Canadian Association of Pathologists–Association canadienne des pathologistes National Standards Committee for High Complexity Testing/Immunohistochemistry

Guidelines for the Preparation, Release, and Storage of Unstained Archived Diagnostic Tissue Sections for Immunohistochemistry

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ABSTRACT

Objectives: Formalin-fixed, paraffin-embedded unstained archived diagnostic tissue sections are frequently exchanged between clinical laboratories for immunohistochemical staining. The manner in which such sections are prepared represents a type of preanalytical variable that must be taken into account given the growing importance of immunohistochemical assays, especially predictive and prognostic tests, in personalized medicine.

Methods: Recommendations were derived from review of the literature and expert consensus of the Canadian Association of Pathologists–Association canadienne des pathologistes National Standards Committee for High Complexity Testing/Immunohistochemistry.

Results: Relevant considerations include the type of glass slide on which to mount the unstained sections; the thickness of the tissue sections; the time from slide preparation to testing; the environment, particularly the temperature at which the unstained sections will be maintained prior to testing; the inclusion of on-slide positive control tissue where possible; and whether patient identifier(s) should be included on slide labels.

Conclusions: Clear communication between requesting and releasing laboratories will facilitate the proper preparation of unstained sections and also ensure that applicable privacy considerations are addressed.
Rationale/Background

Archived diagnostic tissue is frequently used as a substrate for immunohistochemistry (IHC) testing. Archived diagnostic tissue that is released to external laboratories for IHC testing may be in the form of paraffin blocks or unstained slides; this reflects the current variation in institutional policies and practices with respect to the retention of such records.

IHC is a broadly available, powerful technique that, when properly applied, allows for the in situ analysis of protein expression in human tissues. IHC, much like other laboratory assays, has preanalytical, analytical, and postanalytical elements. Even though the preanalytical component is essential for a successful result in IHC, it has been addressed only recently by a national body in the form of a guideline for human epidermal growth factor receptor 2 IHC testing.1 The Canadian Association of Pathologists–Association canadienne des pathologistes (CAP-ACP) followed by issuing additional resources to further highlight the importance of standardizing the preanalytical phase.2,3

Previously published literature relevant to the preanalytical phase of IHC has focused on aspects of tissue processing up to the point at which the tissue has been embedded in paraffin. Once formalin-fixed tissue has been embedded in paraffin, epitopes of interest may be preserved for many decades. For example, breast cancer biomarker IHC targets may be reliably detected up to 7 decades after embedding in paraffin blocks.4

With personalized medicine driving the demand for new clinical IHC tests, especially class II tests, the current clinical laboratory practice of exchanging unstained sections cut from archived diagnostic tissue needs to be addressed. In particular, the handling of unstained slides from the time of their preparation to the time that an IHC test is performed will be of increased importance to the success of IHC testing. Publications that have examined specific factors and specific markers provide some guidance regarding the potential for loss of immunoreactivity once the paraffin blocks are cut and unstained slides are prepared; they also address measures that may help prevent potential adverse events related to the loss of reactivity in unstained tissue sections over time.5–11 The CAP-ACP National Standards Committee for High Complexity Testing/Immunohistochemistry has prepared these guidelines to increase awareness of these often underrecognized components of the preanalytical phase, some of which have direct relevance to technical issues as well as interpretive (ie, postanalytic) and regulatory issues. These guidelines are based on the currently available evidence regarding issues relevant to unstained sections as well as on expert opinion; they are not enforced in Canada but are prepared as a resource for best laboratory practices. The guidelines were approved by the CAP-ACP Executive Committee on January 11, 2014.

Reasons for Requests

There are many reasons why archived diagnostic materials may be requested for IHC, including (1) clinical care requests (pathologist/patient/treating physician–initiated second opinions for diagnostic assistance/confirmation and availability of new targeted therapies), (2) medicolegal requests (lawsuits and investigations by regulatory bodies), (3) quality assurance (QA) requests (mandatory external quality control programs, substrates for test validation, and other QA-IHC activities), (4) research requests (to determine eligibility for clinical trials, translational research projects, and other novel uses of IHC), and (5) education requests. The type of request will determine how the slides will be handled and released (see guidelines below).

Definition of Archived Diagnostic Tissue

Excised human tissue can be broadly classified into diagnostic tissue and research tissue.12 Diagnostic tissues comprise all biomaterials removed for medically indicated procedures that must be submitted to a clinical laboratory for analysis. Research tissues are principally those biomaterials intended for nonclinical investigation that are either a portion of tissue procured prospectively from a larger specimen or a specimen removed solely for research-only procedures. Under appropriate circumstances, the former of these may include postretention diagnostic tissue.

Archived diagnostic tissues are portions of the originally excised tissue that were selected to undergo additional analyses after the initial gross morphologic examination. They include frozen tissue and formalin-fixed (or any other fixative in use for diagnostic purposes), paraffin-embedded (PE) tissue, along with all structural derivatives (eg, sections mounted on glass slides and unmounted sections placed into tubes) and biological derivatives prepared for clinical analyses (eg, DNA, RNA, and protein). Although practices may vary between institutions, unstained sections mounted on glass slides cut from archived PE diagnostic tissue are often shared between clinical IHC laboratories.

Excess diagnostic tissues are portions of the originally excised tissue that did not undergo additional analyses after the initial gross examination. They typically consist of the wet (fixed) specimen that remains after a case has been grossed and blocked. For many antigen targets, this type of tissue may be an appropriate sample for IHC testing once samples are harvested and processed to paraffin blocks.

Because diagnostic tissue (both excess and archived diagnostic tissue) must, by definition, be clearly identifiable to a specific individual, knowledge of the class of tissue being requested and the reason for a request will guide clinical laboratories preparing unstained slides for release in meeting their obligation to patient privacy with respect to the release of personal health information.
Scope

In terms of tissue preparation, IHC may be performed on tissue that has been fresh frozen and tissue that has been fixed in formalin (or other fixatives) and embedded in paraffin (FFPE); for routine clinical practice, the latter is more common than the former. IHC may also be performed on tissue that has been demineralized (bone, bone marrow tissue, and calcified soft tissues). In terms of form, IHC requires that the tissue to be tested be mounted as thin sections on a suitable medium (typically properly prepared glass slides).

The following recommendations are intended primarily for clinical laboratories that prepare unstained sections mounted on glass slides from archived diagnostic FFPE tissue (with or without demineralization) for IHC testing at an external institution. However, they are also readily applicable to FFPE research tissue that will undergo external IHC staining.

Preanalytical Components Relevant to Unstained Slides

Components of the preanalytical phase relevant to the release and the shipment of unstained slides for IHC include (1) the type of glass slide; (2) the nature of the FFPE tissue section placed on the glass slide; (3) time to testing; (4) environment factors, particularly the temperature; (5) positive controls; and (6) privacy issues.

Guidelines

Type of Glass Slide

It is imperative that unstained sections for IHC be mounted on slides suitable for IHC (slides coated with an adhesive medium or slides that are positively charged). Importantly, because most IHC staining in clinical laboratories is performed on automated platforms, the success of any particular IHC protocol may be dependent on the specific type of glass slide on which the tissue is placed; this may be limited to a single manufacturer of the glass slides irrespective of their general category (coated vs positively charged). Slide fluidics may be unfavorable after environmental stress with some slide types. Therefore, clear communication between the releasing institution and receiving institution is necessary in situations where a special type of glass slide may be required for optimal performance of the IHC testing.

Tissue Sections

Although 3- to 4-µm-thick sections are generally recommended for IHC staining, the requirements are different for sections being prepared for shipping. Tissue sections for IHC staining that are prepared for shipping should be of reproducible thickness, with 2-µm-thick sections recommended for the following reasons: (1) thinner sections adhere better to glass slides than thicker sections, (2) the final use of the unstained slides is not always sections, and (3) it is often difficult if not impossible to interpret the results of IHC staining due to overlapping cellular compartments. The latter is particularly important if double or triple IHC staining is performed. However, if thicker sections are specifically required for research purposes, the desired thickness should be clearly communicated to the laboratory providing cutting services.

When 2-µm-thick sections are not possible, laboratories should strive to ensure that sections are no more than 4 µm in thickness. Most important, laboratories preparing unstained slides for an external facility should ensure that section thickness meets the receiving laboratory’s specifications.

Time

The date of tissue sectioning must be recorded on the slides. FFPE tissue sections, once cut, have an expiration date for IHC testing that may vary greatly depending on the epitope in question. This tenet of laboratory practice was emphasized by Jacobs et al., who studied the effect of time on staining intensity in several markers, including p53, ER, Bcl-2, and factor VIII–related antigen. In addition to demonstrating loss of staining intensity in all markers at 12 weeks, they specifically showed data indicating that the greatest loss of immunoreactivity for p53 occurs during the first 2 weeks of slide storage irrespective of temperature or whether the slides were paraffin coated. However, it is important to recognize that neither time-dependent nor temperature-dependent loss of immunoreactivity has been studied for most biomarkers used in routine clinical IHC testing. These attributes have not been addressed for newly discovered biomarkers, many of which are key markers in clinical research trials. Nonetheless, there is evidence that for more than 40% of all markers, the time elapsed and the storage temperature for the period from sectioning to IHC staining will affect the immunoreactivity of the samples. It is reasonable to assume that the time elapsed from section preparation to IHC testing will vary from days to weeks for clinical care and up to months or years for research studies. If the date of sectioning is not recorded on the slides and these slides are archived, then it may be impossible to determine retrospectively whether the results of the future IHC staining will be reliable. This is especially true for IHC tests that require any degree of quantitation.

Temperature

Once tissue is paraffin embedded, even when sectioned and mounted on glass slides, most short-lived temperature variations that occur below 60°C are likely not detrimental. Previous studies show that loss of antigenicity is a function of time and temperature and that both longer time and...
higher temperatures lead to loss of staining in IHC assays. When slides are shipped over long distances by various modes of transportation, the extent of temperature variation and the time of exposure to such temperature shifts are unknown but might reasonably be expected to be significant. It is recommended that unstained slides be kept at –80°C and individually wrapped in aluminum foil, irrespective of whether the slides are dipped in paraffin, if the IHC staining will not be performed in the same week that the slides are cut. Although –20°C was also shown to be better than room temperature, no adverse effects were recorded with –80°C, and therefore, the general approach that slides should be stored at as low a temperature as possible is recommended.6

Exposure to Light and Moisture

Published evidence suggests that exposure to light, specifically UVA rays (both sunlight and artificial light), may lead to loss of immunoreactivity.6 Exposure to dry heat is also detrimental.6 However, increased moisture during storage may also be problematic because it may cause tissue detachment.11 Indeed, the combination of temperatures higher than 25°C and increased humidity is most detrimental to protein integrity.17 Vacuum packing with desiccant protects against protein degradation and thus may be an appropriate intervention when transport or handling outside of appropriate storage conditions may be prolonged.

Positive Controls

It is recommended that positive controls be mounted directly on unstained slides if the IHC test panel that will be performed in the external laboratory is known. It is suggested that the requestor of the slides provide this information to the sender whenever possible. Use of external positive controls that originate in the same institution as the tissue to be tested helps ensure that the preanalytical conditions of the external controls are similar to those of the samples submitted for testing. Positive controls thus prepared will be more informative for the receiving laboratory as a test of IHC protocol calibration in the tissue samples submitted for testing.3

Privacy Issues

Whether personal health information may appear on unstained slides destined for release must be in compliance with local institutional policies and/or applicable privacy legislation. If the underlying reason for a request is that of clinical care (eg, second opinion) or medicolegal investigation, then the slides on which the unstained archived diagnostic tissue sections are mounted should be clearly identifiable to the specific individual/patient. If the underlying reason for a request is not that of clinical care and/or medicolegal investigation but is instead for quality assurance, research, or education, then the clinical laboratories preparing unstained sections for release must ensure that personal identifiers are not included. However, specific relevant information may be included (research study number, educational collection name, or other as appropriate).

Checklists

Checklist for Requestor

1. Do not request unstained slides for IHC testing far in advance of the actual testing date; optimally, unstained slides should be cut no longer than 1 week before the IHC testing will be performed. Once unstained slides are received, IHC staining should be performed as soon as possible; if storage of the slides is required, the unstained slides should be stored in a dry environment, protected from light, and at as low a temperature as possible. Storage at –80°C may be protective for some epitopes for several months.6,7 Although paraffin coating is sometimes requested, due to more recent conflicting reports, the beneficial effects of paraffin coating are less certain, and thus, paraffin coating cannot be universally recommended.9

2. Provide information about the optimal glass slides to be used for cutting; send the optimal glass slides to the releaser if there is a unique requirement and chances are high that the sender will not have such glass slides.

3. Provide information regarding the IHC tests to be performed to enable the sender to cut appropriate positive control sections onto the slides with tissue to be tested.

4. If the request is for research, provide a study/trial-specific identifier to be placed on the slides in place of the clinical identifier.

5. If the request is for quality assurance, slides should be labeled with the information that is appropriate for its intended use (eg, a request for a low progesterone receptor [PR]–expressing tumor section could be labeled as follows: “Institution Name, IHC QA, LowPR”).

Checklist for Releaser

1. Determine when the testing will be performed; the tissue block should be cut in close temporal proximity to the actual release.

2. Determine what tissue thickness is required by the receiving laboratory (if not specified, see item 10).

3. Determine if there are specific glass slide requirements.

4. Ask what tests will be performed, label the slides with the test name, and release with on-slide controls whenever possible.

5. Do not include patient identifiers unless necessary or appropriate.
6. Record on the slides the date when sections were cut.
7. Unstained slides should be released within 1 week of being cut.
8. Instructions for tissue storage should accompany the slides.
9. Sections must be placed onto coated or charged slides.
10. Sections should be cut 2 μm thick and should not exceed 4 μm.

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References