Validation for Immunohistochemistry

[Model Procedure]
Purpose: Validation ensures a test works as intended. Any antibody assay (novel or replacement) must be validated before it is put into use as a diagnostic test.

Applies to: This procedure covers the following samples:

1) Immunohistochemistry for surgical pathology paraffin-embedded and frozen sections.
2) Cytology FNA and cell block samples.

Validation verses Verification.

Validation is a comprehensive workup of a novel or replacement antibody. It is intended to provide all the information necessary to determine whether the assay performs as intended.

Verification is performed when minor changes are introduced for a previously validated antibody. Minor changes entail receiving a new lot, use of different general reagents or change in instrumentation.

When to validate:

1) Introduction of a new/different antibody
2) Change of antigen retrieval method, instrument or reagent
3) Change in detection system reagents (secondary antibody, chromogen)
4) Change in fixative or fixative incubation time
5) Change in tissue processor schedules or reagents

When to Verify:

1) Introduction of a new lot of antibody
2) Introduction of a new lot of detection system
3) Introduction of different general reagents (i.e. buffers, deparaffinization solvents)
4) Change in instrumentation for reagent delivery (i.e. new staining instrument)

Verification procedure:
Run a test with the 3 to 5 established controls, including negative tissue, in parallel with the existing method and the new method. Verification is acceptable when the results are identical with the two methods.

Validation Preparation

Use the form “Validation of Primary Antibody” to organize the materials for validation.

Validation Method:

1) **Determine the antibody to validate**
    a. Literature search
    b. User recommendation (i.e. pathologist determination)
2) **Determine FDA Class of antibody**
    a. **Class I IVD:** an ancillary test that is not a stand-alone determinant treatment (I.e. keratin for determination of type of neoplasm)
    b. **Class II IVD:** A stand alone test of treatment (i.e. Estrogen Receptor for treatment decision)
    c. **Analyte Specific Reagent (ASR):** Class I or Class II. Not FDA-cleared but is regulated by FDA and is available for diagnostic use when comprehensively validated by the laboratory. Validation and determination of use is sole responsibility of the laboratory. No vendor information is supplied concerning intended use or protocols for test method

d. **Research Use Only (RUO):** Not regulated or cleared by FDA. Validation and determination of use is sole responsibility of the laboratory. Vendor may supply information about use and protocols for use.

3) Provide literature supporting the use of the antibody
   a. Vendor literature
   b. Scientific literature

4) Outline a Validation protocol for initial testing
   a. Determine expected expression
   b. Determine rejection criteria
   c. Tissues required for testing
   d. Antigen Retrieval method
   e. Blocking regime
   f. Antibody dilution or predilute
   g. Detection system
   h. Chromogen
   i. Reproducibility testing
   j. Addition testing necessary, determined on a case-by-case bases
   k. Concordance of tests of unknown against a known antibody or test

5) **Determine Expected Expression of the antibody.** The expression description must include the tissue-type, cell-type, cell compartment and whether the antigen is normally-expressed, over-expressed or lost in the target tissue.
6) **Determine rejection criteria.** Describe what type of expression will cause rejection of the test result. For example, generalized background, cytoplasmic stain for a nuclear antigen, etc.
7) **Determine which tissues are required for validation.** May be modified depending on availability of specific and/or rare tissues. Tissues for validation are a mix of positive and negative tissues.
   a. **FDA Class I IVD** (ancillary tests)
      i. 10 tissue samples
         1. 5 to 7 positive tissues, mix of low to high expressers of the antigen
         2. 3 to 5 known-negative tissues.
   b. **FDA Class II IVD** (stand-alone predictive tests)
      i. Estrogen and Progesterone Receptors
         1. Option 1: Follow verification procedures in the manufacturer's FDA-cleared product insert, if manufacturer's method is followed exactly.
            a. ≥ 20 positive specimens, (≥ 5 must be weakly positive)
            b. ≥ 20 negative specimens
         2. Option 2: perform an comprehensive validation if manufacturer method is modified in any way, or if validating a non-FDA-cleared assay.
            a. ≥ 40 positive specimens, (≥ must be weakly positive)
            b. ≥ 40 negative specimens
         3. Validate against a second method
            a. Previously validated antibody to the same target
            b. Tissues previously validated by another laboratory
            c. Dextran-coated charcoal steroid binding assay (DCC)

      ii. Her2
         1. Perform an in-house validation with or without an FDA-cleared assay. More cases required if manufacturer method modified in any way

a. 25 to 100 cases
    i. Range of positive from 1+ to 3+ tissues
    ii. Negative tissues
b. Validate against a second method.
    i. Previously-validated antibody to the same target.
    ii. Tissues previously validated by another laboratory.
    iii. FISH genetic analysis.

8) **Antigen Retrieval (AR) Method.** Antigen retrieval is normally necessary for formalin-fixed tissue but not necessary for non-formalin fixed or frozen tissue. Ideally, test more than one AR method Determine the following factors:
    a. Antigen retrieval type
        i. Digestion
            1. Enzyme
            2. Temperature
            3. Time
        ii. Heat mediated antigen retrieval (HIER)
            1. Buffer type (pH), manufacturer, catalog number
            2. Instrument (pressure cooker, water bath, on-board instrument (i.e. Leica Bond))
            3. Temperature
            4. Time

9) Blocking Regime, if necessary
    a. Protein blocking
    b. Endogenous Peroxidase blocking
    c. Other

10) Antibody Dilution
    a. **Prediluted antibody.** The purpose of prediluted antibody is to avoid the time necessary for dilution. Adjust prediluted antibody intensity using, in order of preference:
        i. Incubation time of primary.
        ii. Detection reagent incubation time.
        iii. Dilution
    b. **Concentrated antibody.** A dilution series is tested to determine the optimal range of dilution.
        i. Consult the vendor datasheet for the suggested dilution and run three slides at ½ recommendation, at recommendation and twice recommendation.
        ii. Determine if one of those is close to desired result
            1. If so, test on suggested control tissue
            2. If not, test other dilutions as determined by the initial test

11) **Detection system.** As much as possible one detection system will be used for all antibodies. Occasionally an antibody may require a specialized detection system.
    a. DAKO Envision + (most antibodies): a single-step polymer detection system
    b. Vector ABC Elite (avidin-biotin complex): a two-step detection system
    c. Leica Refine (for the Leica Bond Max Stainer): a two-step polymer system

12) **Chromogen.** Normally DAB is used as the chromogen. Specialized tests may require other chromogens
    a. Peroxidase-based tests
        i. Diaminobenzidine (DAB)
        ii. AEC
    b. Alkaline phosphatase-based detection systems
        i. Fast Red

13) **Reproducibility testing.** Reproducibility testing involves testing many slides from the same control(s) with the same reagents to determine if the test is identical on all

a. **Intra-run** reproducibility: Test 5 to 10 slides from the same control in a single run. All should have identical results.
b. **Inter-run** reproducibility: Test 5 to 10 slides from the same control separately on 5 to 10 different runs, with different operators. Results should be identical.

14) **Other testing.** Other tests may be necessary to fully validate the test.

15) **Concordance of tests.** Concordance compares the results of the test of an unknown (ie, a new antibody) against a known test (ie, an previously validated antibody, or a different test of the same tissue, i.e. FISH).

a. **Concordance Results**
i. **Class I IVD:** Small sample sets (5-10 cases) of tests require 100% concordance of positive and negative values
ii. **Class II IVD:** Large sample sets for predictive makers require:
1. ≥ 90% positive concordance (sensitivity)
2. ≥ 95% negative concordance (specificity)

References:

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June 2009 edition
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Anatomic Pathology Checklist

Clinical Laboratory Improvement Amendments (CLIA), Centers for Medicare and Medicaid Services, CMS-2226-F: 42 CFR 493 Interpretive Guidelines for Laboratories, Appendix C, Subpart K (Quality systems).
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Recommendations for Improved Standardization of Immunohistochemistry, Goldstein, NS, et.al., and members of Ad-Hoc Committee on Immunohistochemical Standardization, Appl Immunohistochem Mol Morph, 2007 15(2): 124-133

Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry, Yaziji, H, et.al., including Members of the Standardization Ad-Hoc Consensus Committee, Appl Immunohistochem Mol Morphol, Vol. 16, No. 6, Dec 2008

Recommendations for Validating Estrogen and Progesterone Receptor Immunohistochemistry Assays, Fitzgibbons PL, et. al., Arch Pathol Lab Med. 2010; 134(6): 930-935

American Society of Clinical Oncology / College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer, Wolff AC, et.al., Arch Pathol Lab Med 2007;131:18-43

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Laboratory

VALIDATION OF DETECTION KIT

Detection Kit: **Manufacture:** **Catalog Number:**

Run Number	Lot Number	Receive Date	Expiration		Acceptable	Initial	Date
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		
					Yes / No		

Validation of Primary Antibody

Date: _____

Project Inputs and Overall Design(s)

Name of Reagent:	
Clone:	
Labeling*	IVD ASR RUO
Proposed by	
Approved by	

***IVD** = in vitro Diagnostic Device, FDA Approved; **ASR** = Anylate Specific Reagent, FDA regulated, **RUO** = Research Use Only, not
FDA approved or regulated

Intended Use

☐ Diagnostic (IVD, ASR required)	☐ Research
☐ Immunohistochemistry	☐ Immunofluorescence
☐ In situ hybridization	☐ Others:
This product is intended for :	
Description of reagent:	
☐ Expected Staining Pattern:	
☐ Positive Control:	
☐ Others:	
☐ Others:	

Sources of Input	References	Date
☐ Market Information	Company:	
	Labeled: IVD ASR RUO	
	Clone/Animal host:	
☐ Market Information	Company:	
	Labeled: IVD ASR RUO	
	Clone/Animal host:	
☐ Market Information	Company:	
	Labeled: IVD ASR RUO	
	Clone/Animal host:	
☐ Scientific Literature	Title:	
	Reference:	
	Conclusion:	
☐ Scientific Literature	Title:	
	Reference:	
	Conclusion:	
☐ Scientific Literature	Title:	
	Reference:	
	Conclusion:	

Validation of Primary Antibody

Validation Design Input

Describe the validation requirements

Platform (circle one)	**Dako Autostainer Leica Bond Ventana Ultra Manual**
Antibody	
Antigen Retrieval method	
Blocking regime	
Primary Dilution recommendation (initial trial)	
Primary antibody incubation time	
Detection system	
Chromogen	
Reproducibility testing	**None Inter-run (# slides_____) Intra-run (# slides_____)**
Control tissues:	

Tissue	**Case Number**	**Positive element**

Additional testing required:

Approved by	IHC Lead Technologist	Date
	Medical Director, Immunohistochemistry	Date

Design Output: First Trial Evaluation of Antibody

Reagent Source	**Catalog number**	**Lot Number**	**Date**

Test (IHC, ISH, IF)	Date	Pass	Fail	Comments

See attached test records

Validation/Verification Results

Reagent does / does not match criteria detailed in design specification

Describe results:

Approved by	IHC Lead Technologist	Date
		Date
	Medical Director, Immunohistochemistry	Date

Validation of Primary Antibody

Optimization Instructions (First Pass)

Step	Modification	Note	Date

Optimizaton Results

Test (IHC, ISH, IF)	Modification	Date	Pass	Fail	Comments

Optimization Instructions (Second Pass)

Step	Modification	Note	Date

Optimizaton Results

Test (IHC, ISH, IF)	Modification	Date	Pass	Fail	Comments

Optimization Instructions (Third Pass)

Step	Modification	Note	Date

Optimizaton Results

Test (IHC, ISH, IF)	Modification	Date	Pass	Fail	Comments

Validation of Primary Antibody

Optimized Procedure

Platform (circle one)	**Dako Autostainer Leica Bond Ventana Ultra Manual**
Antibody	
Antigen Retrieval method	
Blocking regime	
Primary Dilution recommendation (initial trial)	
Primary antibody incubation time	
Detection system	
Chromogen	
Control tissues:	

Tissue	**Case Number**	**Positive element(s)**

Attach list if extra control tissue necessary

Approved by	Medical Director, Immunohistochemistry	Date
	IHC Lead Technologist	Date

Validation of Primary Antibody

Reproducibility

Intra-Run reproducibility: 5 to 10 identical slides within one run

Test	Date	Pass	Fail	Comments
IHC				
IHC				
IHC				

See attached test records

Inter-run reproducibility: 5 to 10 identical slides on 5 to 10 separate runs

Test	Date	Pass	Fail	Comments
IHC				
IHC				
IHC				

See attached test records

Reproducibility approval

Reagent does / does not meet reproducibility criteria

Approved by		
	IHC Lead Technologist	Date
	Medical Director, Immunohistochemistry	Date

Validation of Primary Antibody

Design Validation

Validation criteria	Y	N	N/A
Do results of internal and / or external (consultants / pathologist) testing meet the requirements and specifications of the reagent?	☐	☐	☐
Are test results on panel of normal and tumor tissues acceptable?	☐	☐	☐
Are reproducibility tests acceptable?	☐	☐	☐

Validation report:

Does reagent meet specification criteria?

Positive staining criteria:

Rejection criteria:

Comments:

Final Approval	Medical Director, Immunohistochemistry	Date
		Date

CoPath Entry

Name		
Abbreviation		
Search terms		
Description		
Label Text		
Entered in Copath	**Date:**	**By:**