Validation for IHC Antibodies

Final Approval: October 2010  Effective: October 2010

Next Review Date: October 2012

List all stakeholder(s) and dates of approval:

<table>
<thead>
<tr>
<th>Stakeholder Name(s):</th>
<th>Date:</th>
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<td>Shelly M. Siegel HT</td>
<td>10/7/10</td>
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<td>Mark Magilner MD</td>
<td>10/7/10</td>
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<td>Shelly Siegel</td>
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Describe briefly the most recent revision made to this policy, procedure or protocol & why:

New Procedure

Purpose/Policy Statement:

Validation of non-waived test systems is mandated by CLIA 88. Since the introduction of Immunohistochemistry (IHC), this test has been used as an adjunct to morphologic diagnosis. For consistent applications of test procedures, each IHC test needs validation prior to patient testing. IHC will be validated on: 10% formalin fixed specimens, processed on a tissue processor, paraffin embedded, cut at 4 microns, placed on (+) charged microslides and placed in the 60 degree oven for 30 minutes before stained on the Leica Bond 3 system.

Validation will occur if:

- Introduction of a new antibody
- Introduction of change in antigen retrieval process
- Change in detection system
- Change in fixative
- Change in tissue processor instrument
- Change in control material

Definitions:

- n/a

STEPS / KEY POINTS

Validation for Routine Antibody (non predictive, non FDA approved)

Single or Dual Staining

1. Establish a protocol for the antibody.

   A. Start a protocol by testing a (+) control with the following:

   - If this is a brand new antibody (never used in this lab), discuss with pathologist what control tissue to use.

   - Vendor recommendation for Leica BOND 3 instrument. If a recommendation is made test 3 (+) control slides by staining 1 with the recommendation, 1 with increased HIER and 1 with decreased HIER. Example: S-100 recommendation is H1-20. 1st slide H1-20, 2nd slide H1-30 and 3rd slide H1-10.

   - If there is not a recommendation -- Call the Leica Field Support Specialist for a recommendation about that particular antibody. They will tell you what other facilities are using
Validation for IHC Antibodies

2. Send stained slides to pathologist to choose which protocol to use.
   A. Pathologist will recommend a protocol or recommend changes.

3. Retrieve a known positive patient case (if applicable) and perform IHC staining with the chosen protocol.
   A. If this is a brand new antibody, discuss with the pathologist appropriate patient tissue for use in validation.
      • If this is a replacement antibody or new clone, etc. then one patient case is sufficient, provided the results match the previous known positive and negative patient tissue.
      • If this is a brand new antibody, more than one case may be needed at the pathologist discretion.
      • Validation with tissue from another lab or staining at another lab may be performed at the pathologist discretion.
      • Choice of strong and focal/weak patient cases is suggested if possible.

4. Send stained slides to pathologist for review.
   A. Concordance with patient tissue and control tissue (or expected staining) should be 100%.
   B. Pathologist will approve or recommend changes.
   C. Approval will be documented.

Validation for HER2 (predictive, non FDA approved)

1. Establish a protocol for the HER2 antibody.
   • Select 5 cases with a known score of grade 3 and grade 1.
   • Vendor recommendation for Leica BOND 3 instrument. If a recommendation is made test 3 (+) control slides by staining 1 with the recommendation, 1 with increased HIER and 1 with decreased HIER. Example: recommendation is H1-20. 1st slide H1-20, 2nd slide H1-30 and 3rd slide H1-10.
   • If there is not a recommendation -- Call the Leica Field Support Specialist for a recommendation about that particular antibody. They will tell you what other facilities are using to guide you in the right direction. Again, test 3 (+) control slides. 1 with the recommendation, 1 higher and 1 lower retrieval time.

2. Send stained slides to pathologist to choose which protocol to use.
   • Pathologist will recommend a protocol or recommend changes.

3. Select 50 to 60 cases whose results are known by FISH.
   • Must be compared to current system and outside facility for FISH studies.
   • Approximately 30% must be known positive by FISH
   • Approximately 60% must be known negative by FISH
   • Approximately 10-20% should be 2+ (IHC) cases with known FISH results

4. Send stained slides to pathologist for review.
   A. The level of concordance is considered acceptable for 95% of the cases or greater.
B. Pathologist will approve or recommend changes.
C. Approval will be documented and validation results will be posted and signed by the Medical Director.

5. Perform PT twice yearly.
6. Consider FISH correlation yearly for ongoing quality monitoring.
7. Pathologists reading HER2 or other predictive markers should review cases, criteria, etc. as necessary or at least on a yearly basis.

**Validation for ISH probe (non predictive, non FDA approved)**

1. Establish a protocol for the probe.
   A. Start a protocol by testing a (+) control with the following:
      - If this is a brand new probe (never used in this lab), discuss with pathologist what control tissue to use.
      - Vendor recommendation for Leica BOND 3 instrument. If a recommendation is made test 3 (+) control slides by staining 1 with the recommendation, 1 with increased HIER and 1 with decreased HIER. Example: Kappa recommendation is H1-20. 1st slide H1-20, 2nd slide H1-30 and 3rd slide H1-10.
      - If there is not a recommendation -- Call the Leica Field Support Specialist for a recommendation about that particular antibody. They will tell you what other facilities are using to guide you in the right direction. Again, test 3 (+) control slides. 1 with the recommendation, 1 higher and 1 lower retrieval.

2. Send stained slides to pathologist to choose which protocol to use.
   A. Pathologist will recommend a protocol or recommend changes.

3. Retrieve a known positive patient case (if applicable) and perform IHC ISH staining with the chosen protocol.
   A. If this is a brand new antibody, discuss with the pathologist appropriate patient tissue for use in validation.
      i. If this is a replacement probe, etc. then one patient case is sufficient, provided the results match the previous known positive and negative patient tissue.
      ii. If this is a new probe, more than one case may be needed at the pathologist's discretion.
      iii. Validation with tissue from another lab or staining at another lab may be performed at the pathologist discretion.
      iv. Choice of Strong and focal/weak cases is suggested if possible.

4. Send stained slides to pathologist for review.
   A. Concordance with patient tissue and control tissue (or expected staining) should be 100%.
   B. Pathologist will approve or recommend changes.
   C. Approval will be documented.

**CALCULATIONS:**
For concentrated antibodies 3 different dilutions may be used for the first step along with 3 different retrieval protocols.

**CALIBRATION:** None

**QUALITY CONTROL:**

**PROFICIENCY TESTING:** College of American Pathologists MK-IHC, Her2, and PM2 proficiency testing.
RESULTS: 

H:\Lab\Anatomic Pathology\Histology\IHC\Validation

PROCEDURE NOTES: N/A

LIMITATIONS OF PROCEDURE: Validation does not include wet slide mount cytological material fixed in 95% alcohol. Only tissue processed paraffin cell-blocks.

SPECIMEN: Paraffin embedded tissue blocks

MATERIALS, REAGENTS: + glass slides, Multi-tissue control blocks, IHC containers, detection kit

INSTRUMENTATION OR EQUIPMENT: Immunohistochemistry is done on the Leica BOND 3.

Form Name & Number or Attachment Name (If Applicable):
Histo21.01

Author Position:
Lead Histologist

Review/Revision Authority (Position Not Individual Name):
Lead Histologist

Expert Consultant Position/s (Not Individual Name/s):
N/A

References:

MANUFACTURER’S PACKAGING BROCHURE/INSERT: NONE.

Is there a Regulatory Requirement? Yes ☐ No ☐
If yes, insert requirement information here:

Review History (No Changes):
N/A

Revision History (Note changes in area under header):
N/A

Computer Search Words:
N/A

Policy, Procedure or Protocol Cross Reference Information:
N/A