Validation and Quality Control for Immunohistochemistry

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Presentation Goals

Upon completion of this presentation, participants will be able to:

1. Explain the term "Quality Control"
2. Discuss QC in the context of a "Quality System"
3. Explain how proper validation leads to quality
4. Outline an IHC Quality System
5. Institute Quality Improvement using QC data.

Presentation Outline

- Quality Management System
- CAP / CLIA Requirements
- Assay Design Specifications
- Assay Validation
- Quality Control
  - BREAK
- ER / PR / Her2 Validation
- Open discussion
Wall Street Journal, January 2008

Bad Cancer Tests Drawing Scrutiny
By ANNA WILDE MATHEWS
January 4, 2008
Thousands of breast-cancer patients may be getting the wrong treatment because of errors in two laboratory tests widely used to determine which drugs are prescribed.

New York Times, April 2010

Cancer Fight: Unclear Tests for New Drug
By SHIVA SIVAPRAKASH
Published: April 19, 2010
Dr. Linda Griffith was at a conference in Singapore in early January when she felt a lump in her breast. She assumed it was nothing — a cyst. And anyway, she had no time for it. She was returning on a Sunday night and the next Tuesday morning was leaving for a conference in Florida.

New York Times, July 2010

Prone to Error: Earliest Steps to Find Cancer
By STEPHANIE SKLAR
Published: July 16, 2010
Monica Long had expected a routine appointment. But here she was sitting in her new oncologist's office, and he was delivering deeply disturbing news.
“Bad Cancer Tests Drawing Scrutiny”

- Her2
  - When locally-tested cases were re-tested at a central lab:
    - 14-16% False Positive
    - 18 - 23% False Negative

- Estrogen Receptor
  - When locally tested cases were re-tested at a central lab:
    - 1.8% False Positive
    - 70% False Negative

Key References

- Quality Assurance For Immuncytochemistry: Approved Guideline
  - Clinical Laboratory Standards Institute (formerly NCCLS) publication MM4-A, Vol. 19, No. 26, 1999

- Quality Assurance in Anatomic Pathology (book)
  - Nakhleh and Fitzgibbons, Ed., College of American Pathologists, 2005

- College of American Pathologists (CAP)
  - General and Anatomic Pathology Accreditation Checklists
  - Current edition is dated June 15, 2009 (as of August 2010)

- Reference handout

Quality Management

- Quality Management
  - Definitions
  - Interaction
CAP Checklists: Quality Management

- General Laboratory Checklist:
  - GEN.13806 - 20369
    - Quality Management Program
    - Quality Monitoring
- Anatomic Pathology Checklist
  - ANP.10000
    - Is Quality Management Program Defined and Documented?

College of American Pathologists

- Three Definitions concerning Quality Management:
  - 1) Quality Assurance (QA)
  - 2) Quality Control (QC)
  - 3) Quality Improvement (QI)
- Together these constitute a "Quality System"
  - Interaction of these elements leads to better quality
  - System must be "worked" to succeed.

CAP Quality Assurance Definition

- 1) Quality Assurance
  - The practice of assessing performance in all steps of the laboratory testing cycle including pre-analytic, analytic and post-analytic phases to promote excellent outcomes in medical care.
... Deconstructed

• "...assessing performance..."
  • Requires a set of performance standards
  • Requires comparison of results against the standards

• "...to promote excellent outcomes..."
  • Requires action if standards are not met

CAP Quality Control Definition

• 2) Quality Control
  • An integral component of quality assurance consisting of the aggregate of processes and techniques to detect, reduce, and correct deficiencies in an analytical process.

... Deconstructed

• "...processes and techniques to detect, reduce, and correct deficiencies..."
  • "...Detect...":
    • Requires procedures that anticipate possible non-conformances.

  • "...Reduce and Correct...":
    • Leads to procedures that reduce or eliminate confounding results.
CAP Quality Improvement Definition

3) Quality Improvement
- The practice of continuously assessing and adjusting performance using statistically and scientifically accepted procedures.

… Deconstructed

- "...continuously assessing and adjusting..."
  - Requires regular review of results data
  - Investigation of, and correction of root cause
- "...statistically and scientifically..."
  - Data trends over a time period
  - Hypothesis of root cause
  - Eliminating variables
  - Testing solutions

Quality Management System Summary

- Quality Assurance
  - Describe the quality process
  - Set standards
- Quality Control
  - Institute methods to identify problems
  - Record and report results
- Quality Improvement
  - Track problem trends identified by QC results
  - Use trend information to correct problems
Validation Process

- Validation
  - Definitions
  - Processes

Validation and Quality Control

- Validation
  - Assay Design Specification
    - Verification
    - Optimization
    - Standardization

- Quality Control
  - Controls identified by validation
  - Standardized procedures
  - Identification of process deficiencies

Validation – Optimization - Standardization

Verification
Optimization
Standardization
CAP IHC Checklist

- ANP.
  - 12425 ASR disclaimer for report
  - 21850 Positive and Negative controls for immunofluorescence
  - 22250 Procedure for each antibody
  - 22300 Documented modifications for fixation other than formalin
  - 22550 Positive control use
  - 22570 Negative control use (tissue, reagent)
  - 22615 Avidin/Biotin blocking/controls
  - 22650 Review/Recording of IHC results
  - 22750 Validation of new antibody (except ER, PR, Her2)
  - 22760 New lot validation for Antibody and Detection system
  - 22800 Automated IHC staining instrument maintenance records
  - 22900 Quality of IHC stains
  - 22997 Her2 validation (IHC and ISH)

Validation Definition (1)

- Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a result or product meeting its predetermined specifications and quality attributes

Validation Definition (2)

- "Establishing documented evidence..."
  - Documentation of validation testing is readily available

- "...which Provides a high degree of assurance..."
  - Studying an adequate number of samples to give confidence that the new or changed process will work in your laboratory
<table>
<thead>
<tr>
<th>Validation Definition (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• &quot;...that a specific process will consistently produce...&quot;</td>
</tr>
<tr>
<td>• Identifying areas of actual or potential weaknesses so improvements can be made prior to implementation.</td>
</tr>
<tr>
<td>• &quot;...a result or product meeting it's predetermined specifications and quality attributes.&quot;</td>
</tr>
<tr>
<td>• Establishing acceptance criteria before initiating the validation study.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Introducing a New Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>• We want to introduce a new antibody...</td>
</tr>
<tr>
<td>• Who wants it?</td>
</tr>
<tr>
<td>• What is it to be used for?</td>
</tr>
<tr>
<td>• When will it be used?</td>
</tr>
<tr>
<td>• Where will it be used?</td>
</tr>
<tr>
<td>• Why do we need it?</td>
</tr>
<tr>
<td>• How will it be implemented?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial Assay Design Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Write a Specification:</td>
</tr>
<tr>
<td>• The need for the assay</td>
</tr>
<tr>
<td>• The &quot;why&quot;/&quot;s&quot;</td>
</tr>
<tr>
<td>• Identify special requirements</td>
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<tr>
<td>• Identify suppliers</td>
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<td>• Determine the expected results</td>
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<tr>
<td>• Determine the validation procedure</td>
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<tr>
<td>• Optimize the assay</td>
</tr>
<tr>
<td>• Develop the standard protocol</td>
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</tbody>
</table>
### Final Assay Design Specification

- **Final outcome:**
  - Validated reagent
  - Controls specified
  - Specify the expected results
  - Specify rejection criteria (tolerance)
  - “QC Data Sheet” to guide interpretation

### Total Test Validation

- **Pre-Analytic**
  - Acquisition, Fixation, Processing, Sectioning, Storage
- **Analytic**
  - Optimization (HIER, Dilution, time, detection, sensitivity, specificity)
  - Technologist training
  - Instruments
- **Post-Analytic**
  - Controls
  - Interpretation
  - Reporting
  - Pathologist performance

(Adapted from: Goldstein NS, et. al., Appl Immunohistochem Mol Morphol 15(2):124-133)

### Validation: Pre-Analytic

- **Pre-Analytic example:**
  - Acquisition: Range of time before sample is put in fixative
  - Fixation: Type and Range of time in fixative
  - Processing: Several processing schedules
  - Sectioning: Section thickness, drying, heating temperature
  - Storage: Range of time periods for unstained slide storage (retention of antigenicity), temperature
  - Range of block storage conditions
Validation, Analytic Phase Terms (1)

- CAP General Validation
- CAP GEN.42020-42163 Test Method Validation
  - Follows CLIA CFR Sec 493.1253
  - Does not apply well to IHC (IHC is usually qualitative)
  - But the general principle applies:
    - The laboratory must have data on each test's accuracy, precision, analytic sensitivity, interferences, and reportable range
    - Unmodified FDA-cleared or approved tests: the lab may use manufacturer information or published reports but lab must verify outside data.
    - Non-FDA cleared: Lab MUST verify or establish analytic accuracy, precision, sensitivity, specificity and reportable range.

Validation: Analytical

- Validate Accuracy with typical cases
  - Sensitivity
    - Expression range of Positive cases, low to high (10-15 cases)
  - Specificity
    - Positive versus expected negative cases/tissues (10-15 cases)
  - Determine best controls
    - Range of expression, similar to expected cases (normal or disease?)
    - Preferably acquired and processed in your institution

- Determine Precision (Reproducibility)
  - Intra-run: 10 slides in one run
  - Inter-run: 10 slides, Ten different runs with one slide each
  - Should have similar staining pattern and intensity on all slides

Validation Analytic Terms Applied to IHC

- Accuracy:
  - Compare results with New antibody to a previously validated antibody
- Precision:
  - Test samples with varying antigen expression
  - Intra-run, Inter-run tests, 10 slides each
- Sensitivity:
  - True Positive vs False Negative (higher % FN = less sensitive)
- Interferences (Specificity):
  - True Negative vs False Positive (Higher % FP = less specific)
  - What could interfere to give a false positive or negative result?
- Reportable Range
  - Establish a scoring system
  - Definition of a positive result
  - Criteria for rejection of the test
**Sensitivity**

- **Analytic Sensitivity:**
  - Lowest amount of substance detectable by the test
  - Can only be done with controls of known concentration

- **Diagnostic Sensitivity:**
  - Ability of the test to determine true diagnostic positive verses false negative (higher % FN = less sensitive)
  - Requires comparison to a previously validated antibody

- **IHC Sensitivity:**
  - Extent to which an antibody can be diluted and still achieve target recognition.
  - NOTE: This is determined by antibody AND detection system!

(adapted from: Theoretical and Practical Aspects of Test Performance, in Immunomicroscopy, Taylor & Cote, 2005)

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**Dilution series for Sensitivity (1)**

- Dilution series to determine sensitivity

  1:25 1:50
  1:100 1:200

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**Dilution series for Sensitivity (2)**

- Dilution series to determine sensitivity

  1:400 1:800
  NEG CONTL
Specificity

- **Analytic Specificity**
  - Accuracy on tests of known positive and negative controls
  - Controls of known concentration
  - Determine what could “interfere” to confound the result

- **Diagnostic Specificity**
  - Ability of a test to determine true diagnostic negative verses false positives (Higher % FP = less specific)
  - Requires comparison to a previously validated antibody

- **IHC Specificity**
  - Ability of an antibody to bind exclusively to its particular antigen in the absence of staining of other molecules
  - Or, staining of other structures in addition to target structures/cells

(adapted from: Theoretical and Practical Aspects of Test Performance, in Immunomicroscopy, Taylor & Cote, 2005)

Optimization Specificity

- “Non-specific” means background or staining irrelevant cells/structures

![Image of specific stain at 1:400 and 1:800 dilutions](image)

Validation: IVD Class I

- **IVD Class I, FDA exempt reagents**
  - Ancillary to one or more other tests
  - Confirm vendor specification
  - Determine optimal protocol (HIER, Dilution, etc)
  - Determine acceptable controls
  - Determine acceptable results
  - Test on a “sufficient” series of positive and negative tissues (ANP.22750)
Validation: Class II

- **IVD Class II FDA reagents**
- Predictive markers (ER, PR, Her2)
  - Confirm vendor specification
  - Determine optimal protocol
  - Determine acceptable controls
  - Determine acceptable results
  - Validate on mix of 20-40 (ER, PR) or 25 to 100 (Her2) or more known positive and negative cases
  - Compare to previously validated tissue samples

(ER, PR: adapted from Arch Pathol Lab Med. 2010; 134(6):930-935)
(HER2: adapted from Arch Pathol Lab Med. 2007; 131:18-43)

Validation: ASR

- **ASR: Analyte Specific Reagent (ANP.12425)**
- "Active ingredient" of a test
- Not validated by vendor
  - Vendor cannot indicate protocol to customer
  - Vendor cannot specify expected results
  - Laboratory is responsible for entire validation
    - Validation procedure (CAP checklist: Method Validation Gen.42020 - 42160)
    - Expected results
    - Statistically valid test cohort (number, type of cases)
    - Comparison to similar test (i.e., another antibody to same target)
    - Documented results

Validation: RUO

- **RUO: Research Use Only**
- FDA Says: Laboratories should not use RUO reagents.
- CLIA says: CLIA-certificated laboratories may use any reagent as long as CLIA validation procedures are followed (CAP is the deemed accrediting agency for Anatomic Pathology under CLIA)
- CAP says: Nothing in current checklist about RUO’s.
- If your laboratory decides to use an RUO reagent:
  - Document unsuccessful search for IVD/ASR reagent
  - Follow ASR validation procedures: Comprehensive validation.
  - CAP Method Validation checklist: Gen.42020 - 42160
Positive Control Tissue ANP.22550

- Ideally, same specimen type as case tested
  - Not always possible, so validation documents acceptable tissue
  - Remember, some antigens are decreased in tumor, not elevated
  - Normal tissue should have consistent level of antigen
    (However, is “normal” tissue really normal?)
- Fixed / processed with same procedures as sample
- Best practice: put on same slide as sample
  - However, one control per run, per antibody is acceptable.
- Internal positive controls may be used,
  - Document for which cases/antibodies it is acceptable
- Low expressers are ideal to avoid false negatives
  - Multi-tissue blocks with positive and negative tissues are ideal

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Negative Control Tissue ANP.22570

- Tissue that is known to lack the antigen
  - Multi-tissue blocks, may have positive and negative tissues
  - Tissue elements within the positive control or test samples that should be negative
  - Separate single negative tissue slide.

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Optimization Overview

- Verify Vendor Specifications
  - “to ascertain the truth or correctness of, as by examination, research, or comparison”
- Vendor literature is a starting point
  - Review the datasheet
  - Review references given by vendor
  - Literature search adds to knowledge
  - Compare with other vendors, users
- Verify/Optimize:
  - Antigen Retrieval
  - Dilution
  - Control tissue
  - Expected results
**Optimization Method**

- **Optimization**
  - Vendor-supplied protocols are recommendations
  - Ideally work it into your regular protocol
  - Antigen retrieval type (Try several)
    - None
    - Digestion (two or more types)
    - HIER (range of pH, temperature, time)
  - Dilution series (bracket vendor recommendation)
  - Detection efficiency
  - Chromogen efficiency
  - Staining method (Manual or Automated)

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**Multi-expression tissue**

- LOW
- MED
- HIGH

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**Optimization Result**

- Write an optimized procedure for each antibody, including:
  - .22250: Test Procedure
    - reagents, dilution, HIER, etc
  - .22300: Fixation
  - .22550: Positive Control
  - .22570: Negative Control
  - .22625: Avidin-biotin Block (if necessary)
Standardization

- **Inter-Laboratory**
- **Intra-Laboratory**

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Inter-Laboratory Standardization

- **Effort to standardize procedures for IHC**
  - Studies show large variations in procedures and results between laboratories (CAP, UK-NEQAS, NordiQC)
  - Long standing effort to promote standardization
    - Largely failed in US due to lack of consequences
    - UK-NEQAS has had success - took 20 years to achieve
  - Oncologists, due to variable Her2 results, are driving current efforts

- **Recommendations for Improved Standardization of Immunohistochemistry**
  - Appl Immunohistochem Mol Morph 2007 15(2);124-133

  - Arch Pathol Lab Med V.131 Jan 2007, pp.18-43.

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Standardization within the Lab

- **Standardization**
  - Research current literature for acceptable protocol and "best" results (also UK-NEQAS, NordiQC)
  - Once validated and optimized the procedures must be followed:
    - *Every time!!!*
    - *By everyone!!!*
  - Record and report deviations from procedure
    - Helps with troubleshooting
    - Train your staff to accept that mistakes or variations will happen, and admitting them is the first step of good troubleshooting.
### Validation: Post-Analytical

**Controls**
- Define correct Scoring / Interpretation of staining
- Define rejection criteria
- Define reporting parameters
- Document pathologist interpretation and performance
- Determine variations from standard over time
  - Reagent performance
  - Interpretation

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### Quality Control

**Processes and techniques to detect, reduce and correct deficiencies** in an analytic process.

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### Quality Control Interpretation

**Detect Deficiencies**
- Positive Control Tissue
- Negative Control Tissue
- Positive Control reagent (primary antibody)
- Negative Control Reagent
QC: Pos / Neg Tissue Controls

- Control Tissue
  - Positive Tissue Control
    - If True Positive: Proves the system works
    - Tests specific reactivity of primary antibody
    - Range of expression levels help determine sensitivity
    - Tests detection system
    - If Negative: indicates some part of the system did not work
      - No indication of what failed!
  - Internal positive control elements
    - Indicates True Positive staining
    - Indicates tissue quality (fixation, processing, storage effects)
  - Negative Tissue Control (negative for antibody)
    - Indicates specificity of primary antibody (cross-reactivity)
    - Indication of detection system problems (background, etc)

QC: Positive Reagent Controls

- Positive Reagent Control: Primary Antibody
  - On Positive Control tissue
    - Indicates primary antibody reactivity, specificity
    - Must be positive on the positive control tissue
    - Controls with range of expression will indicate sensitivity
    - Internal control on patient tissue help determine tissue reactivity
  - Negative control tissue must be negative (ANP.22570)
    - Internal negative elements (Positive control or sample)
    - Separate tissue block known to be negative for antibody
    - At least one negative tissue control per antibody

QC: Negative Reagent Controls

- Negative Reagent Control: (ANP.22570)
  - Replace positive primary antibody with:
    - Pre-immune serum from same animal (very rarely available)
    - Isotype-specific negative control antibody
    - Irrelevant primary antibody from same species (expensive)
    - Non-immune whole serum from same species (most common)
    - Antibody diluent only
    - Wash buffer only
  - Result: Must be negative on positive and negative tissues
    - Indicates specificity (cross reaction)
    - Indicates problems with detection system (non-specific binding)
    - Indicates problems with blocking reagents (not working?)
    - Indicates patient tissue problems (fixation, processing, etc)
### Negative Control Reagent Exception

**Special exception** (comment to ANP.22570):

- If running:
  - Two or more blocks from the same specimen,
  - Received at the same time,
  - Processed at the same time,
  - For the same antibody:
- Only need to run a negative control reagent slide on one block of that specimen.

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### Quality Improvement

**Quality Control Feeds Quality Improvement**

**QC Results:**

- Record results
- Track trends in deficiencies
- Open a Quality Improvement investigation for deficiencies identified.
- Determine a course of action to reduce or correct deficiency identified.

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### Quality Improvement: Reduce Deficiencies

**Pre-Analytic**

- Standardized Acquisition, fixation, processing
- Criteria for rejection of a specimen or sample

**Analytical**

- Procedures detail optimal test protocol
  - Must be followed by everyone to be effective

**Post Analytical**

- Interpretation guidelines
  - Acceptance criteria
  - Rejection criteria
"Correction" entails (for example)
- Do not perform test with inadequate specimen
- Repeating the test
- Determining what caused the failure
- Testing different processes
- Testing different reagents

Example of QC Trend

<table>
<thead>
<tr>
<th>False Negative</th>
<th>Background</th>
<th>Wrong tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue fell off</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Review trends at "defined" intervals
- Monthly? Quarterly? (GEN.20262)
  - Identify most common and most serious issues
  - Determine plan of action to identify root cause
  - Determine action to resolve
  - Test solution(s)
  - Determine if issue is resolved

Review Quality Assurance System (GEN.20369)
- Review annually and determine if system works
  - Determine if improvements are needed
Key Indicators

• GEN:20316:
  • Does the QM program include monitoring key indicators of quality?
    • Some are defined in the CAP checklist
      • Patient/specimen ID accuracy
      • Specimen Acceptability
    • Some identified by trend analysis.
      • i.e. Failure/Repeat rate of a particular test

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Estrogen and Progesterone Receptor

• ASCO/CAP Recommendations, ER, PR
  • VALIDATION of ER, PR:
    • Fitzgibbons, et. al., Arch Pathol Lab Med, June 2010
      Vol. 134(6):930-935
      • Tissue requirements
      • Validation methodology
  • TESTING OF ER, PR:
    • Hammond, MEH, et. Al., Arch Pathol Lab Med, June 2010
      Vol 134(6):907-922
      • Tissue procurement
      • Fixation (6-72 hours)
      • Testing
      • Interpretation and reporting
Estrogen and Progesterone Receptor Validation (1)

- **ASCO/CAP Recommendations**
  - Validate against previously validated tissue samples
    - Another laboratory that has validated its assay against clinical outcomes
  - Validated tissue samples from another lab that uses an FDA-approved assay and has validated the assay using ASCO/CAP testing requirements
  - Tissue samples validated using a separate assay (genetic, ligand binding assay)
  - Tissues used in a proficiency testing program
  - Validated tissues provided by an established program
  - ≥90% concordance with Positive validated samples
  - ≥95% concordance with Negative validated samples

Estrogen and Progesterone Receptor Validation (2)

- **ASCO/CAP Recommendations**
  - Initial test validation of FDA-cleared assays
    - ≥20 positive specimens (≥5 must be weakly positive)
    - ≥20 negative specimens
    - ≤ samples tested in one run (test samples in multiple runs, multiple operators)
  - Or Follow verification procedures in the Assay insert
  - Test must be used unmodified from manufacturers instructions
  - Initial test validation of Laboratory Modified Assays (LMA)
    - If the lab modifies the test in ANY way a more thorough validation is called for
      - ≥40 Positive specimens (≥10 must be weakly positive)
      - ≥40 Negative specimens

CAP Her2 Validation (1)

- **ANP.22997- Her2 test validation**
  - Wolff AC, et. al., Arch Pathol Lab Med 2007;131:18-43
  - 25 - 100 cases, mix of:
    - Variety of expression levels
    - Negative cases
  - Compare to a validated alternative method (one or more)
    - Other antibody
    - FISH
  - Fixation validation, if non-formalin
  - Validation of each change in methodology
**CAP Her2 Validation (2)**

- **ANP.22998** Documented procedure for length of fixation
  - 10% Neutral-buffered formalin (NBF)
  - Minimum 6 hours, maximum 48 hours (under review)
  - Record duration of fixation
  - Qualify negative results of specimens fixed over 48 hours, consider confirmatory FISH testing
  - Outside referral documentation of fixation duration

- **ANP.22999** Does lab use ASCO/CAP her2 scoring criteria?

**Other Controls for IHC**

- Tissue arrays
- Cell Culture slides
- Peptide spots

**Tissue Arrays as Controls**

- Tissue arrays allow extensive testing on one slide
  - Small arrays for particular antibodies
    - Lung, thyroid (pos), tonsil (neg) for TTF-1
    - Panomics Universal Array: 12 tissues: 90% IVD antibodies
      - Brain, tonsil, colon, lung, thyroid, uterus, prostate, breast ca, placenta, melanoma, thymus/thymoma, skeletal muscle
  - Larger arrays for validation
    - "FDA" array with 33 normal tissues, 40 tumors
      - Biochain, Panomics, others
    - 10's or 100's of Ca-types / mix for pos / neg validation
Array Size variety

- Variety of arrays sizes/composition

| Array Size | 24 | 65 | 95 | 150 |

Predictive Test Validated Arrays

- Her2, ER, PR, Validated arrays: 3+, 2+, 1+, Neg


Graduated Expression Arrays

- ER, PR, HER2

### Cell Culture Controls

- Genetically engineered expression level
  - Her2
  - Estrogen / Progesterone Receptor
  - Human papilloma virus
- Fixed, processed in formalin/paraffin
- Excellent for validation of tissue controls

### Cell Culture Control Stain

Positive control cells using no AR has more background in serum than when AR used

<table>
<thead>
<tr>
<th>Positive HER2 Cells W/O AR</th>
<th>Positive HER2 Cells W/ AR</th>
</tr>
</thead>
</table>

### Peptide Spots

- Peptide Spots (concept)
  - Peptide simulates epitope of native antigen
  - Peptides can be blended with several epitopes of different antibodies
  - Peptides do not degrade during deparaffinization and HIER
  - Peptides can be produced synthetically in infinite amounts, with identical quality
  - Can help detect changes in antibody dilution and HIER
Peptide Spots image

- Peptide spots are positive or negative

Peptide Spots vs Tissue Array

Top: Peptide spots (2 rows)

Middle: Cultured Cells

Bottom: Multi-tissue arrays with range of expressions

Peptide Controls in Practice

National HER2 Proficiency Test Results Using Standardized Quantitative Controls

Characterization of Laboratory Failures

- Peptide controls included with CAP 2006 Her2-B proficiency testing survey
  - 18.3% sub optimal staining as judged by peptide control
    - 35% due to HIER errors
    - 25% due to antibody or staining protocol
    - 45% due to combination of the two
- Vani K, et.al., Arch Pathol Lab Med. 2008;132;211-216
Quality Control is only part of the picture
- QC is part of the Quality Management System

Quality Control is dependent on proper Validation
- Validation determines protocols and controls

Quality Control feeds data to Quality Improvement
- Trend analysis identifies problems

Questions?

Thank you.
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:

### Sources of Input

<table>
<thead>
<tr>
<th>Company:</th>
<th>Labeled:</th>
<th>IVD</th>
<th>ASR</th>
<th>RUO</th>
<th>Clone/Animal host:</th>
</tr>
</thead>
</table>

| Company: | Labeled: | IVD | ASR | RUO | Clone/Animal host: |

| Company: | Labeled: | IVD | ASR | RUO | Clone/Animal host: |

| Title: | Reference: | Conclusion: |

| Title: | Reference: | Conclusion: |

| Title: | Reference: | Conclusion: |
### Validation Design Input

Describe the validation requirements

<table>
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<tr>
<th>Platform (circle one)</th>
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<th>Leica Bond</th>
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<td>Antibody</td>
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<tr>
<td>Antigen Retrieval method</td>
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<td>Primary antibody incubation time</td>
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<td>Chromogen</td>
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**Reproducibility testing**: None Inter-run (# slides___) Intra-run (# slides___)

<table>
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<tr>
<th>Tissue</th>
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<th>Positive element</th>
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### Additional testing required:

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

---

**Design Output: First Trial Evaluation of Antibody**

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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
- Medical Director, Immunohistochemistry Date
## Validation of Primary Antibody

### Optimization Instructions (First Pass)

<table>
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### Optimization Results

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### Optimization Instructions (Second Pass)

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### Optimization Instructions (Third Pass)

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### Optimization Results

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Validation of Primary Antibody

Optimized Procedure

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Control tissues:

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Attach list if extra control tissue necessary

<table>
<thead>
<tr>
<th>Approved by</th>
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Approved by | Medical Director, Immunohistochemistry | Date |

Approved by | IHC Lead Technologist                  | Date |
Reproducibility

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

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See attached test records

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

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See attached test records

Reproducibility approval

Reagent does / does not meet reproducibility criteria

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<tbody>
<tr>
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Validation of Primary Antibody

Design Validation

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<tr>
<td>Are test results on panel of normal and tumor tissues acceptable?</td>
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<tr>
<td>Are reproducibility tests acceptable?</td>
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Validation report:
Does reagent meet specification criteria?

Positive staining criteria:

Rejection criteria:

Comments:

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CoPath Entry

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</table>
Validation and Quality Control for Immunohistochemistry
NSH Symposium
Seattle, WA
September, 2010

References

Guidelines and Accreditation Checklists


College of American Pathologists, Commission on Laboratory Accreditation. College of American Pathologists, Northfield, IL, USA, www.cap.org
   September 27, 2007 Edition is the current edition in use (as of April, 2009)
   General Laboratory Checklist
   Anatomic Pathology Checklist


Books


Selected Literature

Validation and Standardization

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Recommended Policies for Uses of Human Tissue in Research, Education, and Quality Control, Grizzle, W, et.al., Arch Pathol Lab Med, 1999; 123: 296-300
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Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study, Mohsin, SK, et.al., Mod Path (2004) 17, 1545-1554


Technical Aspects of Immunohistochemistry, Ramos-Vara, JA, Vet Pathol 42:405-426 (20050


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Test Validation: A Brave new World for Anatomic Pathology, Brown, RW and Sharkey, FE, May 19, 2010, 2010 Laboratory Accreditation Program, College of American Pathologists (www.cap.org)

Quality Assurance / Quality Control


References


Quality control in immunohistochemistry: Experiences with the estrogen receptor assay, Bosmon FT, et.al., J Clin Pathol 1992;45:120-124

Audit and internal quality control in immunohistochemistry, Maxwell P and McIluggage WG, J Clin Pathol 2000; 53:929-932


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My approach to internal quality control in a clinical immunology laboratory, Lock RJ, J Clin Pathol 2006;59:681-684

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Breast Carcinoma-specific QC

Estrogen Receptor


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Her2 Specific QC


Concordance between central and local laboratory HER2 testing from a community-based clinical study, Reddy JC, et.al., Clin Breast Cancer 2006 Jun;7(2):153-7


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Implementation of American Society of Clinical Oncology/College of American Pathologists HER2 Guideline Recommendations in a Tertiary Care Facility Increases HER2 Immunohistochemistry and Fluorescence In Situ Hybridization Concordance and Decreases the Number of Inconclusive Cases. Middleton LP, et. al., Arch Pathol Lab Med. Vol. 133:775-780.

Controls

Tissue Array Controls


Assessment of Interlaboratory variation in the Immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray, Parker RL, et.al., Am J Clin Pathol 2002 May;117(5):723-8

References


**Miniature tissue microarrays for HercepTest® standardization and analysis**, Gulmann C, et.al., J Clin Pathol 2004;57:1229-1231


Validation and Quality Control for Immunohistochemistry

NSH Symposium
Seattle, WA
September, 2010

References

Cell Culture Controls

Her2

Available from
- Dako: www.dakousa.com, slides (available in kits only?)
- Ventana Medical: www.ventanamed.com, slides
- Invitrogen: www.invitrogen.com, slides or block available
- QC Sciences: www.qcsciences.com slides

Estrogen and Progesterone Receptor
- Dako (available in kits only?)
- Invitrogen

Human papilloma virus
- QC Sciences, slides
- Invitrogen, slides or block

Peptide Controls


_National HER2 Proficiency Test Results Using Standardized Quantitative Controls_, Vani, K, et.al., Arch Pathol Lab Med 2008;132:211-216

Quality Assessment Organizations

_College of American Pathologists (USA)._ www.cap.org,
Several quality management tools including Q-probes, Anatomic Pathology Surveys/IHC, Anatomic Pathology Education programs with IHC, HistoQIP program.

_UK National External Quality Assessment Scheme (UK-NEQAS)_.
www.ukneqas.clinic.ucl.ac.uk,
Of great interest are the downloadable pdf’s of the UK-NEQAS Immunocytochemistry Journal, which discusses in detail the results of the IHC surveys the UK-NEQAS conducts each year.
www.ukneqas.clinic.ucl.ac.uk/neckqascc.shtml

_Nordic Quality Control._ www.nordiqcc.org.
A quality control survey program of the Nordic countries. Extensive reviews of antibodies, procedures and suggestions for IHC.