Guidance for Industry

Guidance for Submission of Immunohistochemistry Applications to the FDA

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U.S. Department Of Health And Human Services
Food and Drug Administration
Center for Devices and Radiological Health

Immunology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

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Preface

Public Comment

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GRZAN

SECTION 1. Introduction

1.1 Purpose 1

1.2 Background 1

1.3 Scope 2

1.4 Intended Audience 2

1.5 Document Organization 2

1.6 Relationship to Other Documents 3

1.7 Terminology 3

1.8 Classification: 21 CFR 864.1860 Immunohistochemistry reagents and kits 4

SECTION 2. Level of Concern 4

2.1 Safety and Effectiveness Issues Involved with IHC's 5

2.2 The FDA Pre-Marketing Review of IHC's 5

SECTION 3. Documentation in a Premarket Submission 6

3.1 Valid Scientific Data/Information to be Submitted for Class II IHC's: Types and Adequacy of Data 6

3.2 Analytic Performance Characteristics: Antigen/Antibody Characterization 6

3.3 Validation and Verification of Immune Reactivity of All Classes of IHC's with a Panel of Normal Tissues or Cells 7

3.4 Characterization of Specimens Used in the Panel of Normal Tissues 7

3.4.1 Purpose of the Studies with Panels of Normal Tissues 8

3.5 Validation and Verification of Immune Reactivity of Class II IHC's by Testing Panels of Tissues That Have Been Well-Characterized Clinically or That Have Been Established by Comparison to a Widely-Accepted Reference Method 8

3.6 Class II IHC's with Claims for Quantitative Results for Prediction or Prognosis of a Clinically Significant Outcome 9

3.7 Discrepancies in Comparison Studies 10

3.8 Sensitivity and Specificity Determinations for Class II IHC's 10

3.9 Manufacturers' Recommendations for Verification of IHC Performance by the User 10
3.10 Validation of Class III IHC's 10
3.11 Summary of Reactivity for Package Insert 11
3.11.1 Photomicrographs of Expected Staining Reactivity for Package Insert (optional) 11
3.12 Documentation of Valid Scientific Evidence of Safety and Effectiveness to Support the Intended Use of Class II IHC's 11
3.13 Current Good Manufacturing Practices (cGMP) Are Now Part of FDA Quality Systems Regulations (June 1997) 11
3.13.1 Stability Studies 11
3.13.2 Handling, Storage, and Dilution of IHC reagents 12
3.13.3 Protocol for Quality Control Release Criteria for Manufacturer 12
SECTION 4. Package Insert Instruction for the Manufacturer 12
4.1 Requirements to consider in addition to meeting 21 CFR 809.10 12
4.1.1 Product nomenclature 12
4.1.2 Intended Use 13
4.1.3 Summary and Explanation of the Device 13
4.1.4 Reagents Provided 13
4.1.5 Procedure 13
4.1.6 Limitations 14
4.1.7 Expected Results 14
4.1.8 References Used for Drafting the Suggested Package Insert 14
Appendix A: FDA's Risk-Based Evaluation of IHC Applications 16
Appendix B: Checklist for Completeness of the Submission for Class II IHC's 17
Appendix C: Normal Tissue Required for Establishing the Specificity of IHC's 18
Appendix D: Worksheet for Recording IHC Staining Results 20
Appendix E: Where to Summarize in the Package Insert the Results 21
Appendix F: Template for IHC Antibody Package Insert 22

Appendix G: Checklist for Completeness of Scientific Documentation of IHC Antibody in the Package Insert 35

Table

Table 1: The Purpose of Daily Quality Control 30

GRZAN

SECTION 1. Introduction

1.1 Purpose

This guidance document supersedes and revises the "Draft Guidance For Submission of Immunohistochemistry Applications to the FDA" dated March 28, 1995 according to FDA's "Good Guidance Practices" (GGP's), FR Docket Number 95P-0110, and FDA's Medical Devices; Classification/ Reclassification of Immunohistochemistry Reagents, FR Docket Number 94P-0341.

The use of this guidance is not a mandatory requirement. Alternative methods that comply with the relevant statute or regulations are acceptable. However, the guidance is intended as a guide and explanation for following FDA regulations, in particular, 21 CFR § 809.10(b) and 21 CFR § 864.1860. It does not supersede these regulations. This guidance (1) updates the template for preparation of manufacturers' IHC package inserts previously published by the Biologic Stain Commission and FDA, "Proposed Format: Package Insert for Immunohistochemistry Products"[1]; (2) incorporates the proposed Package Insert template developed by Joint Council of Immunohistochemistry Manufacturers (JCIM)[2] ; and (3) revises the previous version of this guidance, "Draft Guidance For Submission of Immunohistochemistry Applications to the FDA, March 28, 1995"[3].

This guidance document is intended to guide in (1) the processing, content, and evaluation/approval of submissions for immunohistochemistry devices (IHC's); (2) the design, production, manufacturing, and testing of IHC devices; (3) it establishes for FDA personnel and/or the public policies intended to achieve consistency in the agency's regulatory approach; and (4) to serve as the special control for class II immunohistochemistry (IHC) in vitro diagnostic devices (IVDs) under 21 CFR § 864.1860.

As the special control for class II immunohistochemistry devices (IHC's), this guidance document provides (1) the types and amounts of valid scientific evidence a sponsor should provide to support the reasonable assurance of the safety and effectiveness of the sponsor's performance claims for the IHC device, and (2) a template for acceptable language to use in a package insert for IHC devices following 21 CFR § 809.10.

This guidance provides checklists in the appendices to aid IHC manufacturers in preparation of their premarket submissions and for the FDA reviewers who review IHC submissions.

1.2 Background

This is the December 8, 1997 Version of FDA's Immunohistochemistry Guidance. It is being published following review by CDRH management and OGC staff. This version includes FDA's revisions of the March 1995 Immunohistochemistry Guidance in response to comments by the

Joint Council of Immunohistochemical Manufacturers (JCIM), professional organizations, individual pathologists and laboratory scientists, and ODE/DCLD comments. The guidance was

edited to serve as the special control for class II IHC IVDs, and it retains from previous versions suggestions for meeting general control issues for any class of IHC. The document follows FDA's Good Guidance Practices recommendations.

1.3 Scope

This guidance document is based on FDA's experience with IHC device submissions and incorporates scientific and regulatory information presented at the FDA Workshop on Immunohistochemistry: Scientific and Regulatory Issues held on June 28-29, 1994, and at the Hematology and Pathology Devices panel held on October 21, 1994 by industry representatives, scientific organizations, pathologists and other laboratory scientists (laboratorians); and comments to the FR Notice Docket No. 94P-0342 from industry representatives, scientific organizations, pathologists and other laboratorians, and the Small Business Administration.

1.4 Intended Audience

This guidance document is intended for use by scientific reviewers within the FDA CDRH Office of Device Evaluation (ODE), the medical device industry, and other interested parties.

1.5 Document Organization

This document contains 4 sections and 8 appendices.

- Section 1 (Introduction) describes the purpose, background, scope, intended audience, document organization, relationship to other documents, and terminology.
- Section 2 (Level of Concern) explains how the level of concern relates to the review of a premarket submission.
- Section 3 (Documentation in a Premarket Submission) identifies what informational elements should be included in a premarket submission.
- Section 4 (Package Insert Instructions for the Manufacturer) identifies and illustrates the labeling requirements of 21 CFR 809.10.
- Appendix A (FDA's Risk-Based Evaluation of IHC Applications) outlines questions posed in the pre-market risk assessment for IHC's.
- Appendix B (Checklist for Completeness of the Manufacturer's Submission for Class II IHC's) lists the components of pre-market submissions for class II IHC's.
- Appendix C (Types of Normal Tissue Required for Establishing the Specificity of IHC's) lists the types of normal tissue that may be used to establish the specificity of class I, II, or II IHC's.

- Appendix D (Worksheet for Recording IHC Staining Results for Each Tissue Tested to Assist in Preparation of Summary of Testing Results in the Package Insert) is the template for a worksheet to record the staining results from studies sponsored by the manufacturer.

- Appendix E (Where to Summarize in the Package Insert the Results of Class II IHC Staining Reactivity in Tissues) identifies where to place the staining results and performance characteristics of IHC's in the package insert.

- Appendix F (Template for IHC Package Inserts) is a narrative description of 21 CFR 809.10 labeling requirements for package inserts of class II IHC's.

- Appendix G (Checklist for Completeness of Scientific Documentation of IHC in Package Insert) is a list of the items described in Appendix F.

1.6 Relationship to Other Documents

This publication is a guidance document. FDA and device manufacturers use guidance documents to provide a means, among possible others, to meet regulations and policy. Appropriate national and international consensus standards may also be viewed as tools for achieving and demonstrating compliance with regulations.

1.7 Terminology

Identification of Immunohistochemistry Devices (IHC's) and Their Intended Use and Indications for Use

Panel: Hematology and Pathology

Regulation Number: 21 CFR § 864.1860

Identification: Immunohistochemistry reagents and kits

Immunohistochemistry tests systems (IHC's) are in-vitro diagnostic devices consisting of polyclonal or monoclonal antibodies labeled with directions for use and performance claims, which may be packaged with ancillary reagents in kits. Their intended use is to identify, by immunological techniques, antigens in tissues or cytologic specimens. Similar devices that are intended for use with flow cytometry devices are not considered IHC's under this rule.

This guidance document does not apply to (1) analyte specific reagents (ASR's) that are monoclonal or polyclonal antibodies for which the manufacturer makes no analytic or clinical performance claims and provides no instructions for use; (2) nucleotide-based devices for detection of DNA or RNA sequences, e.g., in situ hybridization; (3) flow cytometry products; or (4) IHC's for detection or measuring of etiologic agents of infectious disease in tissue or cytologic specimens. Other classifications and guidance documents cover these product areas.

1.8 Classification: 21 CFR § 864.1860 Immunohistochemistry reagents and kits

Class I IHC's (General Controls). Class I IHC's provide the pathologist with adjunctive diagnostic information that may be incorporated into the pathologist's report, but that is not ordinarily reported to the clinician as an independent finding. These IHC's are used after the primary diagnosis of tumor (neoplasm) is made by conventional histopathology using nonimmunologic histochemical stains such as hematoxylin and eosin. Examples of class I IHC's are differentiation markers, such as keratin. Class I IHC's are subject to general controls including current good manufacturing practice regulations. Class I IHC's are exempt from 510(k).

Class II IHC's (Special Controls/Guidance Document). Class II IHC's are intended for the detection and/or measurement of certain target analytes by immunological techniques in order to provide prognostic and predictive data that are not directly confirmed by routine histopathologic internal and external control specimens. These IHC's provide the pathologist with diagnostic information that is ordinarily reported as independent diagnostic information to the ordering clinician, and the claims associated with these data are widely accepted and supported by valid scientific evidence. Examples of class II IHC's are those intended for semi-quantitative measurement of an analyte, such as hormone receptors in breast cancer.

Class III IHC's (Premarket Approval). These IHC's are IHC's that do not meet the criteria for class I or II, or are IHC's that meet those criteria but raise new issues of safety and effectiveness. Examples are markers used to identify new target analytes in tissues that are claimed to be clinically significant genetic mutations and that cannot be confirmed by conventional histopathologic internal and external controls specimens.

SECTION 2. Level of Concern

FDA/CDRH uses the term "level of concern" as an estimate of the severity of injury that a device could permit as an estimate of the severity of injury that a device could permit or inflict (directly or indirectly) on a patient or operator as a result of latent failures, design flaws, or using the medical device. The extent of the premarket review process pertaining to medical device products is proportional to the level of concern.

2.1 Safety and Effectiveness Issues Involved with IHC's

Immunohistochemistry devices (IHC) are subject to FDA regulation as in vitro diagnostic medical devices. IHC's include a primary monoclonal antibody or polyclonal antibodies intended for use for patient diagnosis, whether packaged in a kit form, or sold separately as a pre-diluted reagent, or as an undiluted (neat) or concentrated primary antibody reagent.

IHC secondary antibody and staining reagents are ancillary reagents to primary IHC antibodies. Although these ancillary reagents are necessary to produce the final immunohistochemistry staining of tissues and cells, they are subject to 21 CFR § 864.1860 only when packaged as components of a complete test system with one or more primary antibodies.

The safety of an IHC relates to whether the diagnostic information that it provides is likely to cause a misdiagnosis of the patient sample. Effectiveness of an IHC device relates to the performance characteristics of how well the device detects and/or measures the intended analyte in the patient sample.

2.2 The FDA Pre-Marketing Review of IHC's

FDA's pre-marketing review of IHC's is based on the risks and benefits of the device. The process is approached as outlined in Appendix A. The following topics are covered in FDA's review:

Documentation that the submission contains adequate and appropriate, valid scientific evidence for reasonable assurance of the safety and effectiveness of the device for the intended use of the IHC. This evidence may be based on (1) test data generated by the sponsor; (2) literature references of research and Investigational studies that are appropriate and sufficient to indicate safety and effectiveness of the same or equivalent IHC devices for similar intended uses; and/or (3) consensus evaluations from future national or international scientific workshops or FDA-sponsored panels.

Class II IHC's qualify for 510(k) clearance if they are documented to be substantially equivalent to a predicate, legally marketed IHC device, i.e., detects or measures immunologically a substantially equivalent antigen in tissues or cells.

The 510(k) substantially equivalent determination requires that there are no new issues of safety and effectiveness for the intended use (Safe Medical Devices Amendments of 1990). The safety and effectiveness of an IHC is tied to the sponsor's claims for the expected or implied use of the IHC, and the clinical indication for the use of the diagnostic information according to the medical understanding of the IHC.

The types and quantity of valid scientific evidence that FDA requires for an IHC (21 CFR § 860.7) depend on (1) the medical decisions that will be made using the diagnostic information from the test as a stand-alone test and/or as a component of a panel of tests; (2) how much relevant literature has been published in the scientific literature about the submitted IHC device, i.e, valid published, supportive information from several independent laboratories documenting the safety and effectiveness of the IHC, and there is no new information that may indicate that additional safety and effectiveness data will be required from manufacturer-supported testing.

Prognostic and predictive markers used as stand-alone IHC diagnostic tests may require additional valid scientific evidence. This evidence may be clinical outcome data from the peer-

reviewed scientific medical literature and/or clinical testing sponsored by the manufacturer to establish the safety and effectiveness of the IHC for the claimed intended use.

SECTION 3. Documentation in a Premarket Submission

3.1 Valid Scientific Data/Information to be Submitted for Class II IHC's: Types and Adequacy of Data

To receive 510(k) clearance for class II IHC's, manufacturers should submit the following valid scientific data/information to support the IHC's analytic performance, safety, and effectiveness for the claimed intended use and indications for use:

Even if the IHC reagent is provided by another manufacturer, it is the responsibility of the sponsor of the 510(k) submission to provide these data/information in the submission.

Appendix B provides a checklist to facilitate review for completeness of the 510(k) submission for the necessary valid scientific data/information of Class II IHC's.

3.2 Analytic Performance Characteristics: Antigen/Antibody Characterization

The protocol for screening/selecting the clone of a monoclonal antibody. (Appropriate peer-reviewed scientific literature references are acceptable.)

Identification of the protein and/or its epitope, if known, recognized by the antibody.

Characterization of the primary antigen including photographs, if possible, e.g., SDS gels, western blots, immunoelectron microscopy, etc., especially to support relevant claims, e.g., Moll number reactivity for cytokeratins, melanosomal reactivity of HMB45, etc.

If documentation is based on valid scientific data in the peer-reviewed literature, the manufacturer should provide copies of pertinent references to the FDA. The manufacturer should provide evidence that the primary IHC monoclonal antibody reagent studied in the published reference is the same clone that is the subject of the submission, and that it is substantially equivalent in reactivity and intended use.

If the IHC is based on a polyclonal primary reagent, there should be information about characterization of the immunoreactivity of the polyclonal reagent, the materials used a the immunogen for the primary reagent, any purification processes, and the materials that were used as the calibrator and controls of the assay.

3.3 Validation and Verification of Immune Reactivity of All Classes of IHC's with a Panel of Normal Tissues or Cells

Validation of the immune reactivity of IHC's requires establishing the performance of the IHC primary antibody, together with an acceptable intended secondary marker antibody, by testing

tissues or intact cells that do and do not contain the target analyte in the same matrix as the intended use of the IHC.

Class I IHC's are not the subject to the special control of this guidance. However, 21 CFR § 809.10 regulations for Class I IHC's do require inclusion of the intended use and performance characteristics of the IHC in the product labeling. The performance characteristics of class I IHC's intended to detect differentiation markers and other analytes should be tested and documented by testing these IHC's against a battery of normal and/or abnormal tissues. Multi-tissue blocks of normal tissues or cells that are fixed and processed in the same manner as the intended specimens are usually more predictable for establishing the reactivity, optimal fixation, and are sufficiently consistent to allow for comparison of lot-to-lot reactivity of antibodies and reagents.

The intended enduser can verify that an IHC meets the manufacturer's specification by testing appropriate positive and negative control tissues or cells in the enduser's laboratory.

The specificity of primary antibodies from all IHC's should be determined on a panel of normal tissues. Appendix C is a checklist of the tissues that should be tested for all primary antibodies. The testing should be performed on samples from three different persons for each tissue listed. Literature references may not satisfy this requirement because many antibodies have not been completely characterized with normal tissues.

3.4 Characterization of Specimens Used in the Panel of Normal Tissues

Use freshly fixed surgical specimens if possible. Freshly fixed autopsy material is permitted if the tissues are well preserved by microscopic criteria (minimal autolysis).

Include validation that the test tissues were adequately fixed (not over or under fixed. If pre-treatment with heat, buffer solutions, etc. is used, document the specific times, concentrations, etc.

Document the source of each test tissue used in the submission including the age and sex of the patient. Do not include specific patient identifiers such as name, social security number, etc.

Multitissue blocks may be used, but each piece of tissue should be large enough to contain an adequate sample, e.g., the liver section should contain at least one portal triad; the brain section contain adequate numbers of representative cell types, etc.

3.4.1 Purpose of the Studies with Panels of Normal Tissues

Interlaboratory precision is a continuing problem with commercialized and in-house developed (home brew) IHC devices. Expected values with panels of normal tissue can guide pathologists

and other laboratorians in their verification of an IHC. Normal tissues are potentially more reproducibility than tumor tissues. Tumors with the same hematoxylin and eosin histopathologic appearance may be heterogeneous in their immunoreactivity and genetic composition.

Because the primary antibody in an IHC device may be used to distinguish whether a poorly-differentiated neoplasm is primary or metastatic, it is useful to test each primary antibody on a wide variety of normal tissues. This testing can help to detect unanticipated cross-reactivity and explain some of the background reactivity of each antibody in many tissues throughout the body. Any unusual or unexpected staining should be summarized in the package insert.

The normal tissue testing should establish the following staining reactivity: staining reactivity or non-reactivity of parenchymal cells of each organ tested, which part(s) of the cells stain [cell membrane (plasmalemma), cytoplasm, nucleus), distribution of the staining within the tissue and within the cell (all of the cells or a fraction of the cells], and, for quantitative and semi-quantitative stains, the intensity of staining. (See Appendix D)

Also, primary antibodies may be used to detect cellular components that characterize the physiologic or pathophysiologic state of cells, e.g., markers for different stages of the cell cycle, secretory products, etc. Documentation of the staining of cells in various functional states within a panel of normal tissues can be used to support the performance characteristics of the IHC primary antibody reagent, e.g., staining reactivity of epithelial cells in crypts of the small intestine, basal cells of the epidermis, etc.

3.5 Validation and Verification of Immune Reactivity of Class II IHC's by Testing Panels of Tissues That Have Been Well-Characterized Clinically or That Have Been Established by Comparison to a Widely-Accepted Reference Method

Class II IHC's are intended to detect target analytes that cannot be controlled by readily available internal and external histopathologic or cytopathologic controls. For example IHC's for certain target analytes whose presence or concentrations and distributions are predictive or prognostic for increased or decreased morbidity or mortality and/or response or resistance to therapy. Validation of performance characteristics for class II IHC's to be used as a predictive or prognostic marker requires testing appropriate samples of tissues or cells that have been well-characterized clinically for the intended clinical outcome for the same intended use. The study should provide information on the performance of the IHC in the intended populations of patients and be of sufficiently large study size. The results with the IHC should be compared with a FDA-approved or cleared IVD, or a well-established, widely-accepted reference laboratory methodology.

Examples of acceptable comparison methods are biochemical tests with uptake of isotopically labeled substances, e.g., estrogen receptor assays, that have been widely accepted as established and validated with specimens from appropriate numbers of patients with the well-characterized clinical outcomes.

If there is no appropriate in vitro diagnostic comparison method, the performance characteristics of an IHC should be established and validated by testing patient samples. These samples should be well-characterized and widely-accepted as scientifically appropriate for the determination or prediction of the clinical outcomes claimed as the end-point of the IHC assay, such as morbidity (e.g., recurrence of tumor), mortality, response or resistance to therapy, etc.

There should be appropriate criteria for obtaining the study samples with statistical controls for selection bias. The amount of data should be adequate to characterize the IHC performance on all of the study samples in comparison with the claimed clinical outcome, i.e., prospectively collected specimens or archival tumor tissues, obtained without selection bias, from patients with short and long natural histories and/or from patients with known responses to particular therapy or combinations of therapy.

3.6 Class II IHC's with Claims for Quantitative Results for Prediction or Prognosis of a Clinically Significant Outcome

The assay results of a quantiative IHC should be testing across the whole assay range (from low to high levels of the claimed activity) in comparison to clinical outcomes, e.g., length of survival with or without therapy; differences in morbidity or mortality rates; etc. Analyze the data using appropriate statistical methods in order to report the results in the Performance Characteristics section of the package insert. The sample size of the validation study is decided clinically. There is no unique statistical answer. The number depends upon the precision (width of the confidence intervals) desired for the proportions (sensitivity, specificity). The sample size is important for calculating confidence intervals for sensitivity and specificity (they are all binomial proportions, assuming each observation of 2 x 2 table distributed independently and randomly, e.g., each patient only sampled once). The studies should be performed on an adequate number of positive and negative specimens to support statistical significance. A statistician may suggest an appropriate number for the study size.

3.7 Discrepancies in Comparison Studies

Discrepancies between the new IHC and the comparison method (either the FDA-cleared predicate device or a reference method) should be investigated further using other reference methods to determine if they are true or false. (The new device may have improved performance characteristics compared to a reference method.)

3.8 Sensitivity and Specificity Determinations for Class II IHC's

The sensitivity and specificity and their 95% confidence intervals should be determined and reported in the Performance Characteristics section of the package insert

3.9 Manufacturers' Recommendations for Verification of IHC Performance by the User

Manufacturers of class II IHC's should provide the intended user with recommendations and directions for materials and methods to verify that the IHC reagent or kit is performing according

to the manufacturer's specification. Usually this is performed by testing known positive and negative tissue controls that are either supplied by the IHC manufacturer or from tissues that have be tested with a reference laboratory method.

Verification by testing with tumor tissue that has not be characterized with a well-characterized and widely accepted reference method is not recommended for prognostic or predictive use of IHC's.

The IHC staining of various markers of different functional states of cells from various normal tissues can be used to support claims for the qualitative performance characteristics of the IHC primary antibody, e.g., staining reactivity of epithelial cells in crypts of the small intestine, basal cells of the epidermis, etc. for evidence of cellular proliferation. However, such IHC results alone would not be validated with the appropriate well-characterized clinically significant samples, and would not support quantitative predictive or prognostic claims for clinical outcome based on IHC results with patient specimens.

3.10 Validation of Class III IHC's

Class III IHC's are not the subject of this guidance, however this guidance may be used by a manufacturer to collect and analyze the valid scientific evidence to establish the analytic and clinical performance characteristics of an IHC which is classified as class III because of the lack of widely-accepted, adequate and sufficient scientifically valid evidence necessary to qualify as a class I or II IHC.

3.11 Summary of Reactivity for Package Insert

Include a summary of the sponsor's staining results in the package insert (See Appendix B for suggested items to document.) A summary of the presence or absence of immunoreactivity with each tissue type should be noted for each IHC device. If the device is intended as a quantitative or semi-quantitative device, e.g., proliferation marker or receptor, documentation of the quantitative reactivity should be submitted.

3.11.1 Photomicrographs of Expected Staining Reactivity for Package Insert (optional)

The manufacturer is encouraged to supply the end user with true color photographs of the expected staining patterns either in the package insert, under separate cover, or refer to references that show examples of the expected staining patterns in addition to the narrative descriptions of the expected and unexpected staining patterns.

3.12 Documentation of Valid Scientific Evidence of Safety and Effectiveness to Support the Intended Use of Class II IHC's

The valid scientific evidence may be taken from literature references when a link can be established between the antibody used in the literature reference and the antibody that is the

subject of the 510(k). If the documentation is based on published literature, provide copies in the submission.

Documentation and description of the immunostaining patterns of the primary antibody in target tissues that are sufficiently characterized to support the intended use and indications for use claims should be submitted. The basis for the documentation may be either with a summary of reactivity taken from literature references using the same clone or manufacturer's product; or if that is not available, with manufacturer sponsored studies summarized in the 510(k) submission.

3.13 Current Good Manufacturing Practices (cGMP) Are Now Part of FDA Quality Systems Regulations (June 1997)

Manufacturers of FDA-regulated medical devices are subject to the current good manufacturing practices (cGMP) requirements of the Quality Systems Regulations. This includes being subject to regular GMP inspections.

3.13.1 Stability Studies[4]:

In the 510(k) submission, IHC manufacturers should provide a summary of studies used to establish the stability time intervals for the IHC reagents stated in the Package Insert.

The FDA Current Good Manufacturing Practices (cGMP) recognizes that accelerated stability methods alone are not reliable for monoclonal antibody reagents. In addition, monoclonal antibody reagents may vary greatly in their stability in an inconsistent manner, particularly after dilution[4].

3.13.2 Handling, Storage, and Dilution of IHC reagents

In the package insert, provide the end user with recommendations for how to handle, store, and dilute all IHC reagents. These recommendations should provide guidance on how to appropriate tissue controls with each test run to verify the stability and reactivity of IHC devices.

3.13.3 Protocol for Quality Control Release Criteria for Manufacturer

The manufacturer should enclose with each submission a copy of the written quality control product release criteria to be used for each production lot. These criteria are intended to ensure that the specificity of the primary antibody reagent will be identical to that of the original clone reported in the literature, and that the specificity of each production lot remains constant.

SECTION 4. Package Insert Instructions for the Manufacturer

The Package Insert section of the submission should follow the 21 CFR § 809.10(b) regulation. Appendices E through G present suggestions on how to prepare the Package Insert. Although it not mandatory, the template in Appendix F is designed to facilitate FDA's 510(k) review and to assure that the package insert meets the labeling regulations found in 21 CFR § 809.10(b). This template updates the document previously published by the Biologic Stain Commission and FDA,

"Proposed Format: Package Insert for Immunohistochemistry Products"[1], and incorporates the proposed Package Insert template developed by Joint Council of Immunohistochemistry Manufacturers (JCIM)[2]. The manufacturer should have data or references available to support all of the statements and claims in the package insert. Appendix G is a checklist to facilitate review of package inserts for IHC products, especially if the template is not used.

The manufacturer may publish the package insert in two parts. One part contains generic information that the manufacturer can apply to any or all of the IHC's that share the same information and procedures. This part does not have to be distributed with each package of IHC IVD but should be easily available and should be incorporated into the laboratory procedure manual. The other part of the package insert contains the specific characteristics, directions and documentation for the product.

4.1 The following are suggestions to consider in addition to those given in 21 CFR § 809.10(b) and the proposed formats[1-3:]

4.1.1 Product Nomenclature

If Cluster Designation terminology, e.g., CDX, is used to characterize an antibody or antigen, documentation that the antibody has been assigned to the relevant cluster by the World Health Organization should be provided. Otherwise, use another name for the antibody, and other characterization for the antigen.

4.1.2 Intended Use

The claims in the package insert for effectiveness should be limited to the types of fixatives (e.g., formalin, alcohol, B5, etc.) used in the manufacturer's studies and in referenced literature where authors used the same clone or source of antibody.

If the product is sold as a prediluted or as an undiluted (neat) primary antibody reagent instead of a kit, the package insert should designate which secondary detection (labeling) antibody systems have been tested with the separately sold prediluted or undiluted antibody reagent.

4.1.3 Summary and Explanation of the Device

The Package Insert should include a brief summary and/or discussion of these literature reports discussing the observed expected and discrepant staining or non-staining in various normal and abnormal tissues.

If literature is not available for the product, summarize the literature for the substantially equivalent product with the same intended use and expected performance.

4.1.4 Reagents Provided

The following information regarding the antibody concentration should be provided:

The total protein concentration.

The measured fraction that represents the specific immunoglobulin (as a percentage of the total protein).

The concentration of irrelevant antibody.

4.1.5 Procedure

The Package Insert of IHC devices sold as a kit should provide instructions that the end user can follow that are based on optimization of the test by the manufacturer. The manufacturer's instructions should include how to fix, process, and stain the specimens.

If the IHC device is sold as a separate primary reagent (pre-diluted; undiluted; neat), the manufacturer should establish or verify protocols and provide instructions to the end user to optimize the IHC IVD in addition to supplying references to textbooks and journals. These instructions should include directions about fixation and processing of tissues, antigen recovery techniques (antigen retrieval techniques), checker board titration techniques to optimize performance including how to make dilutions of primary and secondary antibodies, and storage and handling of undiluted and diluted reagents.

Such instructions in the package insert will enable the end user to follow manufacturer's instructions for almost all circumstances that arise with use of this IHC. These instructions will permit the end user to conform with CLIA '88 requirements that permit less rigorous verification steps by the enduser if the manufacturer's recommendations are followed without "major modifications".

4.1.6 Limitations

The Limitations section of the Package Insert should include these additional limitations, as appropriate, for the particular analyte and the safety and effectiveness claims:

"Immunohistochemistry is a multistep diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selection, fixation, processing, preparation of the IHC slide, and interpretation of the staining results."

"Unexpected negative reactions in poorly differentiated neoplasms may be due to loss or marked decrease of expression of antigen or loss or nonsense mutation in the gene(s) coding for the antigen. Unexpected positive staining in tumors may be from expression of an antigen not usually expressed in morphologically similar normal cells, or from persistence or acquisition of an antigen in a neoplasm that develops morphologic and immunohistochemical features associated with another cell lineage (divergent differentiation). Histopathologic classification of tumors is not an exact science and some literature reports of unexpected staining may be controversial."

4.1.7 Expected Results

Include a summary of tissues tested to establish the specificity of this IHC IVD.

4.1.8 References Used for Drafting the Suggested Package Insert

1. Proposed Format: Package Insert for Immunohistochemistry Products. Biotechnic Histochemistry 1992;67:328-338.

2. Proposed Template for Package Inserts for Immunohistochemistry Products. Joint Council of Immunohistochemistry Manufacturers. 1994. St. Louis, MO (unpublished communication to FDA)

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Appendix A: FDA's Risk-Based Evaluation of IHC Applications

The following questions may be posed by a FDA reviewer when assessing the risks and benefits of an IHC in applications submitted to the FDA:

What is intended use of the IHC?

What is the indication for use of the IHC?

What is the analyte?

Is the target analyte claimed only to be a qualitative, adjunctive differentiation marker that can be verified by internal and external histochemical or cytochemical controls in the specimen?

Is the target analyte a semi-quantitative or quantitative marker for functional activity (cell proliferation, level of hormone receptor, etc.) that cannot be verified by the user with readily available well-characterized internal and external histopathologic or cytochemical controls?

Is the IHC to be used as a stand-alone, independent diagnostic test without a confirmatory test; or is it used as an adjunctive test; or as part of a panel of tests to subclassify a disease or condition?

What are the claimed and implied clinical indications for use of the newly submitted IHC device?

What is the clinical significance of the results?

Is there appropriate and sufficient information in the peer-reviewed scientific literature about the analyte and the intended use to support a reasonable assurance of the safety and effectiveness of the product or will it be necessary to gather more test data to validate the IHC?

Is the clinical-pathologic interpretation of the presence or absence of immunostaining made by a qualified pathologist or other qualified laboratorian?

Are quality control materials and methods readily available to ensure that the pathologist can verify that the IHC procedure meets the manufacturer's specifications for acceptable use?

Appendix B: Checklist for Completeness of the Sponsor's Submission for Class II IHC'S

Manufacturer_____

Full name of antibody_____

Y N N/A (fill in the appropriate blank for each item listed below)

Documentation of Analytic Performance:

1. Antigen/Antibody Characterization

__ __ ____ a) Protocol for screening/selecting the clone of a monoclonal antibody. (Literature references are acceptable.)

__ __ ____ b) Epitope and/or protein recognized by the antibody.

__ __ ____ c) Characterization of antigen.

2. Characterization with a Panel of Well-Characterized Tissue Samples (See Appendix C)

__ __ ____ Adequate number of appropriate samples to provide valid scientific evidence for analytic performance claims

Valid Scientific Evidence For Safety and Effectiveness (Literature references acceptable if the same antibody was used and the characterization testing conditions are documented.)

__ __ ____ 1. Safety and effectiveness evidence provided

__ __ ____ 2. Reactivity with adequate numbers of appropriate samples with well-characterized clinical outcomes to support claims for clinical performance

Stability Studiess

__ __ ____ Summary of studies used to establish expiration date.

Quality Control Product Release Criteria

__ __ ____ Summary of criteria

Appendix C: Sources of Normal Tissue Required for Establishing the Specificity of IHC's

Manufacturer_____

Full name of Antibody_____

Three (3) test samples (1 each from 3 different individuals) for each of the following anatomic sites:

Y N N/A (fill in the appropriate blank for each item listed below)

Central Nervous System:

__ __ ____ Brain, Cerebrum (gray and white matter containing neurons, glia, etc.)
__ __ ____ Brain, Cerebellum

Endocrine:

__ __ ____ Adrenal (cortex and medulla)
__ __ ____ Ovary
__ __ ____ Pancreas (Islets of Langerhans and exocrine pancreas)
__ __ ____ Parathyroid
__ __ ____ Pituitary (adenohypophysis and neurohypophysis)
__ __ ____ Testis
__ __ ____ Thyroid (follicular epithelium, parafollicular cells, colloid, etc.)

Breast:

__ __ ____ Breast (lobules, ducts, myoepithelial cells, etc.)

Hematopoietic:

__ __ ____ Spleen
__ __ ____ Tonsil
__ __ ____ Thymus (juvenile)
__ __ ____ Bone marrow (lymphocytes, monocytes/macrophages, granulocytes, erythroid precursors, megakaryocytes, mast cells, osteoclasts, osteoblasts

Respiratory:

__ __ ____ Lung (bronchi, bronchioles, alveoli, etc.)

Y N N/A (fill in the appropriate blank for each item listed below)

Cardiovascular:

__ __ ____ Heart

Gastrointestinal:

__ __ ____ Esophagus
__ __ ____ Stomach (fundus)
__ __ ____ Small intestine (Ileum, jejunum or duodenum)
__ __ ____ Colon
__ __ ____ Liver (portal triads, hepatic cells, etc.)
__ __ ____ Salivary Gland

Genitourinary:

__ __ ____ Kidney
__ __ ____ Prostate
__ __ ____ Uterus
__ __ ____ Cervix

Musculoskeletal:

__ __ ____ Skeletal muscle

Skin:

__ __ ____ Skin (epidermis, appendages, dermis)

Peripheral Nerve:

__ __ ____ Peripheral Nerve

Mesothelial cells:

__ __ ____ Lining cells from chest wall, abdominal wall, pericardium or from the surface of gastrointestinal, heart and/or lung samples

Appendix D: Worksheet for Recording IHC Staining Results for Each Tissue Tested to Assist in Preparation of a Summary for the Package Insert
(Modified from the Immunoreagent Evaluation Protocol of the Immunostain Quality Control Committee of the Biological Stain Commission):

Identify Protocol Used for Testing Antibody Reagent:

Observer: _____ (Note if observer was blinded or unblinded to identity of antibody while making microscopic observations.)

Primary antibody:

Lot #

Specificity of antibody:

Antigen determinant

Concentration:

Total Protein Concentration: XX.X g/L; Mouse IgG Concentration: XXX µg/mL and as per cent of total protein (not relevant if polyclonal); Concentration of irrelevant antibody: XXX mg/mL

Immunostaining method used:

Antibody used at dilution Y or prediluted in kit form using manufacturer's instructions;

Conditions of testing:

For example, buffers, temperature, incubation times, detection system, etc.

Comparison method used for establish clinical performance:

FDA-approved or cleared device with well-characterized clinical outcomes_____

Well-established reference method based on validation with samples with known clinical outcomes_____

Well-characterized tumor samples with known clinical outcomes_____

Appendix E: Where to Summarize in the Package Insert the Results of Class II IHC Staining Reactivity in Tissues

E.1 "Summary and Explanation" section of the package insert:

Briefly summarize the expected and unexpected positive and negative staining patterns of tissues. Include literature references when available. Clarify which literature references reported results using the same clone or product as the subject of the 510(k), or if unavailable, which literature references discuss generic reactivity or reactivity of the substantially equivalent product.

E.2 "Quality Control" section of the package insert:

Recommend at least one tissue for each primary antibody for use as a positive quality control specimen. Ideally, some of the positive controls should be expressed at a weak level of staining so that early failure of the IHC reagent or methodology can be detected early.

E.3 "Interpretation of Staining" section of the package insert:

Describe the correct staining for the chosen quality control specimens in detail. For example: In small intestine mucosa, the IHC stains cytoplasm (nucleus or both) of the epithelium in the base of the crypts where cellular proliferation is maximal, etc.

E.4 "Limitations" section of the package insert:

Summarize unexpected positive and negative staining results in target tissues. The product specific limitations may be based on the sponsor's testing data with the device and appropriate literature references. The general limitations section also should include pertinent generic literature references for the IHC methodology. Most of these should be found in the package insert template.

E.5 "Performance Characteristics" section of the package insert:

Report a summary of the expected and unexpected staining results for the target tissues that were actually tested by the sponsor. Do not include results from the literature in this section unless the same clone and same assay format were used.

Appendix F: Template for IHC Antibody Package Insert:

(Modified from the template developed by FDA and JCIM, 1994)

F.1 Code No.

F.2 Identity of antibody

(Give full name of antibody) for Immunoenzymatic Staining

F.3 Lot No.

F.4 **Intended Use:** For In Vitro Diagnostic Use

(Name of the antibody) _____(specify if it is a monoclonal or a polyclonal antibody) is intended for laboratory use to qualitatively identify by light microscopy _____ **(antigen/epitope)** in normal and/or pathological _____ ***(list types of tissues supported by test data****: paraffin-embedded and/or cryostat tissues, and/or cytosmears*) processed in _____ ***(list types of fixative included in the test tissues)*** fixative **(If data are available to support it, list cryostat sections and/or cell smears). _____ (Name antibody and clone)** specifically binds to antigens located in the _____ ***(location in the cell)*** of normal _____ ***(type of cells)*** The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

(For neat antibody:) List what immunohistochemical staining methods have been tested and are suitable for use with this antibody.

(For prediluted antibody:) This antibody has been optimally prediluted for use ____ secondary staining systems/kits.)

F.5 **Summary and Explanation:**

The ***(name of antibody)*** antibody has been shown to react with ***(give details about the specificity of monoclonal antibodies used in the product, e.g. molecular weight of co-precipitating protein, nature of the antigen, etc.) Describe/List the normal and abnormal cells/tissues that express the antigen and stain with the antibody. Provide references to support the information, where possible.***

If references are unavailable for the manufacturer's clone, include a summary of the scientific medical literature for the substantially equivalent IHC. Discuss any false-positive and false-negative or controversial reactions. Request that users report to the manufacturer's Technical Service Department any controversial staining detected.

F.6 **Principle of Procedure:**

_____ ***(antibody name)*** may be used as the primary antibody with the ***(manufacturer's recommended immunohistochemical technique***). In general, immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link antibody), an enzyme complex and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light

microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

(If antibody is optimally diluted for a specific visualization system, give specific information concerning that system [manufacturer specific]. For example: For kits based on labeled avidin-biotin (LAB) immunohistochemical staining technology with horseradish peroxidase, the specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity. This initial step is followed by the application of blocking reagent to block non-specific reactions of the tissue with the antibody. The specimen is then incubated with a primary antibody followed by subsequent incubations with biotinylated link antibody and peroxidase-labeled streptavidin. Staining is completed after incubation with a substrate-chromogen (such as 3-amino-9-ethylcarbazole (AEC) or 3,3'diaminobenzidine (DAB) and optional counter-staining.)

(If antibody is intended for use with an automated stainer, include additional information concerning the automated staining)

F.7 **Reagent Provided**:

_____ (***antibody name***) is _____ ***(describe antibody, e.g. a mouse anti-human monoclonal)*** antibody produced as a tissue culture supernatant ***(or ascites fluid or, if a polyclonal, provide the animal source and the preparation, i.e., whole serum or an immunoglobulin fraction).*** This product is supplied in ***(buffer),*** containing ***(Give carrier protein, e.g. fetal calf serum, BSA or etc. and any microbiocidal agent used***). (__mL total volume.)

F.8 Immunogen:

F.9 Clone/Reference: ***(not relevant if polyclonal)***

F.10 Subclass: ***(include light chain identity) (not relevant if polyclonal)***

F.11 Total Protein Concentration: XX.X g/L

Specific IgG Concentration: XXX µg/mL and as per cent of total protein

Concentration of irrelevant antibody: XXX mg/mL (not relevant if polyclonal)

F.12 Specificity: ***(Provide information on the reactivity of the antibody, i.e. antigen determinant)***

F.13 Method: ***(How the antibody is produced and processed)***

(***If applicable***: Nonspecific Negative Reagent Control***: (Characterize and describe ingredients and give the total volume supplied.)***

F.14 **Reconstitution, Mixing, Dilution, Titration (If reagents are sold as optimally diluted for use):**

Optimally diluted for use with (_____) (automated instrument, secondary staining system, etc.) Further dilution may result in loss of antigen staining. The user must validate any such change. Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results necessitating regular performance of in-house controls (see Quality Control section).

F.14 **Materials Required But Not Provided:**

Staining reagents such as ***(name preferred staining reagents, e.g., a labeled streptavidin-biotin (LSAB) Kit or peroxidase-anti-peroxidase (PAP) Kit),*** antibody diluent, nonspecific negative reagent control and ancillary components, including negative and positive tissue control slides, are not provided. ***(give manufacturer and catalog numbers where appropriate) (Or cross reference to package insert of secondary reagents for details.)***

F.15 **Storage:**

Store at 2-8°C or aliquot into convenient volumes and freeze at -20°C. Avoid repeated freezing and thawing.Fresh dilutions of the antibody should be made prior to use and are stable for up (***give length of time)*** at room temperature (20°-25°C). Unused portions of antibody preparations should be discarded after ***(same length of time).*** Frozen antibodies may be stored in small aliquots until periodic assay verifications detect unacceptable changes in reactivity. (See Assay Verification Section.)

(Name of antibody) is suitable for use (***give length of time the product is stable***) from the point of manufacture when stored at 2-8°C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.[23]

Positive and negative controls should be run simultaneously with all patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected***, (Company Name and Technical Service Telephone Number)*** should be contacted immediately.

F.16 **Specimen Preparation:**

Paraffin Sections: Tissues fixed in ***(list fixatives shown to be appropriate for this antibody; provide references if available)*** are suitable for use prior to paraffin embedding. ***(Provide information on any advantages/disadvantages of a specific fixative)***

Osseous tissues should be decalcified prior to tissue processing to facilitate tissue cutting and prevent damage to microtome blades.[2,3] ***(Give any helpful references or specific warnings for specific antibodies/tissues.)***

Properly fixed and embedded tissues expressing the ***(specific antigen)*** antigen should be stored in a cool place. The Clinical Laboratory Improvement Act (CLIA) of 1988 requires in 42 CFR § 493.1259(b) that "The laboratory must retain stained slides at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination."[4]

(Include information on cryostat sections or cell smears if valid scientific evidence is available to support claim.)

Consult the ***(Your favorite handbook on Immunochemical Staining Methods***) or references 2 and 3 for further details on specimen preparation.

F.17 **Treatment of Tissues Prior to Staining**: ***(Give information on the need for pre-treatment and the type recommended for the specific antibody, and what may be observed without pretreatment.)***

F.18 **Precautions:**

The package insert and labeling must bear the label "For in vitro diagnostic use".
Toxic and hazard information of preservative if used in the product. For example: "The sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing."[5,6]

(Warnings concerning toxicity, carcinogenicity, immunological sensitivity etc., specific to any reagents used for tissue preparation or staining. See list of potential compounds and suggested wording in the first Proposed Format for the Package Insert.)

Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.[7] Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.

Minimize microbial contamination of reagents or increase in nonspecific staining may occur.

Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

(If sold in kit form, state whether or not certain reagents can be substituted across kit lot numbers.)

F.19 **Instructions for Use:**

F.19.1 Staining Procedure ***(for Concentrated/Undiluted or Pre-diluted Antibody):***

(Antibody Name) may be diluted from (***Give range of recommended dilutions***) when using labeled avidin-biotin staining procedures on paraffin embedded tissues. More concentrated solutions may be necessary for peroxidase-anti-peroxidase (PAP) and alkaline-phosphatase/antialkaline-phosphatase (APAAP) methods ***(Give dilution recommended***). Consult staining protocols for optimal or suboptimal diluents. ***(List product name and catalog numbers of products that may be suitable for use.)***

Refer to the ***(Company's favorite handbook for Immunochemical Staining Methods*)**, and references 2 and 3, or the package insert of a commercial kit system (if used) for guidance on preparing dilutions and for specific staining protocols.

These recommendations are for guidance only. Each end-user laboratory should determine optimal dilutions and procedures. Improperly diluted primary antibodies may result in non-specific or false-negative staining if solutions are too strong or too weak, respectively.

F.19.2 **Staining Procedure for Optimally Diluted Antibody:**

Refer to the staining procedure section of the recommended visualization kit for which the antibody is optimally diluted. If giving time or temperature, provide an estimate of a range that will be appropriate, i.e., 37 C ± ?, or 2 minutes ± ? seconds/min., to provide the user with an understanding of the steps with wide flexibility from those with no flexibility.

State the stability period of the final reaction product (stained slide) when stored under defined conditions and utilizing specified mounting medium.

F.20 Quality Control:

Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in house controls in addition to the following procedures. Consult the quality control guidelines of the 10. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, Battifora H, Brigati J. Special report: Quality control in immunohistochemistry. Am J Clin Path 1989; 92:836,[8] the Proposed NCCLS guideline for IHC[9] and/or reference 10 for additional information.

Positive Tissue Control: External Positive control materials should be fresh autopsy/biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and proper staining techniques. One positive external tissue control for each set of test conditions should be included in each staining run.

The tissues used for the external positive control materials should be selected from patient specimens with well-characterized low levels of the positive target activity that gives weak positive staining. The low level of positivity for external positive controls is designed so to ensure

detection of subtle changes in the primary antibody sensitivity from instability or problems with the IHC methodology. Commercially available tissue slides such as ***(Give names and catalog numbers of control slides, if available for this antibody)***, or specimens processed differently from the patient sample(s) validate reagent performance only and do not verify tissue preparation.

Make specific recommendations for a tissue(s) to use for as a positive tissue control for each antibody, e.g., adenocarcinoma of breast, lung, etc.

Known positive tissue controls should only be utilized for monitoring the correct performance of processed tissues and test reagents, rather than as an aid in formulating a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Negative Tissue Control: Use a negative tissue control ((known to ***be (name the specific antigen/antibody***) negative)) fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the IHC primary antibody for demonstration of the target antigen, and to provide an indication of specific background staining (false positive staining). Also, the variety of different cell types present in most tissue sections can be used by the laboratorian as internal negative control sites to verify the IHC's performance specifications. List the types and sources of specimens that may be used for negative tissue controls in the Performance Characteristics section of the package insert. for a list of specimens that may be used for a negative tissue control.

Specific recommendations for tissues to use for as negative tissue controls for each antibody, e.g., breast tissue, skin, etc., should be provided.

If specific staining (false positive staining) occurs in the negative tissue control, results with the patient specimens should be considered invalid.

Nonspecific Negative Reagent Control: Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. Ideally, a negative reagent control contains a ***(give isotype of the antibody, e.g., same isotype as primary antibody if monoclonal***) antibody produced from tissue culture supernatant in the same way as the primary antibody but exhibits no specific reactivity with human tissues in the same matrix/solution as the **(antibody name)** antibody. Dilute an ***(give isotype, e.g., IgG_1)*** antibody ***(give name and catalog number if available***) to the same immunoglobulin or protein concentration as the diluted primary antibody using the identical diluent. If fetal calf serum is retained in the neat ***(name of antibody)*** antibody after processing, fetal calf serum at a protein concentration equivalent to the diluted primary antibody in the same diluent is also suitable for use. (refer to reagent provided). Diluent alone may be used as a less desirable alternative to the previously described negative reagent controls. The incubation period for the negative reagent control should correspond to that of the primary antibody.

(***If primary reagent is polyclonal***: Use a negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site. To prepare a negative reagent control, dilute an immunoglobulin fraction ***(or whole serum)*** of normal/nonimmune (***animal source***) serum to the same protein concentration as the diluted primary antibody using the identical diluent.)

When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as a negative/nonspecific binding background control for other antibodies.

To differentiate endogenous enzyme activity or nonspecific binding of enzymes from specific immunoreactivity, additional patient tissues may be stained exclusively with substrate-chromogen or enzyme complexes (PAP, avidin-biotin, streptavidin) and substrate-chromogen, respectively.

F.21 **Assay Verification:**

Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues. Refer to the quality control procedures previously outlined in this section of the product insert and to the quality control recommendations of the CAP Certification Program[8] for Immunohistochemistry and/or the NCCLS IHC guideline[9]). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Performance Characteristics Section are suitable for assay verification.

F.22 **Troubleshooting:**

Refer to the Troubleshooting section in the previously referenced ***(manufacturer's preferred reference***) for remedial action, or contact ***(name of company)*** Technical Service Department at ***(phone number)*** to report unusual staining.

(The authors of Package Insert of IHC Kits that contain the secondary staining system should provide more details on troubleshooting the complete staining system in a troubleshooting section just preceding the Bibliography section of the package insert.)

F.23 **Interpretation of Staining:**

Positive Tissue Control: The positive tissue control stained with ***(name of antibody***) should be examined first to ascertain that all reagents are functioning properly. The presence of a rose-red (3-amino-9-ethylcarbazole, AEC), bright pink (new fuchsin or fast red) or reddish-brown (3,3' diaminobenzidine tetrachloride, DAB) reaction product with the target cell's (location in the cell) is indicative of positive reactivity. ***(Describe the pattern of reactivity in the cell/tissue if well characterized. In particular, describe the staining reactivity of the one or two tissues suggested for use as controls. Note the weakly positive areas upon which to focus quality control.)*** If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid.

The color of the reaction product may vary if substrate chromogens other than those stated are used. Refer to substrate package inserts for expected color reactions. Further, metachromasia may be observed in variations of the method of staining.[11]

(Statement of variability in choice of counterstain, for example): Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results.

Negative Tissue Control: The negative tissue control should be examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. The absence of specific staining in the negative tissue control confirms the lack of antibody cross-reactivity to cells/cellular components***. (Describe the pattern of reactivity in the cell/tissue if well characterized. In particular, describe the staining reactivity of the one or two tissues suggested for use as controls.)*** If specific staining (false positive staining) occurs in the negative external tissue control, results with the patient specimen should be considered invalid.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically.

Patient Tissue: Examine patient specimens stained with ***(Antibody name)*** last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. If necessary, use a panel of antibodies to identify false-negative reactions.

Table 1: The Purpose of Daily Quality Control

Tissue: Fixed & Processed Like Patient Sample	**Specific Antibody & Secondary Antibody**	**Nonspecific Antibody* or Buffer plus Same Secondary Antibody as Used with Specific Antibody**
Positive Control: tissue or cells containing target antigen to be detected (could be located in patient tissue). *The ideal control is weakly positive staining tissue to be most sensitive to antibody degradation.*	Controls all steps of the analysis. Validates reagent and procedures used for staining	Detection of non-specific background staining

Tissue: Fixed & Processed Like Patient Sample	**Specific Antibody & Secondary Antibody**	**Nonspecific Antibody* or Buffer plus Same Secondary Antibody as Used with Specific Antibody**
Negative Control: Tissues or cells expected to be negative (could be located in patient tissue or positive control tissue)	Detection of unintended antibody cross-reactivity to cells/cellular components	Detection of non-specific background staining
Patient Tissue	Detection of specific staining	Detection of non-specific background staining

* = Same source and type as the specific antibody but not directed against the same target antigen. To detect non-specific antibody binding, e.g., binding of Fc portion of antibody by the tissue.

(The manufacturer is encouraged to supply the end user with color photographs of the expected staining patterns either in the package insert, under separate cover, or refer to references that show examples of the expected staining patterns)

Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding (***Antibody Name***) immunoreactivity.

F.24 **General Limitations**:

Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.

Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.[12]

Excessive or incomplete counterstaining may compromise proper interpretation of results.

The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests. It is the responsibility of a qualified pathologist who is familiar with the proper use of IHC antibodies, reagents and methods to interpret the all of the steps used to prepare and interpret the final IHC preparation.

{Kit Only} The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections or cytologic preparation. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

(If data are not available for use of the primary antibody in flow cytometry): This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.)

Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[13]

Reagents may demonstrate unexpected reactions in previously untested tissues. The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues.[14] Contact (***Give company name and technical services telephone number***) with documented unexpected reaction(s).

Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to autoantibodies or natural antibodies.

False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used.[12]

F.25 **Product Specific Limitations**:

(Provide information on limitations specific to the antibody. Discuss the false negatives/positive, cross-reactivity, advantages and disadvantages for using normal and neoplastic tissues as control tissues, etc.)

F.26 Performance Characteristics:

F.26.1 Reproducibility:

Describe the reproducibility testing that was conducted and the results

Intra-run reproducibility of staining was determined by staining (X) slides containing the same normal tissue. ***(Give results of the testing.)*** Inter-run reproducibility of staining was determined by staining slides containing the same normal tissue on (X) number of days/runs. ***(Give results of the testing.)***

F.26.2 **Immunoreactivity:**

The following positive and negative immunoreactivities have been demonstrated in ***(List types of tissues, i.e. paraffin-embedded, cryostat sections, or smears supported by test data) tissues using (secondary staining system(s)).*** The list provided below is not exhaustive but characterizes the types of immunoreactivities observed with the antibody ***(name of the specific monoclonal clone or if primary reagent is polyclonal, provide the source and purification:***

(Report a summary of the expected and unexpected staining results for the tissues actually tested by the sponsor. Do not include results from the literature in the Expected Results section of the Package Insert.)

F.27 **Bibliography**: Use less or more references as appropriate

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2. Kiernan JA. Histological and Histochemical Methods: Theory and Practice. New York: Pergamon Press 1981

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4. Clinical Laboratory Improvement Amendments of 1988: Final Rule, 57 FR 7163, February 28, 1992

5. “Procedures for Decontamination of Plumbing Systems Containing Copper and/or Lead Azides”, Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health, Rockville, MD. 1976

6. Center for Disease Control Manual. Guide: Safety Management, No. CDC-22, Atlanta, GA. April 30, 1976. “Decontamination of Laboratory Sink Drains to Remove Azide Salts.”

7. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P

8. College of American Pathologists (CAP) Certification Program for Imunohistochemistry. Northfield IL. Http://www.cap.org (800) 323-4040.

9. O'Leary TJ, Edmonds P, Floyd AD, Mesa-Tejada R, Robinowitz M, Takes PA, Taylor CR. Quality assurance for immunocytochemistry; Proposed guideline. MM4-P. National Committee for Clinical Laboratory Standards (NCCLS). Wayne, PA. 1997;1-46

10. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, Battifora H, Brigati J. Special report: Quality control in immunohistochemistry. Am J Clin Path 1989; 92:836

11. Koretzik K, Lemain ET, Brandt I, and Moller P. Metachromasia of 3-amino-9-ethylcarbazole (AEC) and its prevention in Immunoperoxidase techniques. Histochemistry 1987; 86:471-478.

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13. Omata M, Liew CT, Ashcavai M, Peters RL. Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen: a possible source of error in immunohistochemistry. Am J Clin Path 1980;73:626

14. Herman GE and Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech & Histochem 1991;66:194

(Company name and address and phone number) Part No.: Revision Date

Appendix G: Checklist for Completeness of Scientific Documentation of IHC Antibody in the Package Insert

Manufacturer_____

Full name of Antibody_____

Intended Use:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ full trade name of antibody
__ __ ____ clone name of antibody
__ __ ____ monoclonal vs. polyclonal
__ __ ____ quantitative vs. qualitative
__ __ ____ target antigen (& epitope, if known)

__ __ ____ test methodology
__ __ ____ specimen types
__ __ ____ fixatives
__ __ ____ instrumentation, if applicable
__ __ ____ secondary staining system(s) for which product is optimally diluted, if applicable
__ __ ____ safety and effectiveness for intended use
__ __ ____ "The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist."

Summary and Explanation:

__ __ ____ history of methodology (usually in secondary antibody package insert)
__ __ ____ merits and limitations of the methodology (secondary antibody package insert)
__ __ ____ antibody specificity, characterization of antigen
__ __ ____ molecular weight of co-precipitating protein
__ __ ____ nature of antigen/epitope
__ __ ____ reference for WHO classification, if claimed
__ __ ____ summary of positive and negative staining in target tissues
__ __ ____ summary of unexpected results
__ __ ____ references where possible for staining in target tissues
__ __ ____ references for unexpected results

Principle of Procedure:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ principle is sufficiently explained

Reagent(s) Provided:

Antibody Reagent

__ __ ____ volume provided
__ __ ____ source (animal species)
__ __ ____ preparation (e.g., ascites, tissue culture)
__ __ ____ purification techniques
__ __ ____ non-reactive ingredients stated (buffer, stabilizer, and preservative)
__ __ ____ immunogen with reference(s) (These references should contain the protocol for screening/selecting the clone of a monoclonal antibody.)
__ __ ____ clone designation with reference(s), (reference(s) for CD designation, if claimed) (not relevant, if polyclonal)
__ __ ____ subclass, including light chain identity with reference(s), if possible (not relevant, if polyclonal)
__ __ ____ total protein concentration
__ __ ____ specific IgG Concentration in micrograms/mL and per cent of total protein
__ __ ____ concentration of irrelevant antibody
__ __ ____ specificity (brief summary, details should be under Summary & Explanation section)

Nonspecific Negative Reagent Control (if present)

__ __ ____ volume provided
__ __ ____ characterization sufficient to demonstrate parallel ingredients to primary antibody
__ __ ____ chemical names of reactive and harmful ingredients
__ __ ____ quantity, concentration or proportion of reactive ingredients
__ __ ____ characterization of buffers, preservatives and stabilizers

Other Non-Antibody Reagents (if present)

__ __ ____ volume provided

__ __ ____ chemical names of reactive and harmful ingredients
__ __ ____ quantity, concentration or proportion of reactive ingredients
__ __ ____ characterization of buffers, preservatives and stabilizers

Reconstitution, Mixing, Dilution, Titration:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ (for reagents optimally diluted for use) "Optimally diluted for use with (_____) *(automated instrument, secondary staining system, etc.)* Further dilution may result in loss of antigen staining. The user must verify any such changes. (Reference: Clinical laboratory Improvement Amendment of 1988, Final Rule 57 FR 7163 February 28, 1992) Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results necessitating regular performance of in-house controls (see Quality Control section)."

Materials Not Provided: (details may be distributed between package inserts for primary antibodies and secondary staining kits)

Reagents, not provided

__ __ ____ neutral buffered formalin
__ __ ____ positive and negative tissue controls
__ __ ____ negative reagent control
__ __ ____ ammonium hydroxide, 15M diluted to 37mM
__ __ ____ wash solution
__ __ ____ secondary staining kit or solutions
__ __ ____ counterstain (recommendation given)
__ __ ____ mounting medium (recommendation given)
__ __ ____ poly L-lysine (or other adhesive material)
__ __ ____ distilled water
__ __ ____ ethanol, absolute and 95%
__ __ ____ xylene
__ __ ____ digestive enzyme (recommendation given)
__ __ ____ hydrogen peroxide
__ __ ____ protein blocking solution

Laboratory equipment, not provided.

__ __ ____ absorbent wipes
__ __ ____ wash bottles
__ __ ____ drying oven capable of maintaining a temperature of 70^{o}C +/-5^{o}C
__ __ ____ automated instrumentation
__ __ ____ microscope slides (specifications given or manufacturer recommended)
__ __ ____ coverslips (specifications given or manufacturer recommended)

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ timer (capable of 3 -10 minute intervals)

__ __ ____ humid chambers (optional)
__ __ ____ staining jars or baths
__ __ ____ light microscope (20-80X)

Storage:

__ __ ____ recommendations for how to handle, store and dilute provided reagents
__ __ ____ recommendations for how to handle and store diluted/prepared reagents
__ __ ____ total shelf life of each reagent provided from date of manufacture
__ __ ____ "Do not use after expiration date stamped on vial. "
__ __ ____ "If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user." (Reference: Clinical Laboratory Improvement Amendments of 1988: Final Rule, 57 FR 7163, February 28, 1992)"
__ __ ____ signs of deterioration, if possible, especially for reconstituted reagents without stated expiration periods

Specimen Preparation: (instructions may be distributed between package inserts for primary antibodies and secondary staining kits)

__ __ ____ specimen type(s) claimed, e.g., paraffin embedded, cryostat tissue, cytocentrifuged, etc.
__ __ ____ reference(s), cross-reference(s) and/or instructions for how to prepare and fix all claimed tissues. Include all acceptable fixatives with recommended concentrations and duration of fixation.

Treatment of Fixed Tissues or Cytologic Specimens Prior to Staining:

__ __ ____ pretreatment of specimens: reference(s), cross-reference(s) and/or instructions for recommended performance and quality control methods for pretreatment of tissue or cytologic specimens, e.g., proteolytic enzyme digestion, decalcification, heat (microwave, steam bath, etc.) and/or buffer treatments of test material, etc. Include concentrations of reagents, duration of treatments, temperature range of any heating steps, verification of acceptable results, troubleshooting, etc.
__ __ ____ storage conditions given (X - X^{o}C)
__ __ ____ stability period of tissue block given
__ __ ____ stability period of sectioned or prepared slides given
__ __ ____ special precautions

Precautions: (See specific wording in "Proposed Format:...[1]")

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ **"For in vitro diagnostic use."**
(Toxic and hazard information of preservative if used in the product).
__ __ ____ "The sodium azide (NaN3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.[21,22]
__ __ ____ "Symptoms of overexposure to ProClin 300 may include skin and eye irritation and irritation to mucous membranes and upper respiratory tract."

(Warnings concerning toxicity, carcinogenicity, immunological sensitivity, etc., specific to any reagents used for tissue preparation or staining, if applicable. See list of potential compounds and suggested wording in the Proposed Format: Package Inserts...[1].)

__ __ ____ AEC
__ __ ____ DAB
__ __ ____ OPD
__ __ ____ "Store OPD and TMB in the dark"
__ __ ____ N,N - dimethyl formamide
__ __ ____ formaldehyde (paraformaldehyde)
__ __ ____ flammability of organic reagents
__ __ ____ "Methanol is a poison. Do not ingest"
__ __ ____ Toxic effects of trace metals
__ __ ____ "Consult Federal, State or local regulations for disposal of any potentially toxic components."
__ __ ____ "Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.[20] Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water."
__ __ ____ "Minimize microbial contamination of reagents or increase in nonspecific staining may occur."
__ __ ____ "Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user."
__ __ ____ ***(If sold in kit, state whether or not certain reagents can be substituted across kit lot numbers.)***

Instructions for Use/Staining Procedure:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ adequate instructions are given, including cross-referencing to secondary staining kits, instrument manuals, or other ancillary labeling or instruction books or references

Quality Control:

__ __ ____ "Controls should be fresh autopsy/biopsy/surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Such a control monitors all steps of the analysis, from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will control for all reagents and method steps *except* fixation and tissue processing."

Positive Tissue Control:

__ __ ____ specific tissue containing weak positive staining recommended
__ __ ____ "A tissue with weak positive staining is more suitable than a tissue with strong positive staining for optimal quality control and to detect minor levels of reagent degradation." Reference: Battifora H. Diagnostic uses of antibodies to keratins: a review and immunohistochemical comparison of seven monoclonal and three polyclonal antibodies." Progress in Surg Path 6:1-15. eds. Fenoglio-Preiser C, Wolff CM, Rilke F. Field & Wood, Inc., Philadelphia.
__ __ ____ frequency of running positive quality control tissue stated.
"One positive tissue control for each set of test conditions should be included in each staining run."
__ __ ____ "If the positive tissue control(s) fail to demonstrate positive staining, results with the test specimens should be considered invalid."

Negative Tissue Control:

__ __ ____ specific tissue(s) recommended for use as a negative control
__ __ ____ "This tissue should show absence of specific staining, and provide an indication of specific background staining. It also should be used as an aid in interpretation of results."
__ __ ____ "The variety of different cell types present in most tissue sections frequently offers negative control sites, but this should be verified by the user."
__ __ ____ "If specific staining occurs in the negative tissue control, results with the patient specimens should be considered invalid."

Nonspecific Negative Reagent Control:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ (Explain the purpose of this control:)"Use a nonspecific negative reagent control in place of the primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow better interpretation of specific staining at the antigen site."
__ __ ____ Recommendation of the ideal control reagent.

__ __ ____ Recommendation of alternative(s)
__ __ ____ "Dilute the negative reagent control material to the same dilution in the same diluent as the primary antibody/antiserum."
__ __ ____ "The incubation period for the negative reagent control should correspond to that of the primary antibody."
__ __ ____ "When panels of several antibodies are used on serial sections, the negatively staining areas of one slide may serve as negative/nonspecific binding background controls for other antibodies."

Assay Verification:

__ __ ____ "Prior to initial use of an antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical performance characteristics representing known positive and negative tissues."
__ __ ____ "These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Tissues listed in the Performance Characteristics Section are suitable for assay verification."

Troubleshooting (Remedial Action):

__ __ ____ "Refer to the Troubleshooting section in the previously referenced ***(manufacturer's preferred reference)*** for remedial action, or contact ***(name of company)*** technical service department at ***(phone number)*** to report unusual staining."

Interpretation of Staining:

Positive Tissue Control:

__ __ ____ Staining of recommended positive control tissue(s) described in detail
__ __ ____ "If the positive tissue controls fail to demonstrate positive staining, any results with the test specimens should be considered invalid."

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ **(Describe appearance of counterstain:)"**Depending on the incubation length and potency of the hematoxylin used, counterstaining will result in a pale to dark blue coloration of the cell nuclei. Excessive or incomplete counterstaining may compromise proper interpretation of results."

Negative Tissue Control:

__ __ ____ **(State the purpose of this control)"**The negative tissue control should be

examined after the positive tissue control to verify the specificity of the labeling of the target antigen by the primary antibody. the absence of specific staining in the negative tissue control confirms the lack of antibody cross-reactivity to cells/cellular components."

__ __ ____ staining of recommended negative control tissue(s) described in detail

__ __ ____ "If specific staining occurs in the negative tissue control, results with the patient specimen should be considered invalid."

__ __ ____ "Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain nonspecifically." (Reference: Nadji M, Morales AR. Immunoperoxidase, part I: the techniques and pitfalls. Lab Med 1983;14:767)

Patient Tissue:

__ __ ____ "Examine patient specimens last. Positive staining intensity should be assessed within the context of any nonspecific background staining of the negative reagent control."

__ __ ____ "As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed."

__ __ ____ "If necessary, use a panel of antibodies to identify false-negative reactions."

__ __ ____ "Refer to Summary and Explanation, Limitations, and Performance Characteristics for specific information regarding immunoreactivity."

General Limitations:

__ __ ____ "Immunohistochemistry is a multistep diagnostic process that consists of specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results."

__ __ ____ "Tissue staining is dependent on the handling and processing of the tissue prior to

Y N N/A (fill the appropriate blank for each item listed below)

staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue."

__ __ ____ "Excessive or incomplete counterstaining may compromise proper interpretation of results."

__ __ ____ "The clinical interpretation of any positive staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence must be complemented by morphological studies and proper controls as well as other diagnostic tests. This antibody is intended to be used in a panel of antibodies. It is the responsibility of a qualified pathologist who is familiar with the antibodies, reagents and methods used to interpret the stained preparation. Staining is to be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls."

__ __ ____ {Kit Only} "The manufacturer provides these antibodies/reagents at optimal dilution for use following the provided instructions for IHC on prepared tissue sections or cytologic preparation. Any deviation from recommended test procedures may invalidate declared expected results; appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances."

__ __ ____ "Unexpected negative reactions in poorly differentiated neoplasms may be due to loss or marked decrease of expression of antigen or loss or mutation(s) in the gene(s) coding for the antigen. Unexpected positive staining in tumors may be from expression of an antigen not usually expressed in morphologically similar normal cells, or from persistence or acquisition of an antigen in a neoplasm that develops morphologic and immunohistochemical features associated with another cell lineage (divergent differentiation). Histopathologic classification of tumors is not an exact science and some literature reports of unexpected staining may be controversial."

__ __ ____ (***If appropriate and if data are not available for use in flow cytometry:)*** "This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry."

__ __ ____ "Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[27]"

__ __ ____ "Reagents may demonstrate unexpected reactions in previously untested tissues.

Y N N/A (fill the appropriate blank for each item listed below)

The possibility of unexpected reactions even in tested tissue groups cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact ***(Give Company Name and Technical Services telephone*** #) with documented unexpected reaction."

__ __ ____ "Normal/nonimmune sera from the same animal source as secondary antisera used in blocking steps may cause false-negative or false-positive results due to auto-antibodies or natural antibodies."

__ __ ____ "False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes), endogenous peroxidase activity (cytochrome C), or endogenous biotin (e.g. liver, breast, brain, kidney) depending on the type of immunostain used.[26]"

Product Specific Limitations: (Product specific limitations are adequately discussed.)

__ __ ____ false negatives
__ __ ____ false positives,
__ __ ____ cross-reactivity, if applicable
__ __ ____ controversies in analytic or clinical testing results

Performance Characteristics:

Reproducibility:

__ __ ____ within run/day and between run/day reproducibility results are adequately presented

Immunoreactivity:

__ __ ____ types of fixatives tested stated
__ __ ____ secondary staining systems tested stated
__ __ ____ summary of tissue reactivity.

Troubleshooting (for secondary staining kit package inserts):

__ __ ____ no staining of any slides
__ __ ____ weak staining of all slides
__ __ ____ excessive background of all slides
__ __ ____ tissue sections wash off slides during incubation
__ __ ____ specific staining too dark

Bibliography:

Y N N/A (fill the appropriate blank for each item listed below)

__ __ ____ references given are appropriate and adequate
__ __ ____ name/place of manufacturer, packer, or distributor
__ __ ____ date of last revision of labeling