Using LEAN to Improve Quality, Patient Safety and Workflow

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Sioux Falls, SD
Disclosure

Presenter has no conflicts of interest in this presentation and endorses no vendor.
Expectations:

- Identify how process Improvement methods can lead to improved outcomes.

- Learn how the application of LEAN principles reduces opportunities for errors.

- See how automation and LEAN complement each other in histology.
Who Are We?
McKannan Hospital and University Health Avera Center

- 510 bed facility located in Sioux Falls, South Dakota
- More than 3,100 employees; 18,000 discharges; 265 employed physicians in 26 specialties and 73 mid-level practitioners located in 50 sites
- Member of Avera Health, a regional healthcare family of more than 100 healthcare facilities in the five state region of South Dakota, North Dakota, Minnesota, Iowa and Nebraska
Base Conditions in Histology
(Where We Started)
Space and Product Flows

- Like most Histology Labs, space was added in a random fashion as it was needed and became available with little attention to flow.

- Specimen/Product flow did not follow the most efficient and effective route due to layout constraints.
Pre-Lean Histology
H & E Slides/Tech/Yr

2002: 8,831
2003: 8,413
2004: 8,020
2005: 9,459
2006: 13,009
2007: 14,179

Linear (Slides/Tech)
Average Surgical TAT
(spec. registration to sign out)

- **Base Condition:**
  - Group Average = 44.5 Hours
    - Range - 23 to 104 hours
    - Time reflects all cases average

- Specimens accessioned upon arrival on day 1. Processed overnight

- Techs start at 0400 to embed, cut and stain, coverslip on day 2. Usually only 1 or 2 remain after 12:30 PM
CURRENT STATE
Equipment Selection

- Sakura Express Rapid Processor
  - 1 and 2 hour protocols in use
- Sakura VIP Conventional Processors
  - Overnight processing for selected tissues
- Conventional Embedding Centers
- Ventana Symphony H&E Stainer
  - Issues with reliability
- Sakura Linear Stainer - backup became primary
Product/Operator Flow

- Cases are processed singly through the grossing step.
- Processed in small batches on the Sakura Express.
- Embedded, Cut and mounted in single case flow to reduce errors.
- Final step is block check to ensure quality and error detection.
- Product Flow is now unidirectional.
Post Lean Workflow
Unique/Innovative Features

- Hazardous Solvent Disposal System
  - Waste from recyclers placed down a dedicated sink to a solvent collection center for removal.

- Recycling Layouts
  - Recycling of Alcohol and Xylene

- IHC/FISH Layout
  - Self supporting section – offers >100 markers

- Transcription
  - Home based transcription - EMR
Post Lean Performance Improvement

- Redistributed Staffing Matrix
  - 2 Lab Technical Assistants for Gross Assistance as well as other support duties.
  - Histology Technical Staff does technical duties plus bone marrow/FNA slide preparation – quality step.
  - Slides/histotech increased to >14,000 per year
Post Lean Improvements (cont).

- Linear workflow means markedly reduced walking and time waste.

- Block Check/Quality Check means less re-work and higher quality.

- In excess of 65% of all specimens are processed using rapid processing.
Current Pathology Turn Around Times

Current Condition (as of 6/08)

- Group Average = 27.3 Hours
  - Range - 6 to 48.5 hours

Receipt into Histology to Sign-Out by Pathologist
Time reflects all cases average
Selected cases available same day as received
Summary

- Lean design improved workflow
- Lean plus Automation allowed for greater productivity with less stress.
- Lean design improved total Pathology Turn Around Time.
- Staff satisfaction is at its historical highest.
  - Both technical staff as well as Medical Staff.
Pearls of Pathology

- Improve processes THEN automate.
- Use improved processes to design area.
- No need to have pathologists read at night if no action is to be taken until morning.
- Look at TOTAL processes not just technical processes.
- Applying Lean Principles takes patience
Questions??

Thank You!!
Syllabus

The most important component in a successful quality assurance and improvement program is a commitment to the quality principle of continuous quality improvement. This is heavily dependent on a department’s ability to collect data without bias and be able to trust the data sufficiently to make changes. Quality assurance and improvement has its edges and some of its core elements imperceptibly intertwined with laboratory management. This is best demonstrated by understanding the cycle of Plan-Do-Study-Act which is emblematic of QA. Traditional QA programs represent the “Study” aspect of this cycle. To make improvements, successful QA programs repeatedly use the entire cycle of “Plan-Do-Study-Act”. As such, the director of quality assurance and the director of the laboratory need to be on the same page, since QA data is likely to drive a good number of management decisions.

Inherent in the QA cycle is the ability to critically analyze processes and redesign them with policies and procedures that reduce errors. In this continuous assessment and redesign, quality checks must be built into processes so that if errors occur they are detected at the earliest possible point. Adopting these principles also addresses patient safety. Simply defined patient safety may be defined as “freedom from accidental injury” but Joint Commission requirements have expanded that definition to “ensuring patient safety involves the establishment of operational systems and processes that minimize the likelihood of errors and maximize the likelihood of intercepting them when they occur.”

The traditional role of quality assurance may be defined as the function of measuring the quality of a laboratory. In regards to anatomic pathology, a quality product is best defined as an accurate, complete and timely report. Therefore, quality assurance programs must measure report accuracy, completeness and timeliness. The core components of quality assurance and improvement should include active monitors in all the disciplines within anatomic pathology, i.e. surgical pathology, cytopathology, autopsy pathology as well as any number of specialty diagnostic laboratories, among them; electron microscopy, immunohistochemistry, immunofluorescence, and molecular testing.

The existence of a QA program is mandated by the Laboratory Accreditation Program’s standards ANP.10000; “Is the quality management program defined and documented for surgical pathology?” and GEN.13806; “Does the laboratory have a documented quality management (QM) program?” Defining a quality management program is best done in a quality management plan that includes:
1. A quality assurance committee with a clear charge,
2. An assessment of the risks facing the laboratories.
3. Individuals responsible for the various monitors of the plan,
4. A list of study monitors that address laboratory risk with a time table for evaluation and discussion of the results.
5. Inclusion of monitors that cover the entire test cycle (pre-analytic, analytic, post-analytic), turn-around times and customer satisfaction.
6. Defined working relationships with other departmental and institutional QA and other committees.
7. Annual review of the plan.

Selection of study monitors is dependent on multiple factors including:
1. Laboratory accreditation requirements.
2. Institutional accreditation requirements
3. Prevalent and persistent problems
4. Satisfying institutional concerns
5. Resources

Numerous monitors are mandated for accreditation; for example CLIA-88 mandates the majority of the QA monitors in cytopathology. The CAP’s LAP mandates that monitors cover the pre and post-analytic phases of the test cycle. A number of standards also mandate a specific turn-around time for specimens including frozen section results, surgical specimens, autopsy reports and cytology.

Occasionally, there are monitors that satisfy multiple factors. A good example is specimen identification. The Joint Commission has placed heavy emphasis on patient identification which includes specimen identification. This is an opportunity to address a pre-analytic problem that is prevalent and persistent and has become an institutional accreditation concern. Addressing specimen identification also requires working with other departments and enhances better integration of pathology QA activity with other departments.

The LAP standard GEN.20368 states “Have referring physicians' or patients' satisfaction with laboratory service been measured within the past 2 years?” Customer satisfaction surveys are central in the determination of quality of most products in a host of industries. In some instances it is the only determinant of quality. In pathology it is a relatively new tool, although, many pathologists know too well the importance of clinician’s happiness with pathology reports and services. Surveys are particularly helpful when changes or new services are introduced.

Most important in selecting monitors is the choice of an analytic monitor and the method for determining an error rate. Nearly all laboratories monitor frozen section – permanent section discrepancies as well as cytology – histology discrepancies. However, there is no agreement on the best method for determining diagnostic error of the final diagnosis. Follow up is the ultimate judge of diagnostic error, but this is impractical as a QA monitor. By default, peer review has become the standard for judging diagnostic error. But there is no agreement on the best method to review cases. There is also no evidence to demonstrate that one type of review is superior to others. It is difficult to estimate the actual diagnostic error rate without expert second review of all of the cases. This is, however, impractical and time consuming and is only performed in a few laboratories. Most laboratories use multiple methods of directed case reviews such as review of cases for conferences. Many use amended reports for revised diagnosis as a surrogate measure of diagnostic error.

One important aspect of monitors is to set a reasonable range for results while at the same time try to drive improvement. Expectation for QA results should be set by examination of
benchmarks that may be available in the literature. In using published benchmarks, pathologist should be careful to use the same measurement methods.

Multiple new aspects of quality assurance are being introduced and mandated by accrediting agencies including:
1. Proficiency testing in anatomic pathology (cytology, HER2),
2. Maintenance of certification,
3. Emphasis on actual improvement,
4. Emphasis on extra-departmental communication
5. Patient safety and error prevention

In the short term, this has increased the interest in QA programs. In the long term, it is unclear how these new demands will impact the practice of pathology. It is clear that tolerance for the status quo is diminishing as more and more groups and institutions demand improvement.

Reference:

Core Components of a Comprehensive Quality Assurance Program in Anatomic Pathology

Raouf E. Nakhleh, MD, FCAP
Mayo Clinic, Florida
Disclosure

• None
Learning Objectives

• Address basic concepts of quality and patient safety
• Present the core components of a traditional QA plan
• List the test cycle segments and global measures that should be monitored in a QA program
• Understand new points of emphasis in quality assurance
Agenda

- Overview
- Core components
- QA monitors
- Points of emphasis
Overview

• Quality assurance program
  – A system to monitor the quality of complex processes

• Quality in Anatomic Pathology
  – Accurate diagnosis
  – Complete report
  – Timely delivery
Overview

• Quality management
  – Integration of quality principles into the core values and philosophy of an institution
  – Integration of quality principles and quality monitors into the design of processes
Overview

• Patient safety
  – Renewed focus with JC and CAP goals
  – Addressed within the QA program

• Definition: Freedom from accidental injury; ensuring patient safety involves the establishment of operational systems and processes that minimize the likelihood of errors and maximize the likelihood of intercepting them when they occur.
Quality by Design

• Commitment to quality principles
  – Continuous quality improvement
  – A Systems approach
• Critically analyzing processes and redesigning them to reduce errors
• Build quality checks into processes so that errors are detected at the earliest possible point
Core Components

- Quality Management Plan
  - Quality assurance committee with a clear charge
  - Individual responsibility
  - Risk assessment
  - Monitors with a time table for reporting
  - Relationship to other institutional Quality Programs
  - Patient safety
  - Annual review
Risk

• The Doctors Company
• 166 (61%) false negative
• 73 (27%) false positive
• 10 (4%) frozen section
• 22 (8%) operational
  – 13 mix-ups
  – 3 floaters
  – 2 mislabeled biopsy site
  – One transcription error, “no” omitted before malignant cells
Core Components

• Address all disciplines
  – Surgical pathology
  – Cytology
  – Autopsy

• Address all laboratories
  – Gross room
  – Histology
  – Immunohistochemistry
  – Molecular
  – Electron microscopy
QA Monitors

- Pre-analytic
- Analytic
- Post-analytic
- Turnaround time
- Customer satisfaction
Factors in Selecting Monitors

- Laboratory accreditation requirements
  - CLIA-88, LAP
- Institutional accreditation requirements
  - JC Patient safety goals
- Prevalent and persistent problems
  - Specimen identification
- Satisfying institutional concerns
- Resources
Pre-analytic

- Specimen identification
- Specimen collection
- Specimen labeling
- Specimen fixation
- Specimen transport
- Accessioning
Analytic

- Grossing
- Sectioning and Block labeling
- Tissue processing
- Embedding
- Cutting and slide labeling
- Microscopic interpretation
- Special stains
- Immunostains and other ancillary testing
Analytic

- Frozen section – permanent section correlation
- Cytology – histologic correlation
- Final diagnostic errors
  - amended reports
Post-Analytic

- Communication of significant diagnoses
- Gross and microscopic dictation
- Transcription
- Verification and finalization
- Report completeness
- Report delivery (electronic and paper)
- Comprehension
Turnaround Time

• Frozen section
• Small biopsies
• Large resections
• Autopsy: GAD, FAD
• Cytology: GYN, non-GYN

• Receipt of specimen to report delivery
Customer Satisfaction

- Overall satisfaction
- Diagnostic accuracy
- Frozen section timeliness and accuracy
- Report timeliness
- Report completeness
- Pathologist availability
- Recent changes
Customer or Clinician Satisfaction

- Opportunity to monitor and manage expectations
- Opportunity to understand institutional concerns
- Opportunity to inform and educate
- Opportunity to integrate and be recognized more fully as a service within the larger institution
Measuring Quality

• Benchmark
  – Performance standard most commonly determined by literature

• Trending
  – Performance of laboratory over time
Points of Emphasis

- Proficiency testing
  - HER2
  - Cytology
- MOC?
- Emphasis on continuous improvement
- Emphasis on extra-departmental communication
- Emphasis on patient safety and error prevention
Summary

• Tried to demonstrate the need to integrate quality and patient safety principles into every day processes
• Brief overview of the core components of a comprehensive quality assurance program
• Need to demonstrate improvement
Questions?
Thank you for participating!

Please be sure to complete the course evaluation online after the conference.
Patient Safety and Next Steps for Improving Quality and Service in Surgical Pathology

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Disclosure

• No conflicts of interest relevant to this presentation
Patient Safety and Next Steps for Improving Quality and Service in Surgical Pathology

Objectives

At the end of this presentation attendees will understand

• the problem of patient safety in surgical pathology, and

• practical solutions for addressing safety and quality problems
“The goal of this report is to break the cycle of inaction. . . . Despite the cost pressures, liability constraints, resistance to change and other seemingly insurmountable barriers, it is simply not acceptable for patients to be harmed by the same health care system that is supposed to offer healing and comfort.”
Six Specific Aims for Improvement

Health care should be:

• Safe
• Effective (i.e. avoiding underuse and overuse)
• Patient-centered
• Timely
• Efficient (i.e. avoiding waste)
• Equitable
U-M lab cancer mistake verdict upheld

In 2003, a laboratory mix-up at the University of Michigan Hospitals caused Theresa Karwoski’s doctor to incorrectly diagnose her with breast cancer.

Karwoski went through a partial mastectomy, removal of seven lymph nodes, drugs and testing to fight a cancer she didn’t have.

In February, a jury awarded the 51-year-old Ann Arbor woman and her husband more than $3 million in damages in a lawsuit filed against the medical center. The verdict was upheld in the court of claims by Washtenaw Circuit Judge Timothy F. Connors, who late last month ordered U-M to pay the Karwoskis’ attorney’s fees and interest.

“$3 million in damages . . . attorney’s fees and interest.”
Gambling with Your Life

Millions of medical mistakes happen in the lab. Here’s how to protect yourself.

By Pamela F. Gallin, MD, and Joseph K. Vetter

Lenore Janecek was headed toward her Chicago home on a September afternoon in 2000 when she received a call on her cell phone that would change her life forever. It was her doctor. He told her that the test results from her routine colonoscopy two weeks earlier revealed she had intestinal cancer. Stunned, Janecek, 61, pulled over. “There must be a mistake,” she insisted. But the doctor, a gastroenterologist, assured her there was no mistaking the diagnosis. Janecek would need immediate surgery.

There are few things more dreaded than a cancer diagnosis. But for Janecek, the news was doubly traumatic: She had been successfully treated for intestinal cancer ten years earlier, so the thought that the disease had come back was terrifying.

On September 26, in a procedure that lasted three hours, the surgeon made an incision running the length of
Types of Error

**active** errors — occur at the level of the frontline operator

Types of Error

**active** errors — occur at the level of the frontline operator

**latent** errors — tend to be removed from the direct control of the operator (e.g. poor design, poorly structured organizations, etc.)
Types of Error

**active** errors — occur at the level of the frontline operator

**latent** errors — tend to be removed from the direct control of the operator (e.g. poor design, poorly structured organizations, etc.)

the “blunt end” of complex health care system

modified from Woods *et al.*: *Behind Human Error: Cognitive Systems, Computers and Hindsight.* Crew Systems Ergonomic Information and Analysis Center, WPAFB, Dayton, OH.
Every system is perfectly designed to achieve exactly the results it gets.

Don Berwick’s “Central Law of Improvement”
preventable misdiagnosis that results, or has the potential to result, in patient harm
Error Rates in Anatomic Pathology
Diagnostic Disagreement as a Measure of Error

Prospective/Blinded Re-Review
(Significant errors – 0.3%-1.2%)

Total disagreements
Significant errors

Whitehead et al 1984: 1.0%
Safrin and Bark 1993: 0.3%
Lind et al. 1995: 1.2%
Weydert et al 2005: 0.3%
Renshaw and Gould 2006: 0.8%
Error Rates in Anatomic Pathology
Diagnostic Disagreement as a Measure of Error

Interinstitutional Re-Review (“consults”)
(Significant errors – 1.4%-5.8%)

- Abt et al 1995: 5.8%
- Kronz et al 1999: 1.4%
- Weir et al 2003: 3.7%
- Tsung 2004: 3.8%
Error Rates in Anatomic Pathology
Diagnostic Disagreement as a Measure of Error

Retrospective Re-Review
(Significant errors – 0.4%-3.2%)

- Total disagreements
- Significant errors

Focused subspecialty based review

5% random review

Whitehead et al 1986: 2.1%
Ramsay et al 1992: 1.2%
Raab et al 2005: 1.0%
Raab et al 2008: 0.4%
Raab et al 2008: 3.2%
Error Rates in Anatomic Pathology
Impact of “Major” Diagnostic Disagreements*

Impact on Patient Care | Random Review (n=26) | Focused Review (n=10)
--- | --- | ---
No harm/ near miss | 15 (58%) | 3 (30%)
Minimal harm | 5 (19%) | 7 (70%)
Mild harm | 4 (15%) | 0
Moderate harm | 2 (8%) | 0
Severe harm | 0 | 0

No, minimal, mild harm = 34/36 (94%) cases
(0.4% of 7,824 cases reviewed)

Moderate, severe harm = 2/36 (6%) cases
(0.03% of 7,824 cases reviewed)

Error Rates in Anatomic Pathology
Measuring Frequency of Diagnostic Discordance


Raab et al. Arch Pathol Lab Med 2005
Error Rates in Anatomic Pathology

- Patient and/or clinician generated (18)
  - 2 errors
  - 33% error rate
  - 6 errors followed up

- Pathologist generated (55)
  - 8 errors
  - 57% error rate
  - 14 errors followed up

- Incoming (35)
  - 15 errors
  - 79% error rate
  - 19 errors followed up
“. . . diagnostic disagreement is not the same as error.”

Error Rates in Anatomic Pathology
Amended Reports as a Measure of Diagnostic Error*

Amended Report Frequency (per 1,000 cases) by Year

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
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<td>Frequency (per 1,000 cases)</td>
<td>2.8</td>
<td>2.6</td>
<td>3.4</td>
<td>4.8</td>
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</tbody>
</table>

*from Meier et al. Am J Clin Pathol 2008; 130: 238-46
Errors in Medicine
Application to Surgical Pathology*

Diagnostic
✓ Error or delay in diagnosis
✓ Failure to employ indicated tests
✓ Use of outmoded tests or therapy
✓ Failure to act on results of monitoring or testing

Treatment
Preventive
Other
✓ Failure of communication

Objectives

At the end of this presentation attendees will understand

• the problem of patient safety in surgical pathology, and
• practical solutions for addressing safety and quality problems
Designing for Safety in Health Care
IOM Committee on Quality of Health Care in America

• Provide leadership
• Respect human limits in process design
Respect Human Limits in Process Design

- avoid reliance on memory
- use constraints and forcing functions
- avoid reliance on vigilance

- provider order entry
- standardized workflow
- prespecified targeted review enforced by LIS

- bar code/RFID driven patient-specimen ID
- standardized results reporting
- real time monitoring of diagnostic trends
Respect Human Limits in Process Design

- avoid reliance on memory
- use constraints and forcing functions
- avoid reliance on vigilance
- simplify key processes
- standardize work processes
The Henry Ford Production System
Measures of Process Defects and Waste in Surgical Pathology as a Basis for Quality Improvement Initiatives

Designing for Safety
Application of Lean Tools


Visual data display poster showing data entries by pathologists that captured detail on misidentifications arising in the surgical pathology process.
• Provide leadership
• Respect human limits in process design
• Promote effective team functioning
Medical Errors – “Very Important” Causes

- insufficient time spent with patients
  - MDs: 37%
  - Public: 72%
- overwork, stress, fatigue
  - MDs: 50%
  - Public: 70%
- failure to work together or communicate as team
  - MDs: 39%
  - Public: 67%
- understaffing of nurses
  - MDs: 53%
  - Public: 65%
- complexity of care
  - MDs: 38%
  - Public: 62%
Designing for Safety in Health Care

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<tr>
<th>Patient Safety Culture Composites</th>
<th>Average % Positive Response</th>
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<tr>
<td>1. Teamwork Within Units</td>
<td>79%</td>
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<tr>
<td>2. Supervisor/Manager Expectations &amp; Actions Prompting Patient Safety</td>
<td>75%</td>
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<td>3. Management Support for Patient Safety</td>
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<td>4. Organizational Learning-Continuous Improvement</td>
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<td>5. Overall Perceptions of Patient Safety</td>
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<td>6. Feedback &amp; Communication About Error</td>
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<td>7. Communication Openness</td>
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<td>8. Frequency of Events Reported</td>
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<td>9. Teamwork Across Units</td>
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<td>10. Staffing</td>
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<td>11. Handoffs &amp; Transitions</td>
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<td>12. Organizations Response to Error</td>
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web-based service team calendars
Designing for Safety in Health Care

Communication

INTRANET
Forms

Faculty Forms
- Absence Form
- Change of Address
- Effort Reporting Form
- Faculty Disclosure
- Travel Expense
- Travel Request

Resident Forms
- Absence Form
- Autopsy Form
- Change of Address
- CME Request
- Resident Evaluations
- Travel Expense
- Travel Request

Staff Forms
- Change of Address
- PA Evaluation Form
- Travel Expense
- Travel Request
- Clinical Laboratory Travel Request

Clinical Forms
- Amended Diagnosis QA/QC Review
- Clinical Conference QA/QC Review
- Fire Critique Form
- Occurrence Report
- Patient Safety
- SBARC Communication Form
- Slide and Block Request Form
- Specimen Procurement
- Surgical Pathology 1p/13q Send Out
- Surgical Pathology Problem Resolution
- Second Room Accessioning

Research Forms
- AP Research Application
- NIH Grant Forms
- Research Supplies Requisition

SBARC Communication Form

- Accession #
- Patient:

- Situation:

- Background:

- Assessment:

- Recommendation:

- Calibrate:

- Requestor:

- Email:

- Anatomical Pathology
- Clinical Pathology

- Assigned to:

Submit Form
Designing for Safety in Health Care
Communication
web-based service team calendars

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<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
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<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
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<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
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<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
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<td>30</td>
<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
</tr>
<tr>
<td>31</td>
<td>2:00 PM-3:00 PM ROOM 1:MATT #13634</td>
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</tbody>
</table>

- 1: Office setting
- 2: Office setting
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- 10: Office setting
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- 20: Office setting
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- 22: Office setting
- 23: Office setting
- 24: Office setting
- 25: Office setting
- 26: Office setting
- 27: Office setting
- 28: Office setting
- 29: Office setting
- 30: Office setting
- 31: Office setting
• Provide leadership
• Respect human limits in process design
• Promote effective team functioning
• Anticipate the unexpected
Error Rates in Anatomic Pathology
Impact of Prospective Peer Review

Anticipate the unexpected

### Communicating (“Critical”) Diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Routine report delivery</th>
<th>Phone call within 24 hrs</th>
<th>Phone call ASAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasculitis</td>
<td>14%</td>
<td>31%</td>
<td>55%</td>
</tr>
<tr>
<td>Neoplasms causing paralysis</td>
<td>20%</td>
<td>20%</td>
<td>60%</td>
</tr>
<tr>
<td>New diagnosis of malignancy, with clinical suspicion</td>
<td>62%</td>
<td>28%</td>
<td>10%</td>
</tr>
</tbody>
</table>
• Provide leadership
• Respect human limits in process design
• Promote effective team functioning
• Anticipate the unexpected
• Create a learning environment
Create a Learning Environment

- encourage *reporting of errors* and hazardous conditions
- ensure *no reprisals* for reporting of errors
Designing for Safety in Health Care

Staff feel like their mistakes are held against them.

When an event is reported, it feels like the person is being written up, not the problem.

Staff worry that mistakes they make are kept in their personnel file.
Create a Learning Environment

- encourage *reporting of errors* and hazardous conditions
- ensure *no reprisals* for reporting of errors
- develop a *working culture* in which communication flows freely regardless of authority gradient
- implement mechanisms of *feedback and learning from error*
Designing for Safety in Health Care

Staff will freely speak up if they see something that may negatively affect patient care.

- 76%

Staff feel free to question the decisions or actions of those with more authority.

- 47%

Staff are afraid to ask questions when something does not seem right.

- 63%

6. Feedback & Communication About Error: 62%
7. Communication Openness: 62%
8. Frequency of Events Reported: 60%
Designing for Safety in Health Care

Creating and nurturing a culture of safety!
Quality and Safety in Surgical Pathology

Key Points

• diagnostic variance with potential to cause patient harm occurs at low but variable rates in surgical pathology

• defects (latent errors) in pre-analytical, analytical, and post-analytical processes contribute disproportionately to errors

• quality is a systems problem

• solutions must be non-punitive, systematic and process oriented
Quality, Patient Safety and Error Reduction in Cytopathology

Jan F. Silverman, MD, FCAP
Allegheny General Hospital
Pittsburgh, PA
Quality, Patient Safety & Error Reduction in Cytopathology

**GYN Cytology**

- 10% vs. Partial Rescreening
- ASCUS Rate
- ASCUS:SIL Ratio; LG, HG, & CA Rate
- Cyto/Histo Correlation
- Monolayer vs. Conventional; Imaging
- Retrospective Review
- HSIL follow-up letters
- Monitor professional and tech performance to overall lab
- Monitor referral rate for cytotechs
- Monitor unsatisfactory rate and no endo cx. component
- Monitor 2 step discrepancy between tech and pathologists
Quality, Patient Safety & Error Reduction in Cytopathology

- Errors
- 2nd Opinion
- Critical Value
IOM Report
Deaths from Medical Errors

• Colorado & Utah studies – 44,000 deaths – NY study – 98,000 deaths
• 8th leading cause of death. More than MVA (44,000), Breast CA (43,000) & AIDS (17,000)
• Total national costs – lost income, lost household production, disability & health care costs of preventable adverse events (M.E. resulting in injury) – $17 – 29 billion, (1/2 of which is health care costs)
Pathology & Lab Testing

- 70% of medical decisions that affect or change clinical course related to lab data
- 240 million PAP tests/year. 60 million SP specimens
- >97% CA dx based on pathology specimen dx
Error, IOM Definition

- Failure of a planned action to be completed as intended (execution error)
- Use of wrong plan to achieve an aim (planning failure)
Errors in Anatomic Pathology


Troxel. AJSP 28:1092, 2004
### A Total of 218 Surgical Pathology Claims From 1995, 1996, and 1997

<table>
<thead>
<tr>
<th>Specimen Category</th>
<th>Total Claims</th>
<th>% (no.) False Negative (Ca)</th>
<th>% (no.) False Positive (Ca)</th>
<th>% of Total Claims</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscellaneous surgical pathology</td>
<td>101</td>
<td>50 (51)</td>
<td>17 (18)</td>
<td>46</td>
</tr>
<tr>
<td>Breast biopsy</td>
<td>31</td>
<td>54 (17)</td>
<td>35 (11)</td>
<td>14</td>
</tr>
<tr>
<td>Melanoma</td>
<td>23</td>
<td>70 (16)</td>
<td>4 (1)</td>
<td>11</td>
</tr>
<tr>
<td>FNA, miscellaneous</td>
<td>16</td>
<td>19 (3)</td>
<td>56 (9)</td>
<td>7</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>16</td>
<td>75 (12)</td>
<td>19 (3)</td>
<td>7</td>
</tr>
<tr>
<td>FNA, breast</td>
<td>12</td>
<td>58 (7)</td>
<td>33 (4)</td>
<td>6</td>
</tr>
<tr>
<td>Frozen sections</td>
<td>10</td>
<td>40 (4)</td>
<td>40 (4)</td>
<td>5</td>
</tr>
<tr>
<td>Prostate biopsy</td>
<td>9</td>
<td>22 (2)</td>
<td>78 (7)</td>
<td>4</td>
</tr>
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</table>

### A Total of 272 Surgical Pathology Claims From 1998 Through 2003

<table>
<thead>
<tr>
<th>Specimen Category</th>
<th>Total Claims</th>
<th>% (no.) False Negative (Ca)</th>
<th>% (no.) False Positive (Ca)</th>
<th>% of Total Claims</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscellaneous surgical pathology</td>
<td>48</td>
<td>65 (31)</td>
<td>19 (9)</td>
<td>18</td>
</tr>
<tr>
<td>Melanoma</td>
<td>44</td>
<td>95 (42)</td>
<td>4.5 (2)</td>
<td>16</td>
</tr>
<tr>
<td>Breast biopsy</td>
<td>42</td>
<td>48 (20)</td>
<td>52 (22)</td>
<td>15.5</td>
</tr>
<tr>
<td>Gynecologic pathology</td>
<td>31</td>
<td>74 (23)</td>
<td>16 (5)</td>
<td>11.5</td>
</tr>
<tr>
<td>Operational error</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>15</td>
<td>80 (12)</td>
<td>20 (3)</td>
<td>5.5</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>14</td>
<td>57 (8)</td>
<td>43 (6)</td>
<td>5</td>
</tr>
<tr>
<td>Lung pathology</td>
<td>12</td>
<td>42 (5)</td>
<td>58 (7)</td>
<td>4.5</td>
</tr>
<tr>
<td>Gastric biopsy</td>
<td>12</td>
<td>42 (5)</td>
<td>58 (7)</td>
<td>4.5</td>
</tr>
<tr>
<td>FNA, miscellaneous</td>
<td>10</td>
<td>40 (4)</td>
<td>60 (6)</td>
<td>3.5</td>
</tr>
<tr>
<td>Prostate biopsy</td>
<td>9</td>
<td>67 (6)</td>
<td>33 (3)</td>
<td>3</td>
</tr>
<tr>
<td>FNA, breast</td>
<td>5</td>
<td>40 (6)</td>
<td>60 (3)</td>
<td>2</td>
</tr>
<tr>
<td>Bladder CIS</td>
<td>5</td>
<td>100 (5)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Branchial cleft cyst</td>
<td>3</td>
<td>100 (3)</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Errors – Patterns & Trends

- Melanoma misdx increased from 11 – 16% of total claims – misdx as Spitz, unrecognized desmoplastic, etc.
- Breast bx, breast FNA, FS – most common cause of pathology malpractice claims
- Extranodal lymphomas
- Less FNA claims
- PAP smears decreased from 17 – 11% of total claims
- Operational errors increased from 1.8 – 8%
Breast FNA Errors

- 6% of claims from 1995 – 97
- Majority false negative due to benign dx of FCC or negative in sparsely cellular, non-dx specimens & no recommendation to repeat FNA or tissue bx
- Recommend statement to apply triple test strategy. i.e. – correlate FNA with mammogram/ultrasound and clinical examination
- False positive usually due to interpretative errors, esp. fibroadenoma classified as CA.

Troxel DB
Case 1

• 32-YEAR-OLD WOMAN WITH THYROID NODULE
  – Thyroid, left lobe
  – FNA of 7 cm nodule
DIAGNOSIS

Papillary thyroid carcinoma

Comment: Tissue confirmation is indicated
Case 2

- 82-YEAR-OLD MAN WITH LUNG NODULE
  - FNA cytology and cell block of right lung mass
PRELIMINARY DIAGNOSIS

? Neuroendocrine neoplasm

IHC for synaptophysin, chromogranin - Negative
Specimen(s) Received
LUNG, RIGHT, FINE NEEDLE ASPIRATION, CYTOLOGY AND CELL BLOCK

Clinical History

Cancer History:
BREAST
SKIN

Treatment History:
RADIATION
CHEMOTHERAPY: 4 YEARS AGO

Other Clinical Conditions:
SIZE OF LESION: 1.5 CM

Date Ordered: 2/8/2005
Date Complete: 2/8/2005
Date Reported: 2/9/2005
FINAL DIAGNOSIS

Metastatic adenocarcinoma, consistent with a breast primary
Interinstitutional 2nd Review

- 777 patients / 9.1% discordant dx
- Change in Rxmet – 5.8%
- Cytology & FNA discrepant – 21%
- S.P. discordant – 7.8%

Abt et al.
Archives Pathol Lab 119:514, 1995
Cytology Discrepancies on Interinstitutional 2nd Opinion

- University of Utah School of Medicine and Duke University Medical Center
- 146 cases underwent second opinion review for 2 year period
- 24 disagreement, 11 major
- 16% disagreement rate, similar to s.p. but 8% major (slightly higher than s.p.)
- At Duke – disagreement occurred more frequently in thyroid and liver FNA’s, & cervix smears

Layfield et al.
Di Ci 26: 45-48, 2002
**Mandatory Second Opinion in Cytopathology Referral Material**

- Mandatory policy at Univ. of Iowa
- 499 second opinion cytology cases
- No dx disagreement, minor disagreement or major
- Major – 2 step difference or potential for change in Rxment or prognosis
- 37 (7.4%) major disagreement & 55 (11%) minor
- 6 cases had change in clinical management: thyroid FNA (3 cases), GYN (2 cases) & paratoid FNA (1 case) (1.2% of cases)

Lueck et al.
Lab Invest 88:356, 2008
Interinstitutional 2nd Review

Case 3

77 Y.O. MALE WITH LEFT LUNG BASE LESION

Outside FNA followed by left lower lobe segmental resection at treating institution.
Diagnosis:

FNA DX (Outside Hospital):

ADENOCARCINOMA
Mandatory Interinstitutional Pathology Consultation (IPCs) a.k.a. – Second Opinion

- ADASP recommend adoption of IPC as “institutional policy” when patients are referred to a second institution. (AJSP 17:743, 1993)
- No consensus or national guidelines due to perceived cost, delay and/or value
- However, following Institute of Medicine’s 1999 report – 2nd Conference – ASCP – June 2000 affirming ADASP recommendation supporting mandatory review of extramural dx “for which major therapeutic intervention are planned based on a tissue or cytologic dx” at the treating institution (AJCP 714:329, 2000).
Critical Value

• Concept introduced by Lundberg (1972) – “pathophysiological derangement at such variance with normal as to be life-threatening if therapy is not instituted immediately”

• Standard of practice with well established guidelines for clinical pathology
Critical Value Notification

• CLIA 88 – Section 493.1109
• JCAHO Standard LO3.2:1
• JCAHO 2005 - 2007 National Patient Safety Goals
• CAP
Critical Values in Surgical Pathology

Telma C. Pereira, MD,¹ Yulin Liu, MD, PhD,¹,² and Jan F. Silverman, MD¹,²

Key Words: Critical value; Surgical pathology; Physician notification
Prevalence of Cytology CV

- 2000 cytology reports from AGH and Mayo Clinic – 200 GYN, 400 non-GYN, 400 FNA each institution
- CV cases: Unexpected malignancy, disagreement between preliminary FNA & final dx, orgs. in non-GYN & FNA
- 52 CV (2.6%), including 0.25% (1/400) GYN, 1.88% (15/800) non-GYN & 4.5% (36/800) FNA
- Most (42 cases) were unexpected malignancies, 5 disagreement, 5 orgs.
- 30/52 documented phone call

Clinician & Pathologist Cytology CV Perception

• 13 pathologists and 13 clinicians at AGH and Mayo Clinic and 9 national senior cytopathologists

• 18 different CV’s with grading of urgency for phone call (1) no phone call (2) notify within 24 hours (3) ASAP

• Most agree on new dx of malignancy (esp. of unexpected or involving critical site), microorgs in immunosuppressed, and disagreement between preliminary FNA & final dx

• Greater differences of opinion with new METS in known 1°, orgs. in immunocompetent & no phone call needed for urine polyoma virus, new HSIL
Cytology CV

Additional CV’s

• Herpes in pregnant female PAP smears
• AGUS
• Amended report
• Very unusual tumor
• Disagreement with outside slide review
• Anticipated delay in dx (need to consult, etc.)
ADASP AP Critical Value Survey Results

Surgical Pathology
• Survey 225 ADASP members for grading 17 possible S.P. CVs.
• No phone call necessary, call within 24 hrs, phone ASAP.
• List additional CVs.
• 68/73 supported AP CV concept.

Cytology
• Survey ADASP members for grading 18 CVs/57 responses.
• 53/57 supported CVs concept

Good agreement in many CVs, but differences in opinion for some diagnosis.

LiVolsi, Pereira, Fletcher, Frable, Goldblum, Swanson, Silverman
ADASP Survey of Critical DX (Critical Value in SP & Cytology)

57 respondents for cytology CV cases

• Unexpected malignancies
• Malignancy in critical sites i.e. – paralysis
• Disagreement between immediate & final FNA
• Fungi in FNA of immunocompromised patients
• Microorganism in any patients
  – bacteria or fungi in CSF
  – pneumocystic, fungi, virus in BAL, wash or brush

Pereira et al.
AJCP 130:731, 2008
ADASP

**Critical Diagnosis (Critical Values) in AP**

- AdHoc Committee proposed guidelines based on ADASP surveys.
- Consultation with relevant clinical services is important.
- CV guidelines should be used as template, customized at individual hospitals requiring medical staff approval.
- Avoid overuse and eliminate non-critical diagnosis.

AJSP 30: 897-899, 2006
Human Pathol 37: 982-984, 2006
AJCP 125: 815-817, 2006
ADASP Critical DX (Critical Values) in AP

• Cases that have immediate clinical consequences
• Unexpected or discrepant findings
• Infections
SUMMARY
Strategies for Cytology Error Reduction

• Identify Problem Prone Cases
• Structure QA Programs to Identify & Correct Random & Systematic Errors
• Value of interinstitutional and internal 2nd Opinion
• Cyto/Histo Correlation
• Prel/Final FNA Correlation
REFERENCES:


Introduction

This presentation will discuss the assessment of prognostic biomarkers by immunohistochemistry (IHC), using HER2 and estrogen receptor alpha (ER) in breast cancer as examples to illustrate important issues relating to utility, validation, problems and solutions. In general, assessing almost any prognostic biomarkers by IHC is more challenging than assessing diagnostic biomarkers because life-saving treatment decisions in every patient rely on highly accurate, reproducible, and quantitative results, which enzymatic IHC is not particularly well suited for [1]. Nonetheless, nearly all testing for HER2 and ER in breast cancer is currently based on enzymatic IHC, and it is our responsibility as pathologists to ensure that they meet the highest standards possible.

HER2 Testing by IHC

HER2 (also referred to as HER2/neu and c-erbB2) in a proto-oncogene located on chromosome 17. It encodes a tyrosine-kinase protein residing on the outer cell membrane. Many studies during the past 20 years have shown that the HER2 gene is amplified in 15-30% of IBCs (closer to 15% today, which is probably due to screening mammography), and that amplification is highly correlated with over-expression of the protein [2-4]. The relationship between HER2 status and clinical outcome is complex. There is a weak but significant association between poor outcome and “positive” (i.e. amplified and/or over-expressed) HER2 in patients receiving no additional therapy following initial surgery [5, 6]. Most patients receive some type of adjuvant therapy and the association between HER2 status and outcome in this setting appears to depend on the type of therapy. Currently, the most important are antibody-based therapies targeting the HER2 protein, such as trastuzumab, and many studies (including randomized clinical trials) show that HER2 positive tumors are responsive to trastuzumab in both adjuvant and metastatic settings [7, 8]. Because of these beneficial clinical results, HER2 testing is now mandatory in all patients with breast cancer [9, 10], and IHC (which measures protein over-expression) and fluorescent in situ hybridization (FISH; which measures gene amplification) are the two primary methods being utilized, especially IHC. International proficiency testing programs and some of the same studies which validated the clinical efficacy of trastuzumab noted that the error rate in testing with IHC was as high as 20% (including both false-negative and false-positive results) [11-13]. The error rate associated with FISH was less clear, but there was an impression held by many that it was more reliable than IHC. It soon became fairly clear that this unacceptably high error in HER2 testing by IHC was widespread, which motivated many professional medical societies to develop strategies to improve it. Among the most notable outcome of these efforts to date was the publication of testing guidelines in late 2007 which were developed jointly by the College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) [14, 15]. These guidelines, which addressed both IHC FISH, were based on a comprehensive review of the medical literature by panels of experts, and have resulted in many changes for pathologists performing these tests (see Figure 1). For example, laboratories performing HER2
testing are required to strictly follow the guidelines and perform comprehensive ongoing quality assurance programs to obtain CAP accreditation. Hopefully, due to these and other efforts, we will see a dramatic improvement in the quality of HER2 testing in the near future. It is very clear that meeting the guidelines and obtaining accurate results requires substantial training and experience, and that laboratories lacking either should not be performing the tests at all.

Figure 1. Algorithms for HER2 testing in breast cancer by immunohistochemistry (left) and fluorescent in situ hybridization (right) [14, 15].

**ER Testing by IHC**

ER is a nuclear transcription factor activate by estrogen to regulate growth and differentiation of normal breast epithelial cells [16-18]. These pathways remain operative to varying degrees in IBCs, including estrogen-stimulated growth of tumor cells expressing ER, which is detrimental [17-19]. ER expression has been measured in IBCs for almost 40 years. During the first 20-25 years it was measured by radio-labeled biochemical ligand (i.e. estrogen)-binding assays (LBAs) on whole tissue extracts prepared from fresh-frozen tumor samples, which was costly and difficult. Many studies using LBAs in large randomized clinical trials demonstrated that ER was a relatively weak prognostic factor but a very strong predictive factor for response to hormonal therapies such as tamoxifen [19], which is one of the most widely used types of hormonal therapy. Tamoxifen, which binds ER and blocks estrogen-stimulated growth, was shown to significantly reduce disease recurrence and prolong life in patients with ER-positive IBCs [19, 20], which is also true for newer types of hormonal therapies such as the aromatase inhibitors [21, 22]. The primary reason for assessing ER is its ability to predict response to hormonal therapies.

Although the clinical utility of assessing ER was initially based almost entirely on studies using technically standardized LBAs, in the mid-1990s laboratories around the world began abandoning LBAs in favor of IHC, and IHC is used for nearly all testing today, and the validation of this strategy is problematic and evolving. Notwithstanding, there are several advantages associated with IHC compared to LBAs, especially its ability to measure ER on routine formalin-fixed paraffin-embedded samples, eliminating the need for fresh-frozen samples and the onerous infrastructure required to provide it. Other advantages include lower cost, better safety, and superior sensitivity and specificity in the sense that the assessment of ER is restricted to tumor cells under direct microscopic visualization, independent of tumor
cellularity or the presence of benign epithelium, which could be problematic for LBAs. For all these reasons and more, IHC was approved by the CAP and ASCO for routine clinical use [9, 10]. Despite these approvals, however, there are significant problems with IHC that persist today, including the widespread use of diverse staining procedures of unequal quality and varied often arbitrary methods of interpreting results, resulting in an overall error rate estimated to be as high as 20% [23-26] - which has created a "crisis" in ER (and PgR) testing in the minds of many [27]. In response, the CAP and ASCO recently convened another panel of experts to review the literature and develop guidelines for ER (and PgR) testing by IHC. Although the results are still forthcoming, it is likely that they will be similar to the HER2 guidelines. In general, all good guidelines agree that tests used in routine clinical practice should be based on sensitive and specific reagents, standardized laboratory procedures and, especially, calibrated to relevant clinical outcome in a comprehensive manner [9, 28, 29].

References


CAP Companion Society Meeting at USCAP 2009

Quality Assurance, Error Reduction, and Patient Safety in Anatomic Pathology

Immunohistochemistry in Routine Clinical Testing of Prognostic Biomarkers

Utility, Validation, Problems, and Solutions

Examples:
HER2 and ERα in Breast Cancer

D. Craig Allred, MD, FCAP
Department of Pathology and Immunology

Washington University in St. Louis
School of Medicine

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Outline

Define validation of routine prognostic factors.

Example: HER2 testing by IHC

- Overview/Utility
- Validation
- Problems
- Solutions (CAP/ASCO Guidelines 2007)

Example: ERα testing by IHC

- Overview/Utility
- Validation
- Problems
- Solutions (impending CAP/ASCO Guidelines 2009)

General principles and practices of “good” testing
General Guidelines for Evaluating Prognostic Factors in Routine Clinical Practice

Clinical Validation: The factor(s) should identify groups of patients with significantly different risks of relapse, survival, or treatment response – ideally demonstrated in multiple randomized studies.

Technical Validation: The assays should be specific, sensitive, reproducible, calibrated to clinical outcome, interpreted, and reported in a relatively uniform manner. There should be comprehensive ongoing quality assurance.

Useful: Actually used by physicians to make important treatment decisions.

Overview
Testing for HER2 in Breast Cancer

Many Assays
- W.Blot
- IHC
- ELISA
- S.Blot
- FISH
- CGH
- CISH
- N.Blot
- RT-PCR
- Others...

Many Assays/ABs
- HercepTest (PAb)
  - CB11
  - Tab250
  - PAB1
  - 21N
  - SP3
  - Others...

Protein Expression

Gene Amplification

RNA Expression

Highly Correlated

Many Purposes
- Prognostic
  - Untreated (+ = bad)
- Predictive
  - CMF (+ = bad)
  - Anthracylines (+ = good)
  - Endocrine (+ = bad)
  - Taxanes (+ = good)
  - Herceptin (+ = good)

Amp=↑ Exp

Detection Systems
Non-Linear Amplification

HER2
**HercepTest IHC Scoring Method**

<table>
<thead>
<tr>
<th>Staining Pattern</th>
<th>Score</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No staining.</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>Faint incomplete staining of cell membrane in &gt;10% of tumor cells.</td>
<td>1+</td>
<td>Trace Negative</td>
</tr>
<tr>
<td>Weak to moderate complete staining of cell membrane in &gt;10% of tumor cells.</td>
<td>2+</td>
<td>Weak Positive</td>
</tr>
<tr>
<td>Strong complete staining of cell membrane in &gt;10% of tumor cells.</td>
<td>3+</td>
<td>Strong Positive</td>
</tr>
</tbody>
</table>

*Arbitrary strategy which does not convey the true (substantial) heterogeneity of expression, and prone to false-positive results due to many technical artifacts.*
Expected “True” Distribution of IHC (HercepTest) Results

<table>
<thead>
<tr>
<th></th>
<th>0/1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo Clinic (n=1,556)</td>
<td>73%</td>
<td>14%</td>
<td>13%</td>
</tr>
</tbody>
</table>

A Potential Problem with IHC (HercepTest): False-Negatives

<table>
<thead>
<tr>
<th>Central Lab IHC =3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Lab IHC &lt;3+ (n = 268): 15%</td>
</tr>
</tbody>
</table>

HER First Trial; First-line Herceptin + other in metastatic/HER2+ pts
See: Breast Cancer Res Treat 76:S68(abst#235), 2002

*Consequence = Under Treatment*
A Potential Problem with IHC (HercepTest): False-Positives

<table>
<thead>
<tr>
<th>Central Lab IHC &lt;3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1Local Lab IHC =3+ (n = 110): 21%</td>
</tr>
<tr>
<td>2Local Lab IHC =3+ (n = 146): 26%</td>
</tr>
</tbody>
</table>

1Intergroup Trial N9831; First-line Herceptin + other in node+/HER2+ pts J Clin Oncol 22:567, 2004 and 24:3032, 2006


*Consequence = Over Treatment*
HER2 Testing in NSABP-B31

“Inexperienced” vs. “Experienced” Labs

Central QC first 104 pts (JNCI 94:852, 2002)

<table>
<thead>
<tr>
<th>Outside Lab Results</th>
<th>Central Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC=3+/Small labs (n=52):</td>
<td>IHC&lt;3+ 19%</td>
</tr>
<tr>
<td></td>
<td>FISH=Neg</td>
</tr>
<tr>
<td>IHC=3+/Large labs (n=28):</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>FISH=Pos/All labs (n=27):</td>
<td></td>
</tr>
</tbody>
</table>

19% false positives by IHC from “small” labs!
HER2 Testing in NSABP-B31

Results after QA Program

Laboratory Approval Based on Large Volume and/or High Concordance IHC vs. FISH

Central QC next 240 pts (BCRT 76:abst #9, 2002)

<table>
<thead>
<tr>
<th>Outside Lab Results</th>
<th>Central Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC=3+ (n=104):</td>
<td>FISH=Neg</td>
</tr>
<tr>
<td></td>
<td>2%</td>
</tr>
</tbody>
</table>

False positives decreased from 19% to 2%.

Experience/QA = Improved Performance!
HER2 Testing by FISH

Orange = HER2
Green = Chromosome
Ratio = #HER2 ÷ #C17
Normal = 1
Amp > 1 (variable Hx)

Problematic issues with FISH:
Expensive
Difficult and time consuming
Diverse arbitrary cutoffs
Errors (~10% overall; esp. low-level amplification)
Other...
## Studies Comparing IHC (HercepTest) vs. FISH (PathVysion)

<table>
<thead>
<tr>
<th>Study</th>
<th># Pts</th>
<th>0%</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am J Clin Pathol 113:852, 2000</td>
<td>100</td>
<td>0%</td>
<td>0%</td>
<td>17%</td>
<td>89%</td>
</tr>
<tr>
<td>Proc ASCO 19:#294), 2000</td>
<td>142</td>
<td>0%</td>
<td>0%</td>
<td>31%</td>
<td>100%</td>
</tr>
<tr>
<td>Ann Oncol 12”597, 2001</td>
<td>142</td>
<td>0%</td>
<td>0%</td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td>J Clin Oncol 19:354, 2001</td>
<td>79</td>
<td></td>
<td></td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td>J Clin Oncol 19:2714, 2001</td>
<td>145</td>
<td></td>
<td></td>
<td>13%</td>
<td>75%</td>
</tr>
<tr>
<td>JNCI 94:855, 2002</td>
<td>119</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>68%</td>
</tr>
<tr>
<td>J Pathol 199:418, 2003</td>
<td>426</td>
<td>0%</td>
<td>1%</td>
<td>48%</td>
<td>94%</td>
</tr>
</tbody>
</table>

- ~ 70% = IHC 0/1+ (0% FISH+)
- ~ 15% = IHC 2+ (30% FISH+)
- ~ 15% = IHC 3+ (95% FISH+)
- ~ 95% Overall Agreement
Non-Linear Correlation Between Gene Amplification and Protein Expression

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>#Genes/Ratio</th>
<th>#Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA231</td>
<td>2.4/1.1</td>
<td>~20,000</td>
</tr>
<tr>
<td>MDA175</td>
<td>3.0/1.3</td>
<td>~50,000</td>
</tr>
<tr>
<td>MDA453</td>
<td>5.2/2.4</td>
<td>~250,000</td>
</tr>
<tr>
<td>SKBR3</td>
<td>15.3/4.5</td>
<td>~2,500,000</td>
</tr>
</tbody>
</table>

High Correlation Surprising!

6-Fold Increase          125-Fold Increase

Courtesy Dr. Ken Bloom
Clarien, Inc.
**Misconception: FISH > IHC**

*Oncology Drug Advisory Committee (ODAC) data leading to FDA approval for Herceptin*

IHC vs. FISH and Response to Herceptin

### H0648 (Chemo ± Herceptin)

<table>
<thead>
<tr>
<th>Test Results</th>
<th>RR TTP</th>
<th>95% CI</th>
<th>#Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+</td>
<td>0.42</td>
<td>0.32-0.55</td>
<td>349</td>
</tr>
<tr>
<td>2+</td>
<td>0.82</td>
<td>0.54-1.24</td>
<td>120</td>
</tr>
<tr>
<td>FISH+</td>
<td>0.44</td>
<td>0.34-0.75</td>
<td>325</td>
</tr>
<tr>
<td>FISH-</td>
<td>0.66</td>
<td>0.45-0.99</td>
<td>126</td>
</tr>
</tbody>
</table>

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<th>RR TTP</th>
<th>95% CI</th>
<th>#Pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>3+/FISH+</td>
<td>0.42</td>
<td>0.32-0.55</td>
<td>293</td>
</tr>
<tr>
<td>3+/FISH-</td>
<td>0.40</td>
<td>0.19-0.87</td>
<td>43</td>
</tr>
<tr>
<td>2+/FISH+</td>
<td>0.72</td>
<td>0.31-1.64</td>
<td>32</td>
</tr>
<tr>
<td>2+/FISH-</td>
<td>0.86</td>
<td>0.53-1.38</td>
<td>83</td>
</tr>
</tbody>
</table>

IHC≈FISH in H0649 (second/third line Rx)

IHC≈FISH in H0650 (first line Rx)
**Misconception: FISH > IHC**

*Oncology Drug Advisory Committee (ODAC) data leading to FDA approval for Herceptin*

IHC vs. FISH and Response to Herceptin

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**H0648 (Chemo ± Herceptin)**

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</tr>
<tr>
<td>2+/FISH-</td>
<td>0.99</td>
<td>0.45-0.99</td>
<td>126</td>
</tr>
</tbody>
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“Direct comparative statements of equivalence or superiority between IHC and FISH cannot be made.”

Publications combined IHC 3+/2+ versus FISH+ ⇒ biased comparison and primary source of misconception that FISH better than IHC.

IHC≈FISH in H0650 (first line Rx)
Purpose: To develop a guideline to improve the accuracy of human epidermal growth factor receptor 2 (HER2) testing in invasive breast cancer and its utility as a predictive marker. Methods: The American Society of Clinical Oncology and the College of American Pathologists (CAP) convened an expert panel, which conducted a systematic review of the literature and developed recommendations for optimal HER2 testing performance. The guideline was reviewed by selected experts and approved by the board of directors for both organizations. Results: Approximately 20% of current HER2 testing may be inaccurate. When carefully validated testing is performed, available data do not clearly demonstrate the superiority of either immunohistochemistry (IHC) or in situ hybridization (ISH) as a predictor of benefit from anti-HER2 therapy. Recommendations: The panel recommends that HER2 status should be determined for all invasive breast cancer. A testing algorithm that relies on accurate, reproducible assay performance, including newly available types of brightfield ISH, is proposed. Elements to reliably reduce assay variation (for example, specimen handling, assay exclusion, and reporting criteria) are specified. An algorithm defining positive, equivocal, and negative values for both HER2 protein expression and gene amplification is recommended: a positive HER2 result is IHC staining of 3+ (uniform, intense membrane staining of > 30% of invasive tumor cells), a fluorescent in situ hybridization (FISH) result of more than 6 HER2 gene copies per nucleus, or a FISH ratio (HER2 gene signals to chromosome 17 signals) of more than 2.2; a negative result is an IHC staining of 0 or 1+, a FISH result of less than 4.0 HER2 gene copies per nucleus, or a FISH ratio of less than 1.8. Equivocal results require additional action for final determination. It is recommended that to perform HER2 testing, laboratories show 95% concordance with another validated test for positive and negative assay values. The panel strongly recommends validation of laboratory assay or modifications, use of standardized operating procedures, and compliance with new testing criteria to be monitored with the use of stringent laboratory accreditation standards, proficiency testing, and competency assessment. The panel recommends that HER2 testing be done in a CAP-accredited laboratory or in a laboratory that meets the accreditation and proficiency testing requirements set out by this document.
2007 ASCO/CAP Guidelines for IHC Testing
J Clin Oncol 25:118 and Arch Pathol Lab Med 131:18

Assumes the use of accurate, standardized, and validated assays.

CLIA/CAP laboratory accreditation will require continuous comprehensive monitoring and validation.
2007 ASCO/CAP Guidelines for FISH Testing

J Clin Oncol 25:118 and Arch Pathol Lab Med 131:18

Assumes the use of an accurate, standardized, and validated assays.

CLIA/CAP laboratory accreditation will require continuous comprehensive monitoring and validation.
History of Measuring ER\(\alpha\) and PR


1975 $\downarrow$ 1980 $\downarrow$ 1985 $\downarrow$ 1990 $\downarrow$ 1995 Today

EXAs/LBAs...

...taught us that ER\(\alpha\) and PgR are weak prognostic factors in untreated patients but strong predictive factors for response to hormonal therapy.

IHC replaced EXAs/LBAs on FFPE Samples.

Is this good?
Advantages of Assessing Hormone Receptors by IHC vs. LBA

LBA
- large specimens
- frozen specimens
- radioactivity
- difficult
- expensive
- signal any cells

IHC
- any size (small)
- frozen or fixed
- none (safe)
- easy
- cheap
- signal tumor cells

⇒ not so easy
Evaluation of ER\(_{\alpha}\) and PgR is necessary in all primary breast cancers and both LBA and IHC are approved for routine clinical use (Category I).

Acknowledged persistent problems with IHC such as lack of standardization and validation...
Crisis in ERα and PgR Testing in Breast Cancer by IHC

What is the Problem? 
*Inaccurate Test Results*  
*Primarily False-Negatives*

What are the Consequences?  
*Improper Therapy*  
*Primarily Under-Treatment*  
*Potentially Catastrophic*

What is the Magnitude?  
*Largely Unknown*  
*1000s of Tests each Day in 1000s of Laboratories Worldwide*  
*Monitoring of Quality is Rare*  
*Estimated at 10-20% for ERα...Higher for PgR?*
ERα and PgR Testing in Breast Cancer by IHC

Evidence and Magnitude of Problem

Personal Experiences of Health-Care Providers:

*Largest and Most Convincing*

*Example: Re-testing (2nd opinion) for ERα reveals ~30% false-negative rate in consult practice of DC Allred.*

News Reports and Scientific Publications of Uncovered Mistakes:

*Rare but Compelling Examples:*

**BIG 1-98 Clinical Trial: Predictive power of ERα and PgR by IHC (central vs. local labs) in receptor-positive, postmenopausal, early breast cancer randomized to adjuvant letrozole vs. tamoxifen.**


69% false-negatives for ERα by IHC (n~100)!
44% false-negative for PgR by IHC (n~1,200)!

**Debacle in Newfoundland and Labrador, Canada**

40% False-Negatives in >2,000 originally ER-Negative Breast Cancers tested between 1997-2005!!!

Hede A. JNCI 100:836, 2008
ERα and PgR Testing in Breast Cancer by IHC

Evidence and Magnitude of Problem

Published Results of Quality Assurance Programs:

Rare but Compelling Examples:

UK National External Quality Assessment Service (NEQAS).
Comprehensive proficiency assessment (accuracy/reproducibility) of testing for ERα by IHC in 150 laboratories in 26 countries worldwide.

ERα Test Cases

- low pos (~25% cells)
- med pos (~50% cells)
- high pos (>75% cells)

Each lab’s “in-house” assay

Reviewed by experts

Labs finding >10% pos cells

- 40%
- 85%
- 99%

~20% Overall Error

Antigen retrieval most problematic technical error.

Example of Validating an IHC Assay for ER\(\alpha\)

Harvey et al. J Clin Oncol 17:1474, 1999

- Fixed/archival samples
- Antibody 6F11 (ER\(\alpha\) specific)
- Tris pH 9 HIER
- SAHRP\(\alpha\)x detection system
- \(\text{H}_2\text{O}_2/\text{DAB/OsO}_4\) chromogen
- Methyl green counterstain

- Calibrated to clinical outcome
## Choice of ER\(\alpha\) Antibody
(pilot study 200 IBC by PS IHC)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% cases &gt;0% pos cells</th>
<th>% agreement with LBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBA</td>
<td>68</td>
<td>-</td>
</tr>
<tr>
<td>H222</td>
<td>53</td>
<td>70</td>
</tr>
<tr>
<td>1D5</td>
<td>66</td>
<td>79</td>
</tr>
<tr>
<td>LH2</td>
<td>54</td>
<td>68</td>
</tr>
<tr>
<td>6F11</td>
<td>71</td>
<td>87</td>
</tr>
</tbody>
</table>

(sensitivity)

(specificity)
Scoring Immunostained Slides

Proportion Score (PS)

0 = negative
1 = weak
2 = intermed
3 = strong

Intensity Score (IS)

0 = negative
1 = weak
2 = intermed
3 = strong

Total Score (TS) = PS + IS (range 0-8)

Example of Scoring

Proportion Score = 4 (1/3\textsuperscript{rd} to 2/3\textsuperscript{rds} positive cells)
Intensity Score = 2 (average intensity “intermediate”)
Total Score = 6/8
Interpreting ER by IHC

Requires comprehensive studies calibrating content with response to hormonal therapy.

<table>
<thead>
<tr>
<th>IHC Score</th>
<th>LBA (fmol/mg)</th>
<th>#Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>517 (26%)</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>67 (3%)</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>117 (6%)</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>190 (10%)</td>
</tr>
<tr>
<td>5</td>
<td>104</td>
<td>320 (16%)</td>
</tr>
<tr>
<td>6</td>
<td>141</td>
<td>370 (19%)</td>
</tr>
<tr>
<td>7</td>
<td>193</td>
<td>318 (16%)</td>
</tr>
<tr>
<td>8</td>
<td>282</td>
<td>83 (4%)</td>
</tr>
</tbody>
</table>

Near-Linear Correlation with LBA

Wide Distribution of Expression

Positive
>1% ER+ cells!

Direct Correlation with Response to Tamoxifen
## ERα by IHC vs. LBA Predicting Clinical Outcome

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Rx (n=688)</td>
<td>HR: 0.90</td>
<td>HR: 0.76</td>
</tr>
<tr>
<td></td>
<td>p-val: 0.48</td>
<td>p-val: 0.06</td>
</tr>
<tr>
<td>Chemo Only (n=404)</td>
<td>HR: 1.01</td>
<td>HR: 0.97</td>
</tr>
<tr>
<td></td>
<td>p-val: 0.96</td>
<td>p-val: 0.86</td>
</tr>
<tr>
<td>Chemo+Endo (Tam) (n=260)</td>
<td>HR: 0.56</td>
<td>HR: 0.50</td>
</tr>
<tr>
<td></td>
<td>p-val: 0.008</td>
<td>p-val: 0.004</td>
</tr>
<tr>
<td>Endo Only (Tam) (n=517)</td>
<td>HR: 0.47</td>
<td>HR: 0.35</td>
</tr>
<tr>
<td></td>
<td>p-val: 0.0008</td>
<td>p-val: 0.0001</td>
</tr>
</tbody>
</table>
Additional Studies
Using Same/Similar IHC Assay for ERα

**In Adjuvant Setting:**

Love et al. J Clin Oncol 20:2559-2566, 2002 (OvEx ± Tam)
Horiguchi et al. Oncol Rep 14:1109, 2005 (Misc Endo Rx)

**In Neoadjuvant Setting:**

Ellis et al. JNCI 100:1380, 2008 (Tam vs. Al)

**In Advanced Disease:**

*Elledge et al. Int J Cancer.* 2000;89:111 (Tam)
Arpino et al. Clin Cancer Res 10:5670, 2004 (Tam)
Comprehensively Validated IHC Assays for Measuring Hormone Receptors in Breast Cancer

For ERα:
Harvey et al. J Clin Oncol 17:1474, 1999  

For PgR:
Mohsin et al. Modern Pathol 17, 1545, 2004  

Shortcoming of all is Absence of Untreated Cohorts to Separate Prognosis from Prediction
...Relatively Minor Since Prognostic Power so Small?
Elements of Good Testing in Clinical Practice (Highly Inter-Related) NOT Easy to do Right!

**True Expertise (most important issue and largest problem)**
- Understand clinical utility (formal study; multidisciplinary conferences, etc.)
- Understand technology (formal study; workshops; experience, etc.)
- Don’t assume expertise just because you’re a “pathologist” (most with little training in IHC)

**Technically Validated Assays**
- Follow a clinically validated assay
- Standard operating procedures (don’t fiddle with the assay...)
- Optimal sample preparation (e.g. 10% NBF 6-48 hours), identification, and tracking
- Demonstrate and maintain evidence of adequate sensitivity, specificity, and reproducibility

**Clinically Validated Assays**
- Technically validated assay calibrated to COMPREHENSIVE clinical outcome
  - Multiple randomized clinical trials (difficult)

**Comprehensive Reports**
- Proper identification (patient, physician, times of collection-testing-reporting, etc.)
- Quantitative results (proportion and intensity of positive cells)
- Interpretation of results (positive vs. negative based on clinical benefit)
- Critical ancillary information
  - Criteria for scoring and interpretation
  - Key references regarding clinical utility overall and of assay utilized
  - Key reagents (esp. primary antibody)
  - Assurance of ongoing quality assurance program

**Quality Assurance Program**
- Uniform and stable scoring and interpretation (within and between pathologists)
  - Ongoing confirmation of competency
- Uniform and stable quality of slides (especially appropriate positive and negative controls)
- Accurate and stable distribution of results
  - Ongoing monitoring (reassurance that problems are identified and solved in time)
  - Internal and external confirmation of accuracy

**Comprehensive Record Keeping**
Quality, Patient Safety, and Error Reduction in Molecular Diagnostics
Christopher A. Moskaluk, MD, PhD, FCAP
Associate Professor of Pathology, University of VA
March 7, 2009

Learning objectives:

- Understand how procurement issues & target heterogeneity in tissue samples impacts the quality of molecular diagnostics
- Understand how protocol complexity & non-uniformity in molecular diagnostics can lead to errors
- Understand how application of quality assurance & quality control procedures in molecular diagnostics can reduce error

Syllabus

Introduction
The practice of Anatomic Pathology (AP) has for over 100 years been based primarily on the gross and histologic analysis of tissues. In the past few decades, the assessment of specific molecular constituents of tissue has been adopted into AP practices primarily through the histology-based in situ assays of immunohistochemistry and in situ hybridization. More recently it has become necessary to evaluate specific molecular constituents of tissue in solution or gel-based analytic techniques that require the extraction of biomolecules from tissue samples. It is this aspect of molecular diagnostics that this session will cover.

Pre-analytic variables in AP molecular diagnostics: Tissue and molecular target integrity
The traditional practice of anatomic pathology basically had to prevent the autolysis of tissue which precluded optimal histologic analysis. This allowed a relatively leisurely pace of tissue processing since autolysis typically does not become histologically evident for hours to days after excision. Moreover, the standard histologic practice of formalin fixation halts the process of autolysis and stabilizes the tissue for ambient temperature storage, and for histologic processing into paraffin blocks.

The analysis of specific molecular targets in tissue is much more problematic. Certain molecular constituents, particularly those involved in signal transduction, have a dynamic half-life that is significantly altered in the state of cellular stress incurred by devascularization that occurs as a result of surgical resection or biopsy. In particular, certain protein phosphorylation events have been shown to dramatically change levels within minutes of excision (1, 2). Formaldehyde fixation may preserve the original phosphorylation status, but only in areas of tissue that undergo rapid formalin penetration, making the assessment of protein phosphorylation in large organ resections problematic if rapid biopsy-type sampling is not undertaken (3). Other molecular constituents that are known to be particularly labile in devascularized tissue are certain proteins (particularly regulatory proteins) and RNA transcripts (4). One exception to this general rule is genomic DNA, which is generally resistant to marked degradation that would preclude molecular analysis. Since many of the initial techniques being employed in tissue diagnostics is the detection of specific gene mutations in genomic DNA, this is both a blessing and a curse. The blessing is that the majority of samples available in an AP laboratory will be amenable to analysis without altering customary practices and workflow. The curse is that as molecular assessment of
more labile analytes becomes important, AP practices will not have been appropriately attuned to the necessary attention to rapid tissue procurement.

Some of the degradation of molecular targets is beyond the control of AP laboratories. In particular, the time of “warm ischemia” that occurs in an organ undergoing resection – the time between which the surgeon has severed the blood supply to a particular organ area and the time that the specimen is completely removed from the patient, cannot neither be controlled, nor often easily documented. However all steps after that, including the time of transport to the AP laboratories and the time of processing of the specimen (gross examination, inking, sectioning, etc.) need to be reviewed and reduced to the minimum time possible. It will become necessary to set up a triage system such as that now exists for specimens requiring intra-operative frozen section diagnosis, to identify cases needing or likely to need molecular diagnosis and have them processed and sampled in an expedient manner. Alternatively, changes to clinical practice, such as intra-operative biopsy of tissues for molecular diagnostics may need to be employed. Finally, techniques to rapidly stabilize samples that are complementary to the technique to be employed (e.g. snap freezing, emersion in “molecular friendly” fixatives) will have to employed for the analysis of labile analytes.

Having made the general statement that speed is of the essence is obtaining tissue samples for molecular diagnostics, there is some data to suggest that a reasonable time period of 1-2 hours exists after the excision of tissue in which the global RNA transcript profile is relatively stable (5-7). However this not true of all transcripts, and the parameters for any individual analyte needs to be determined for appropriate specimen handling.

There is a substantial literature documenting the changes of biomolecules in tissue as a result of formalin fixation and paraffin embedding (8-12). Primarily, formaldehyde causes covalent cross links to occur within and between biomolecules. While the consequent stabilization of cellular and tissue structures is desirable for histology, this event precludes the complete solubilization of individual biomolecules that is required for many molecular analyses. In addition, the process of paraffin embedding causes additional denaturation and fragmentation of biomolecules, particularly nucleic acids. While this process does not preclude some molecular analyses, for many molecular technologies, formalin-fixation and paraffin-embedding (FFPE) precludes robust and/or accurate measurements.

Since it may not be possible to preclude that some tissue samples being subjected to a molecular diagnostic test do not have sufficient quality to yield an accurate result, there is a requirement to ascertain the molecular integrity of the sample. This may occur as either a separate pre-analytic assessment, or may be integrated by the addition of appropriate controls during the analysis. One of the most common of pre-analytic assessments is the determination of RNA quality. Generally, total RNA isolation is performed from tissue samples, yielding a mixture of processed messenger RNA, ribosomal RNA (rRNA) and heterogeneous nuclear RNA. The quality of the two major rRNA species (18s and 28s), as determined by their absolute and relative amount, has been shown to be generally reflective of the quality of the transcript population. Among the most common analytic methods to assess rRNA and global RNA quality is the use of microcapillary electrophoresis systems, and various computer algorithms have been developed to yield numerical scores of RNA quality that can be used as benchmarks or thresholds for subsequent analyses (13). Again, it is difficult to generalize for molecular diagnostics, and the minimum specimen quality required for any analyte and/or analytic platform needs to be determined on an assay by assay basis.

For those analyses not utilizing pre-analytic specimen integrity testing, generally an internal positive control is developed to ascertain sample quality. For instance, in a quantitative reverse transcriptase polymerase chain reaction assay (qRT-PCR) for a cancer-specific translocation gene product, an internal qRT-PCR for a “housekeeping” gene that is expected to be present in the sample will also be performed. If the concentration of this internal control gene
Quality, Patient Safety, and Error Reduction in Molecular Diagnostics
Christopher A. Moskaluk, MD, PhD, FCAP

does not reach a threshold level, then the sample will be rejected as being insufficient to confidently render a result.

Pre-analytic variables in AP molecular diagnostics: Tissue heterogeneity and the role of histology in molecular diagnostics

In general, molecular diagnostics concerns itself with the analysis of a specific analyte in a specific cell type (e.g. gene mutation in cancer cells). The inherent heterogeneity of tissue samples, precluding accurate assessment of cell type and cell viability of tissue specimens by gross assessment, and the vagaries of tissue sampling by biopsy methods requires some assessment of the type of constituent cells. At this juncture, histology remains the most universal and robust of methods to achieve this goal.

Two things are required to address this issue in AP molecular diagnostics:
1) During the development phase of the assay, a determination must be made as to minimum percentage of target cells is required in order to deliver an accurate assessment. This is typically done by “mixing experiments” using 2 populations of cell culture model systems, in which one cell line represents the target cell population and the other represents “non-target” cells.
2) There must either be a histologic assessment of the input tissue to determine if the target cell population meets the tolerances of the assay, or some sort of dissection technology needs to be employed to enrich the target cells to meet these tolerances.

Thus at a minimum, a matched FFPE tissue section needs to be examined to know what the input material is to a molecular assay. Alternatively, a histologic section is taken of the input sample (frozen or FFPE), and areas enriched for target cells are dissected. This may be performed as bulk or “macrodissection” in which the target areas are dissected from the block, or scraped from histologic sections (14). While this does not typically yield completely pure cell populations, for many samples >70% enrichment may be achieved.

For more pure cell populations, laser microdissection may be employed. This techniques may employ differing technologies, but what is in common is that a laser beam somehow captures discrete populations of cells from a histologic section by direct observer control through a microscope (15). Though capable of obtaining pure or near pure populations of cells, the drawbacks include that the amount of captured material is very small, too small for some types of analysis, and that the technique is relatively slow and cumbersome, taking up too much time of histopathology-trained personnel to be realistically deployed for the majority of molecular assessments in a clinical laboratory.

Patient safety issues: sample misidentification

Sample misidentification is a problem that is inherent to all laboratory testing. In addition to the standard problems of inaccurate pre-analytic and post-analytic identification of a sample or laboratory result, many molecular diagnostic methodologies suffer from numerous container transfers during both the biomolecule extraction phase and the analytic phase, increasing the odds of inappropriate specimen labeling. Often container labeling may be carried out manually, without computer generated labels or with bar-coding. These factors are often the result that many molecular diagnostic techniques are either emerging technology and/or are primarily research technique being newly applied to diagnostics. Such immature technologies often are not supported by robotic platforms requiring large amounts of manual specimen manipulation. A second problem is that some molecular tests may be carried out in hybrid research and clinical labs in which personnel are carrying out similar techniques using different standard operating procedures, leading to lapses in or confusion about the standards applied to clinical testing.
Solutions include moving to robotic platforms whenever possible. While this may not be possible for many assay types, at least for biomolecule extraction, many platforms exist that support the multi-step isolation of DNA, RNA and/or proteins from tissue samples. Whenever possible, bar coding systems should be in place to track specimens in the pre-analytic and analytic process. And finally, appropriate personnel training and quality check procedures should be in place to maintain and verify the accuracy and reproducibility of specimen tracking through the process.

**Quality Assurance and Quality Control procedures**

While all of the preceding discussions have involved aspects of quality assurance (QA) and quality control (QC), this section will discuss the general implementation of these procedures in a molecular diagnostics laboratory. One definition of QA is: the policies, procedures and systematic actions established for the purpose of providing and maintaining a high quality of test integrity and accuracy. Quality control (QC) generally refers to the procedures in place to verify the accuracy of individual assay results (e.g. pre-analytic thresholds for specimen quality, positive and negative controls, etc.). QA/QC procedures involve steps to ensure all the steps involved in pre-analysis, analysis and post-analysis are being performed accurately and are being documented (see Table 1). Most of these procedure categories are not novel to molecular diagnostics; the standard QA/QC paradigm that governs the best practices of all clinical laboratories applies to molecular diagnostics (16, 17). The increased attention to tissue heterogeneity and integrity has already been covered. The specifics of appropriate quality control measures that pertain to the individual assays are unique, but the discussion of the controls used in the myriad types of molecular diagnostic assays is beyond the scope of this discussion.

**Table 1:** Areas that quality programs must address

<table>
<thead>
<tr>
<th>Pre-analytical Variables</th>
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<tbody>
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<tr>
<td>• Specimen acquisition</td>
</tr>
<tr>
<td>• Specimen transport</td>
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<tr>
<td>• Specimen processing</td>
</tr>
<tr>
<td>• Presence/purity of target cells</td>
</tr>
<tr>
<td>• Specimen integrity</td>
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<tr>
<td>• Instrument maintenance</td>
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<table>
<thead>
<tr>
<th>Analytical Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Competency</td>
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<tr>
<td>• Controls</td>
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<tr>
<td>• Methodology/ procedure validation</td>
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<table>
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<tr>
<th>Post-analytical Variables</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>• Result reporting</td>
</tr>
<tr>
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<td>• Monitoring of materials</td>
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<table>
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<tr>
<th>Documentation</th>
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</thead>
<tbody>
<tr>
<td>• Standard Operating Procedures (SOPs)</td>
</tr>
<tr>
<td>• Assay logs</td>
</tr>
<tr>
<td>• Equipment maintenance records</td>
</tr>
<tr>
<td>• Training records</td>
</tr>
<tr>
<td>• Results/diagnoses rendered</td>
</tr>
</tbody>
</table>
A quality assurance program must create standard operating procedures (SOPs) for all aspects of specimen handling, analysis and report generation that all personnel must have access to and are familiar with. The program also devises, implements and documents training and retraining programs for all personnel involved in all of these aspects. Quality control measures are built into the procedures to ensure accuracy. Finally, a quality assurance program entails periodic quality monitoring (proficiency testing) of the system to ensure that quality measures are being adhered to and that accurate test results are being generated.

In order for a test to be considered valid, it must have at least first-line and second-line controls (18). First-line controls consist of the internal controls that ensure the accuracy of each assay (e.g. “no template” negative PCR control, a positive cell line DNA control for k-ras mutation assay, etc.). Second-line controls refer to local proficiency testing and quality monitoring. Examples of this would include giving a technician two aliquots of the same colon cancer to test for microsatellite instability to ensure the reproducibility of the assay, or supplying all technicians with the same cohort of known samples for k-ras mutation analysis to document the consistency of the entire laboratory.

It is also most desirable for the laboratory to participate in third-line controls. This refers to the analysis of externally validated samples. This typically takes place under the aspect of external proficiency testing under the auspices of an accrediting agency or organization (19). Unfortunately for many emerging molecular diagnostic tests, the clinical implementation of tests may outpace the availability of external proficiency testing from accrediting organizations. Such challenges have been faced in the past by clinical labs performing genetic testing, and which have been addressed by cooperative sample sharing between laboratories, or by quality assurance materials being validated and made available to members of regional cooperative groups. Similar arrangements may be made between cooperating molecular AP labs.

The issue of external proficiency testing is important in this area. As mentioned, molecular diagnostics is an emerging field. The result of this fact is that diagnostic tests for the same analyte are carried out using varying technologies, protocols and materials. This invariable will result in assays with varying sensitivity and specificity being deployed. The availability of external quality assurance materials being supplied in a cooperative, non-punitive manner is essential to determine for the field what technology and protocols yield the most accurate results, and to have these findings being disseminated widely.

To close with an evidence-based discussion of this topic, results from two reports of the experience of the Molecular Oncology Survey of the College of American Pathologists will be presented here. In the first study, the detection of the t(14;18)(q32;q21) translocation in samples of follicular lymphoma (FL) by PCR analysis of genomic DNA was studied (20). 24 well-characterized specimens were sent to participating laboratories in a proficiency testing program between 1997-2000 in which 25 to 61 laboratories participated annually. During the course of this program, 819 major breakpoint region and 323 minor cluster region determinations were performed, with an overall correct response rate of 91% and 94%, respectively. It was determined that no significant differences were detected in the detection rates between frozen and FFPE tissue samples, which confirms the previous assertion of DNA stability in this document. Interestingly, despite the overall good performance of the labs, in response to a survey of laboratory methods it was determined that no two laboratories in the survey carried out the analyses using exactly the same method, with the design of PCR primers showing the widest heterogeneity. This underscores the variability in technique inherent to emerging molecular diagnosis. The major deficiency that the survey uncovered was that most laboratories had not determined a sensitivity threshold for their assay. However, it was actually the laboratories that had determined the greatest level of sensitivity that reported false positive results, suggesting that “pushing” the sensitivity of PCR-based analysis may result in false positive results.
The second report deals with the identification of immunoglobulin heavy chain (IGH) clonal gene rearrangement in the diagnosis of B cell lymphoproliferative disorders and malignancies (21). 39 well-characterized specimens were sent to participating laboratories in a proficiency testing program between 1998-2003 in which 161 laboratories participated. 944 results were reported using Southern blot technology, with a success rate of 95%. 2349 results were reported using PCR methodology, with a success rate of 72%. Since PCR methodology also varied widely in this survey, it could be determined that a specific grouping of genomic primers yielded a greater success rate (82%). It is of interest that though Southern blot technology appears to be a superior method, there appears to be wider spread adoption of PCR based methodologies. This may be due to the fact that PCR methodologies require less technician time and is a simpler procedure. PCR based methods also allow the analysis of FFPE material, which generally are precluded from Southern blot analysis. However when matched frozen and FFPE tissues were evaluated side by side by PCR analysis, the success rate for frozen material was 78% and for FFPE material was 66%. Participation in external proficiency testing such as this, with dissemination of results, is important for the diagnostic community to determine optimal protocols and techniques, and for diagnosticians and clinicians to understand the limitations of the assays when results are used in clinical decision-making.

References


Quality, Patient Safety, and Error Reduction in Molecular Diagnostics

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University of Virginia
Disclosure

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• Additional salary support from NIH grants
• Grant support from the National Institutes of Health, Department of Defense, Virginia Tobacco Settlement Foundation, Adenoid Cystic Carcinoma Research Foundation and the National Organization of Rare Disorders
• No support from or privately-held interest in biomedical/health/pharmaceutical industry
Learning Objectives

• Understand how procurement issues & target heterogeneity in tissue samples impacts the quality of molecular diagnostics
• Understand how protocol complexity & non-uniformity in molecular diagnostics can lead to errors
• Understand how application of quality assurance & quality control procedures in molecular diagnostics can reduce error
Agenda

• Quality
  – Pre-analytic considerations for tissue samples
  – Tissue heterogeneity & target selection
• Patient Safety / Error reduction
  – Issues inherent to emerging technology that may contribute to sample misidentification
• Error reduction / Standardization of analytic techniques
  – Typical QA/QC must be applied to AP molecular diagnostics
Focus on “non-in situ” techniques

Specimen

Samples

FFPE

Fresh frozen

Histologic section

Extract biomolecules (DNA, RNA, protein)

Solution or gel-based assays
-RT-PCR
-GeneChips™
-array CGH
-microsatellite PCR
-mass spectroscopy
-Western blots
-Northern blots
-Southern blots
-etc.

in situ assays
-immunohistochemistry
-in situ hybridization
-in situ PCR/RT-PCR

Specimen

Extract biomolecules (DNA, RNA, protein)
Pre-analytic parameters: Target degradation

• Anatomic Pathologists are aware of tissue autolysis that degrades histologic analysis
  – Hours to days depending on tissue type and temperature
  – Formaldehyde-fixation stabilizes tissue architecture and cell morphology
• Current AP clinical practice concerning tissue handling is based on this “leisurely” time table and on the almost universal use of formalin
## Pre-analytic parameters: Target degradation in FFPE tissue

<table>
<thead>
<tr>
<th></th>
<th>Formalin-fixed paraffin-embedded</th>
<th>Fresh/Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleic acids</strong></td>
<td>Cross-linked, sheared to ~200 base pair (bp)</td>
<td>Intact (thousands to millions of bp)</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td>Cross-linked, denatured</td>
<td>Intact</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Excellent, best for clinical analysis based on morphology</td>
<td>Adequate for some but not all clinical assessments</td>
</tr>
<tr>
<td><strong>Storage of archival samples</strong></td>
<td>Cheap, common</td>
<td>Expensive, uncommon</td>
</tr>
</tbody>
</table>
Pre-analytic parameters:
Molecular target degradation

• Many targets of molecular assays are labile!
  – Post-translational protein modification (phosphorylation, etc.)
    • minutes-hours
  – Levels of regulatory protein
    • minutes-hours
  – Levels of RNA transcripts
    • minutes-hours
• DNA is an exception
Pre-analytic parameters: Myriad & some “uncontrollable”

Tissue in patient

Resected tissue (clinical area) → Tissue (received in lab) → Sample stabilized → Sample analyzed

Surgical devascularization: - time variable - variable tissue sensitivity

Transport - time variable - temp. variable - stabilization method variable

Processing - time variable - temp. variable - fixation time

Storage - stabilization method variable - temp. variable - time variable

Rate of degradation (generalities): Rapid increase immediately after devascularization. Rate stabilizes or decreases for a few hours, especially if specimen cools. Rate then rapidly increases as cell membranes breakdown.
Pre-analytic parameters: Testing of specimen integrity

• Degradation in some samples will be unavoidable

• Assessment of overall molecular integrity or specific target integrity
  – Pre-analytic test
  – Integrity assessment may be incorporated into the analytical procedure

• Rejection of specimen/analytic results based on tissue/analyte quality
RNA integrity tests

- RNA integrity is one of the most common “pre-analytic” tests performed.
- Integrity of 28s & 18s ribosomal RNA is commonly used as a global assessment.
- Microcapillary electrophoresis.
Examples of intra-assay specimen integrity checks

• Threshold level for qRT-PCR of translocation gene product
  – Upper limit set for $C_t$ of housekeeping gene

• PCR amplification of c-kit gene for sequencing
  – Minimum PCR product concentration after set number of PCR cycles
Pre-analytic parameters: Recommendations

- Review & streamline specimen transport & handling
  - May require increase in personnel & changes in work flow patterns
- Create triage system that identifies specimens requiring molecular analysis
- Integrate early specimen stabilization as much as possible
  - Freezing
    - LN$_2$ & -80°C ~equivalent, -20°C inappropriate
    - Stabilization solutions (probably not formalin!)
Pre-analytic parameters: Tissue heterogeneity

- Gross assessment does not accurately predict tumor cellularity and viability
- Major impediment to quantitative assays of molecular constituents

Sampling of same ovarian tumor

Areas of >70% tumor cellularity circled
Target selection: Histology guided macrodissection

- Frozen tissue embedded in OCT
  - Frozen histologic section taken and stained. Examined microscopically & areas enriched for target cells are circled.
  - Using the histologic section as a guide, the selected tissue is dissected from the OCT block.

Alternatively, selected areas of tissue can be scraped from several histologic sections.

Biomolecules are extracted from dissected tissue.
Target selection: Laser microdissection

- Several platforms & technologies
- Capture/propulsion/dissection of tissue from histologic section with direct microscopic visualization
  - Precise capture of target tissue
  - Very small sample size
- Laborious, cumbersome & finicky technique
  - Difficult to deploy in high throughput clinical setting with adequate cost-recovery
Pre-analytic parameters: Tissue heterogeneity

• Every tissue based molecular assay must in its development phase be tested for tolerances of “non-target” cells
  – Mixing experiments with tissue culture models
• Knowledge of these tolerances must be then applied in the sample input in the clinical testing phase
Patient Safety: Sample misidentification

Typical RNA isolation:
4 tube exchanges

1. Tissue is lysed & EtOH added
2. Place in spin column & centrifuge
3. Add wash buffer & centrifuge
4. Add elution buffer & centrifuge

- Many molecular techniques have attributes that can be sources of mislabeling error
  - Manually performed
    - Manual container labeling
  - Many container changes
  - Manual loading of gels/instruments
  - May be performed in hybrid research/clinical labs with varying standard operating procedures being performed in parallel
Patient Safety: Sample misidentification

• Remedies
  – Use robotic platforms whenever possible
    • Biomolecule extraction technology most robust at present time
  – Use computer-generated human-readable and bar coded labels whenever possible
    • Complexity of labeling software & hardware and variability in number and size of protocol containers are major obstacles
  – Quality assurance practices
    • Personnel training and retraining
    • Quality checks of protocol adherence
Quality issues in AP Molecular Diagnostics

• Many (most?) assays are:
  – newly emerging technologies
  – or are established research techniques being newly applied to anatomic pathology (emerging application)
Quality issues inherent to emerging technologies

• Protocols derived from research laboratories are focused on assay development
  – Manual
  – Often multi-step
  – Prone to being “operator-dependent”
  – Instrumentation tends to be “highly-tunable”, “tweakable”, hence complex
  – Emphasis is *not* on run-to-run reproducibility
Quality issues inherent to emerging technologies

• For the same analyte:
  – Different detection methodologies may be used by different laboratories
  – Even if the same basic detection method is used, protocols, reagents and instrumentation differ widely
Error elimination: QA/QC

• Quality Assurance (QA)
  – The policies, procedures and systematic actions established for the purpose of providing and maintaining a high quality of test integrity and accuracy.
  – Most standard QA procedures in place for clinical pathology laboratories apply to molecular diagnostics for anatomic pathology specimens

• Quality Control (QC)
  – Techniques that monitor performance parameters of individual assays
  – Are specific to the assays being performed
Typical QA/QC procedures

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</table>

Documentation

• Standard Operating Procedures (SOPs)
• Assay logs
• Maintenance records
• Training records
25

Error Reduction:
QC/QA validation of analysis

• 1st-line (internal) controls
  – Check of results of each assay performed
    • e.g. “no template” negative PCR control
    • e.g. positive cell line DNA control for k-ras mutation assay

• 2nd-line controls (internal proficiency testing, quality monitoring)
  – Check on the spread of results produced in a lab
    • e.g. compare results of DNA isolation and microsatellite instability testing from adjacent sections of a colon cancer specimen
    • e.g. all technicians in a lab given known positive and negative samples of lung cancer for EGFR mutation analysis
  – Needs to be performed in validation-deployment phase of assay, and then periodically post-deployment
Error Reduction: QC/QA validation of analysis

- 3rd-line controls (external proficiency testing, quality monitoring)
  - essentially same as 2nd-line controls except performed with externally-validated materials
  - Often in context of accreditation
    - CAP is major source in U.S.A. for molecular genetic testing
  - Not available for all analytes
Error Reduction: Validation of analysis

- In the absence of validated reference materials from an accreditation agency:
  - Specimen sharing between collaborating laboratories
  - Development of external quality assessment (proficiency testing) materials by regional consortia groups
Summary

- Tissue handling procedures must be optimized for preservation of molecular targets
- Integrity of tissue specimens should be determined pre-analytically or concurrently
- Target selection or assessment by histologic analysis should be incorporated in most cases
- Protocol procedures should be optimized to prevent specimen misidentification
- A quality assurance program must be put in place to monitor assay performance and adherence to standard operating procedures
Pearls of Pathology

• Garbage in - garbage out
  – Molecular analysis of tissue in the absence of histologic input is fraught with problems of target cell validation

• Don’t forget to take out the garbage
  – The rigorous practices of quality control and quality assurance that are well established in standard clinical pathology practice must be transferred to new molecular assessments of tissue specimens
Questions?

Relevant quotations from Yogi Berra:

If you ask me anything I don't know, I'm not going to answer.

There are some people who, if they don't already know, you can't tell 'em.

I never said most of the things I said.
Thank you for participating!

Please be sure to complete the course evaluation online after the conference.

It ain't over till it's over.

Yogi Berra

It’s over!