the concentration of the antibody. When prediluted antibodies are used, the duration of antibody incubation or intensity of AR can be manipulated to optimize the IHC assay. Regardless of whether concentrated or prediluted antibody is used in the IHC assay, several different IHC assays should be established when one antibody is being used in multiple clinical settings, which cover a broad range of antigen expression levels.

Recommendation 6

The specific antibody clone selected for the IHC assay should be selected on the basis of intended clinical use of the IHC assay and the established record of immunoreactivity in published studies.

Comment

Antibodies bind to a small region of the target antigen. Different antibody clones bind to regions on a target antigen that may be distinct and separate areas, overlap to some extent, or be nearly identical. For many antigens, there are no detectable differences in immunoreactivity between antibody clones when validated against a limited number of specimens. However, for some antigens, the antigenic binding site of the specific antibody clone is of crucial importance. Examples of this issue include: WT1 in desmoplastic small round cell tumors versus ovarian serous carcinoma and mesothelioma,[58–60] the pattern and extent of membranous and cytoplasmic E-cadherin immunoreactivity in ductal versus lobular breast carcinomas,[61–66] and the marked difference in immunoreactivity between the M2-7C10 and Melan-A/A103 clones of MART1 in the PEComa neoplasm group (perivascular epithelioid cell tumors, angiomyolipoma, lymphangiomyomatosis, and clear cell lung neoplasms) and endocrine cell neoplasms.[67–70]

Recommendation 7

New antibody optimization should include serially testing and modifying if necessary, AR, antibody concentration, and chromogen detection system. Serial tissue sections from one tissue block should be used to facilitate the comparison of stain appearances. A minimum of 2 AR buffer solutions, 5 different antibody concentrations, including at least one above, at, and below the manufacturer's recommended concentration, and at least 2 types or incubation times of chromogen detection systems. Each component procedure should be modified accordingly. The combination of component procedures modifications that results in the most consistent and best immunoreactivity is the optimal IHC assay for an individual antibody.

Comments

Laboratories differ greatly in their fixation and tissue processing factors. There is also a broad spectrum of different types and forms of AR and IHC staining procedures. An antigen often has several antibody clones, produced by different manufacturers, and packaged in several different forms. The combination of component procedure parameters that results in the best IHC stain is unique to each individual laboratory. No single IHC assay produces, consistent, high-quality IHC assays across all antigens, every antibody, and in all types of tissues. Recognition of the inherent variability of IHC makes optimization of new antibodies mandatory, regardless of the extent, type, and complexity of the automated IHC platform used by a laboratory. The optimization process often requires several rounds of testing and modifying the component procedures. Occasionally, an antibody cannot be optimized, regardless of the modifications made to the component procedure parameters. Changing antibody clones will frequently remedy this problem. The avidity of most antibodies is influenced by the pH of the AR buffer solution and diluent and have optimal signal intensity within a relatively narrow pH range.[54,71–75]

AR

Testing of a low-pH (example: citrate at pH = 6) buffer solution and high-pH (example: ethylenediaminetetraacetic acid or ethylenediaminetetraacetic acid-trishydroxymethylaminomethane at pH = 8 or 9) buffer solution is recommended.

Antibody

Serial dilutions of the antibody including at least one antibody concentration above and below the manufacturer's recommended concentration. These antibody concentrations should be applied to at least 2 sets of tissue sections, each of which were antigen retrieved in a different buffer solution. The optimal combination of antibody concentration and AR buffer should be selected. If necessary, additional rounds of serial dilutions of more finely graded antibody concentrations should be performed.

Chromogen Detection System

All antibodies (prediluted ready-to-use or concentrated) should be evaluated in at least 2 types of chromogen detection systems. Some antibodies, including some hybridized rabbit monoclonal antibodies produce a

strong antigenic signal using a modified avidin-biotin or 2-step polymer detection system whereas 1-step polymer detection systems result in a thin weak signal (unpublished data, personal experience).

Recommendation 8

Detailed documentation of the antibody optimization parameters and factors, type of tissue used to optimize the antibody, and the tissue block case number should be maintained in the laboratory manual. Records of the set of conditions used, when and how an assay was modified should be maintained as a part of the laboratory manual pertaining to the individual antibody. It is not unusual, over a period of years for a new clone to be introduced, requiring a new set of assay conditions. For an individual case, laboratories should be able to document what antibody clone and assay conditions were in place when the IHC assay stain was performed.

Antibody Validation

Recommendation 9

For IHC assays that are interpreted as a categorical, positive/negative manner, a minimum of 25 separate tissue specimens (samples) tested by an alternative validated method in the same laboratory or by a validated method performed in another laboratory should be evaluated. At least 10 samples should have high levels of the target antigen, 10 samples should have intermediate to low levels of target antigen, and 5 samples should have no IHC evidence of the target antigen.

Additional validation and more restrictive standardized IHC assay parameters are required for therapeutic predictive IHC assays, such as HER2, estrogen receptor, and progesterone receptor.[76–80] Predictive IHC assays are strongly influenced by factors such as length of formalin fixation.[22,81] Genetically engineered, rabbit monoclonal antibodies, such as clone SP1 ER antibody (Lab Vision Co, Fremont, CA) have greater avidity compared with mouse monoclonal antibodies.[82] Validation must be to tissues with known levels of estrogen receptor protein expression or valid clinical/ therapeutic end point to avoid miscalibration.[83–88] Parallel staining comparison with immunoreactivity of a different but similarly, uncalibrated IHC assay is insufficient in this regard. As complexity of interpretation increases, the number and spectrum of specimens required for validation must increase accordingly.

Comments

Each tissue specimen is considered to be a single tissue sample for validation purposes, regardless of the number of tissue blocks from the case. In this context, multiple tissue blocks from a large neoplasm are considered as a single tissue specimen. Three levels of target antigen are selected to confirm that the signal threshold was set appropriately and define the IHC assay parameters. The purpose for the validation procedure is to evaluate the IHC assays performance across a range of clinical samples in terms of relative specificity and sensitivity of the IHC reaction. For this reason, it is important that the samples used for the validation procedure be handled in terms of the preanalytical variables described above, as close as possible to the clinical samples from the laboratory on which the new test might potentially be offered. Tissue microarrays if available are acceptable for the purpose of validating a new IHC assays, and depending on how they have been constructed, have the potential for the survey of a number of different normal tissues and multiple tumor types with the new IHC procedure.

Recommendation 10

A new IHC assay should show a high level of concordance with the validated assays to which it is being compared. Data should be evaluated and discrepancies investigated to determine their origin. A systematic approach of corrective actions should be taken.

Recommendation 11

A log of the validation specimens' samples and the IHC assay results should be kept with the antibody package insert material in the antibody section of the laboratory manual.

NEW LOTS OF ACTIVE IHC ASSAYS

Recommendation 12

All validated IHC assays need to be completely revalidated if significant changes are made to the assay procedure. When new antibody lots of validated active IHC assays are brought into the laboratory, 3 validation test samples should be tested. At least 1 sample should have a high level of the target antigen, 1 sample should have intermediate to low level of target antigen, and 1 sample should have no IHC evidence of the target antigen. Validation of new antibody lots should be sufficient to determine the performance parameters of the IHC assay.

POSTANALYTIC FACTORS

Interpreting IHC Assays

Recommendation 13

IHC assay immunoreactivity should be assessed with a quantitative scoring system.[5,84] All scoring systems should have 2 separate components: the extent or proportion of the target antigen that was immunoreactive and the intensity of the immunoreactivity. Immunoreactivity of an IHC assay should be assessed first. After this assessment, a positive result cut-point threshold should be applied to the result that is clinically appropriate (ie, contextual) and takes into account the expected level of immunoreactivity, specimen size, and amount of target antigen in the specimen.

Comment

There is no universal IHC scoring system. An example of a results classification system with broad clinical utility and used by several members of the committee is to classify 0% to 20% immunoreactivity as "focally reactive," 21% to 80% as "variably reactive," and >80% immunoreactivity as "uniformly reactive." Another system being used in clinical predictive IHC assays is the IHC score (range, 0 to 400) which is the product of staining intensity (range, 1 to 4) and percentage of immunoreactive target antigens.[89–92] There is also no universal cut-point threshold for a positive result that can be applied across all clinical situations and types of specimens. Substantial variability in interpretation is introduced by the clinical situation, type of specimen, amount of available target tissue in the specimen, and adequacy of specimen fixation and processing.[93]

Interpreting IHC assays in small specimens differs from their interpretation in large specimens.[94] In small specimens, the extent and intensity of immunoreactivity should be assessed in the regional area with the highest proportion of target immunoreactivity. The positive result cut-point should increase (right shift) as the amount of the target antigen available for interpretation decreases. For low-sensitivity antibodies or focally expressed antigens, any level of immunoreactivity can be significant. Numerous factors can complicate the determination of whether an IHC assay result is classified as positive or negative. Separating the assessment and scoring of immunoreactivity assessment from the determination of whether an IHC assay result is positive or negative is recommended to improve reproducibility and accuracy of IHC assay interpretation.

Reporting IHC Assays

Recommendation 14

The following information should be included in the IHC assay report. Each antibody should be reported separately.

1. Antibody clone. Given that the performance of different antibody clones against the same antigen can vary significantly affecting the end result of immunoreactivity, it is critical to report the clone name and type of antibody (polyclonal or monoclonal). For polyclonal antibodies, reference to the vendor's catalog number should be provided.
2. The subcellular localization (nucleus, cytoplasm), which is immunoreactive, pattern of antigen expression (granular, dot, linear, homogeneous), intensity of immunoreactivity within the cellular compartment, and the proportion of cells demonstrating this pattern of immunoreactivity.
3. The scoring system that was applied and the immunoreactivity cut-point threshold of a positive result.
4. Whether there is an internal positive control cell or structure present on the test (patient) slide and descriptive documentation regarding the intensity and proportion of internal control cells or structures that were immunoreactive.
5. Specimen number and block used for IHC slides.
6. Type of fixative and length of fixation.
7. Tissue specimen anatomic location and type of specimen.

Comments

Ideally, all IHC assays would be interpreted using a single, universal standardized scoring system.[75] The different cellular and structural antigenic targets, broad spectrum of clinical situations in which IHC is used, and the lack of consistent, reliable, external and internal control standards, makes this an unrealistic goal.[95,96] Many oncologic-related nuclear regulator antigens are normally present in the nucleus and cytoplasm. It is important that pathologists understand the transport pathways of these proteins and restrict interpretation to the relevant subcellular structures as reported in the literature by that specific antibody clone.[97–99] In the absence of a universal IHC assay scoring system, responsibility rests with the pathologist to provide this information in the report. Documentation also provides a method of verifying the interpreting pathologist evaluated immunoreactivity in the appropriate antigenic target and applied appropriate cut-points thresholds.

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