



cap
Advancing Excellence

Laboratory Accreditation Test Validation:

Every
number
is a life.™

A Brave New World for Anatomic Pathology

Francis E. Sharkey, MD, FCAP
University of Texas Health Science
Center, San Antonio, TX

Richard W. Brown, MD, FCAP
Memorial Hermann Healthcare
System, Houston, TX

© 2010 College of American
Pathologists. Materials are used
with the permission of the faculty.

www.cap.org



Francis E. Sharkey, MD, FCAP



Richard W. Brown, MD, FCAP

Seminar Outline

Dr. Sharkey

- Validation concepts and terminology

Dr. Brown

- Validation and optimizing procedures
- Positive and negative controls
- Predictive markers

Dr. Sharkey

- Automated imaging systems

Validation Scenario

HER2 immunohistochemistry: After reviewing their performance on proficiency testing samples, the group of pathologists at this laboratory changed their scoring criteria to more closely match the ASCO-CAP guidelines. Do they have to revalidate their process?

Validation Scenario

In this laboratory, the controls for a digital image analysis system are run each day of use by the lab supervisor, and the analyses of patient specimens are performed by residents. The final interpretation is performed by the supervising staff pathologist.

Validation Scenario

An inspector asks for the lab's procedure for scoring HER2 immunohistochemistry slides and is shown the recently published ASCO-CAP journal article.

Validation Scenario

This laboratory is using an image analysis device to test for HER2 by IHC. The system has an option that allows the operator to override the machine scoring. The procedure manual describes how to activate the override, but does not describe the conditions under which override should occur.

Definition of Test Validation

Demonstration that a test system works in the manner in which it was intended.

Guidelines for Test Validation

- Use manufacturer's instructions
- Use high-quality test materials with known target values
- Test materials should be of similar type as patient samples
- Complete documentation, including procedure and results
- Lab director review/approve results

Qualitative vs. Quantitative Testing

- Qualitative test: Bimodal test result (e.g., positive/negative; high/low)
 - If the *amount* of analyte is relevant to the interpretation, then the test is quantitative
- Quantitative test: Continuous range of test results
- Fundamentally different validation procedures

Qualitative Test Validation

- Sensitivity: (+) condition = (+) test result
- Specificity: (-) condition = (-) test result
- Predictive value: Probability that condition = test result
- Example: Gram stain, mucin stain

Quantitative Test Validation

- Accuracy - test quantity correct
- Precision - test quantity consistent
- Linearity - direct relationship between analyte concentration and test quantity
- Reportable range - range of analyte concentrations that can be accurately measured
- Reference intervals - normal values
- Example: Er/Pr immunohistochemistry

Validation of Histochemical Stain

Questions to Ask Yourself

Does the change in test methodology affect:

- The Pathologist's ability to make the correct diagnosis? (*Accuracy*)
- The consistency with which excellent technical preparations can be produced? (*Precision*)
- Relationship between amount present and amount detected? (*Linearity*)
- Conditions in specimens, processing, or reporting that will interfere with the quality of the result? (*Analytical interferences*)
- How normal tissues will be interpreted? (*Reference range*)

What Does Validation Require?

- Repeat testing of (as applicable):
 - Different specimen types
 - Different specimen conditions
 - Different fixation and processing conditions
 - Different staining conditions
 - Different concentrations of analyte
 - Interpretation by different (& same) pathologists
- Laboratory Director must identify the conditions that validation must verify

FDA vs. Non-FDA Approved Systems

- FDA approved:
 - May use data from manufacturer or from published reports
 - Must verify Sensitivity, Specificity /Accuracy, Precision, Reportable Range
- Non-FDA approved:
 - Must establish accuracy, precision, analytic sensitivity, interferences & reportable range
 - *Includes modified FDA-approved systems!*

In Vitro Diagnostic Product (IVD)

FDA Definitions

- "...reagents...intended for use in diagnosis of disease...(in) specimens taken from the human body."
- For immunohistochemistry:
 - Class I: "...provide the pathologist with adjunctive diagnostic information..."
 - "...minimal potential for harm to the user."
 - Class II: "...provide prognostic or predictive data..."
 - "...mandatory performance standards..."

Analyte Specific Reagents (ASRs)

- Non-IVD diagnostic reagent
- Not bundled with other materials in a kit
- Not subject to FDA preclearance or special controls
- Product literature makes no claims regarding use or performance
- Examples: Hepatitis B, p504s

Validation of IHC Tests

(Dr. Brown)

- General concepts of IHC Validation
- Appropriate use of controls
- Antibody optimization
- Validation of new antibodies and reagent lots
- Validation of predictive markers
- ASCO/CAP Guidelines HER2

Validation of IHC Tests

- Antibodies and detection systems are FDA-approved for IVD use
- With rare exceptions (e.g. Herceptest) complete test systems are not FDA-approved
- Laboratory must validate the performance (accuracy, precision, sensitivity, specificity) of each antibody test

Elements of IHC Validation

- Begin with FDA-approved antibodies and detection system and single SOP
- Obtain test and control tissues fixed and processed identically to patient tissue
- Determine and document bimodal tolerance limits for the test
- Optimize primary antibody, then validate the test system

Elements of IHC Validation

- Any departures from routine (FFPE, 10% NBF) specimen require a separately validated procedure or documentation of equivalent staining
 - Alternative fixatives
 - Decalcified tissues
 - Frozen sections
 - Cytology preparations

Elements of IHC Validation

- Medical director discretion is expected
- Each validation must be individualized to reflect
 - The differential diagnostic applications of the antibody (keratin vs. tumor marker)
 - Availability of positive cases (e.g. ALK)
- Validation size must assure adequate sensitivity and specificity

Controls

- Fixed and processed according to routine laboratory protocol
- Tissues known to possess or lack the target antigen
- Positive controls should include low levels of antigen expression

Controls

- Tissue selection should address all differential diagnostic applications of the antibody (e.g. p63)
- Multi-tissue positive/negative controls represent best practice in validation and daily quality control

Tolerance Limits

- New concept for anatomic pathology
- Must be documented in procedure manual for each antibody
- What should and should not stain with the antibody and what represents an unacceptable result
- Applied in initial validation and in daily review of QC slides

Antibody Optimization

- Determine optimal dilution using “standard” antigen retrieval; begin with manufacturer's recommendation
- Test different methods that may improve antibody performance
 - Antigen retrieval techniques
 - Antibody incubation time
 - Enhancement techniques (e.g. heat)

Antibody Validation

- Must follow antibody optimization as a separate step
- Apply the optimized technique to a panel of positive and negative tissues; document sensitivity and specificity
- Precision is established by repeated staining of same positive/negative control (10 x 2 protocol)

Performance Assessment

- New reagent lots must be validated before they are placed in clinical use
- Proficiency testing (PT programs, tissue exchange)
- Daily review of positive and negative controls
- Any changes to the procedure require a new validation

Validation of Reagent Lots

- The performance characteristics of all new reagent lots (enzyme, antibody, detection system) must be assured prior to use on patient tissues
- Serial sections from a small number of pos/neg tissues are stained in parallel using old and new lots

Daily Quality Control

- Includes a positive and negative tissue control for each antibody
- Each case/block requires a negative reagent control using most stringent retrieval technique
- Control sections (+/-) on each slide represent best practice; batch controls are acceptable if available to all

IHC Predictive Markers

- Antibody staining results used to predict response to therapy (e.g. ER, HER2)
- Requires a more rigorous validation as
 - Treatment decisions are made based directly on the results
 - The results are quantitative
- LAP currently has specific validation requirements only for HER2

ASCO/CAP Guidelines HER2

- Initial validation must be performed before test is offered
- 25-100 samples tested by validated method in same or different laboratory
- 95% concordance in (+) and (-) categories required
- Ongoing validation should be done biannually

HER2 Validation

- Possibilities for initial validation
 - IHC vs. FISH in the same laboratory
 - IHC vs. IHC or FISH in a different laboratory with documented validation
 - PT materials in which the vendor provides assessment by an approved laboratory
- “Biannual” validation
 - Audit percent positive at least annually
 - Periodic comparison with validated assay

ASCO/CAP Guidelines HER2

- Ongoing internal QA procedures
- Participation in external PT (2 testing events/year, 90% correct)
- Current accreditation by a valid agency, including biennial on-site inspection
- Specify 10% NBF 6-48 hours; alternative fixatives/times must be validated with 95% concordance

HER2 QA Procedures

- Ongoing quality control using standardized materials (4 level)
- Any changes to the procedure require revalidation
- Ongoing competency assessment of pathologists with 95% concordance
- Documented training and competency assessment of testing personnel

IHC Validation - Summary

- Validations must be appropriate to the testing performed; no “standard”
 - Medical director discretion is required
- All methods and antibodies must be validated and addressed in the procedure manual prior to clinical use
 - Validations must be documented
 - No variations without separate validation

Digital Image Analysis

(Dr. Sharkey)

- New terminology
- Validation procedure
- Personnel issues
- Reporting of patient results

New Terminology

- Calibration
- Matrix
- Calibration verification
- Analytical measurement range
- Controls

Calibration

- Ensures that instrument settings yield an appropriate test result.
- Settings include such parameters as:
 - Color and intensity of light
 - Cleanliness of optical equipment
 - Sensitivity of light detector
 - Stage manipulator
 - Software settings for detection of analyte

Matrix

- Environment in which the substance to be measured is embedded
- May affect the test result
- Examples (for a tissue specimen):
 - Inflammatory and stromal cells
 - Hemorrhage / necrosis / fibrosis
- Procedure must identify what aspects of specimen matrix need to be controlled

Calibration Verification

- Periodic assurance that calibration settings yield a correct test result
- Calibration verification materials should have same matrix as patient samples
 - Prefer previously assayed patient samples
- Failure of calibration verification requires recalibration

Analytical Measurement Range

- The range of analyte values that can be directly measured by test system
- To validate, test specimens at low, middle, and high end of range

Frequency of Calibration / Calibration Verification / Analytical Measurement Range

- Change of major system components, including new reagent lots
- QC fails to meet established criteria
- Major maintenance or service
- When recommended by manufacturer
- At least every 6 months

Controls

- Tested each day of patient sample testing
- More than one level (high/low; pos/neg)
- Tested in same manner as patient samples
- Tested by same person(s) as test patient samples
- Results of controls verified before reporting patient results
- Reviewed at least monthly to detect trends

Personnel

- System operator qualified for high-complexity testing
- Supervisor:
 - Qualified for high-complexity testing
 - Trained by qualified lab director for 1 year
- Lab Director or designee:
 - Review of procedure manual
 - Review of validation, calibration and PT records
 - Monthly review of QC records

Result Reporting

- Correlation with other studies (H&E, special stains, etc.)
- Details of reagents, methodology, instrument, etc.
- Name and address of lab where:
 - Image analysis was performed
 - Final interpretation was performed
- Interpretation by the pathologist

Validation Scenario

- HER2 immunohistochemistry: After reviewing their performance on proficiency testing samples, the pathologists at this laboratory change their scoring criteria to more closely match the ASCO-CAP guidelines. Do they have to revalidate their process?
- Answer: Yes!

Validation Scenario

- In this laboratory, the controls for a digital image analysis system are run each day of use by the lab supervisor, and the analyses of patient specimens are performed by residents. The final interpretation is by the supervising staff pathologist.
- Answer: Controls must be run by operators.

Validation Scenario

- An inspector asks for the lab's procedure for scoring HER2 immunohistochemistry slides and is shown the recently published ASCO-CAP journal article.
- Answer: "Package insert" rule - Lab must detail and document its own procedure.

Validation Scenario

- This laboratory is using an image analysis device to test for HER2 by IHC. The system allows the operator to override the machine scoring. The procedure manual describes how to activate the override, but does not describe the conditions under which override should occur.
- Answer: Conditions must be in procedure.

Final Summary

- New challenges:
 - Terminology
 - Quantitative testing
 - Predictive markers
 - Automated instrumentation
- Regulatory environment
 - FDA approval
 - Proficiency testing

Assistance

<http://www.cap.org>

Email: accred@cap.org

800-323-4040, ext. 6065