Breast Cancer Predictive Factor Testing:

The Challenge and Importance of Standardizing Pre-Analytic Variables

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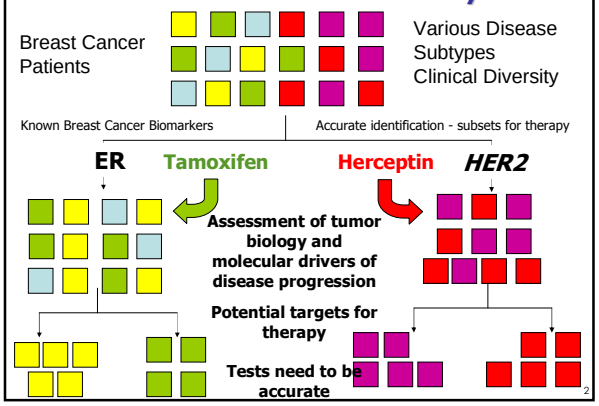
Breast Cancer Diversity



Introduction of Biomarkers into Clinical Practice: (ER, PR, HER2 and Beyond)

- Paradigm shift in breast cancer diagnosis
- Tissue samples no longer used only for microscopic interpretation
- With introduction of breast cancer predictive assays, tissue becomes an analyte
  - Accuracy, precision and quantitative read-out clinically important
- Specifications of tissue quality
  - **Important element of validated assays**
  - **Standardization important component**

Goldstein, NS. Am J Clin Pathol. 2010;133:681-83

3

Standardized Predictive Receptor Assays: *Begins with Tissue Handling*

- Breast resection specimen should be placed in fixative **as soon as possible** after excision
- **Challenges**
  - Excised specimens may need specimen radiograph
    - Insure all Ca^{++} and biopsy clip removed
  - Specimen should be oriented by surgeon
  - Carefully ink for margin assessment & sectioned for gross exam
- Specimen should be placed in formalin within **<1hour*** from removal to facilitate initiation of tissue fixation (good morphology & biomarker testing)

**ASCO/CAP ER, PR Testing Guidelines JCO and Arch Path Lab Med 2010*

Specimen Handling and Tissue Ischemia

- Clinical use of biomarkers has led to increasing emphasis on optimal tissue preparation (Sherman 2010)
- Surgical disruption of blood flow
  - Progressive tissue ischemia, hypoxia and the degradation of macromolecules
  - Cold ischemic time - specimen removal to specimen fixation
  - Nucleic acid and protein changes occur during this interval (Hewitt 2008)
- Current clinical practice of tissue handling and specimen preparation is diverse and lacks strict standardization
  - Significant variability in the quality of formalin fixed paraffin embedded clinical samples
  - Ischemic interval can vary from minutes to hours

Specimen Handling and Tissue Ischemic Time

- Magnitude of ischemic changes and effects on tissue quality are poorly understood
  - Differing lability for DNA, RNA, proteins, phospho-proteins
  - Some of these molecules are potential biomarkers and therapeutic targets
- How much variability in tissue handling exists is unknown in most institutions
  - How much is to much?
  - What is tolerable for accurate biomarker evaluation?

★Tissue Handling Project
★Goal of the Project: Standardize tissue handling

U of R Specimen Handling Project

- **Step 1, Establish Baseline:** Perform audit of time from tissue collection to fixation start time (2 weeks)
  - Specimen transported to tube station by OR nurse at end of each case and sent to pathology
  - Baseline tissue handling procedure and degree of variability
- **Step 2, Intervention:** Pathology lab personnel where place in OR with cell phone
  - When specimen available, pathology staff notified
  - Staff would go to OR to acquire specimen, check all requisitions and labeling and transport ASAP to pathology

Comparison: Baseline Process vs Active Tissue Retrieval

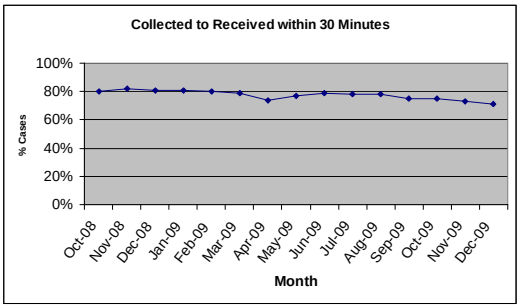
	Baseline specimen monitoring (nurse transport)	Trial with pathology technician pickup (pathology transport)
Collection to receipt in lab (average, minutes)	120 min.	24 min.
Collection to fixation (average, minutes)	138 min.	41 min.

U of R Specimen Handling Project

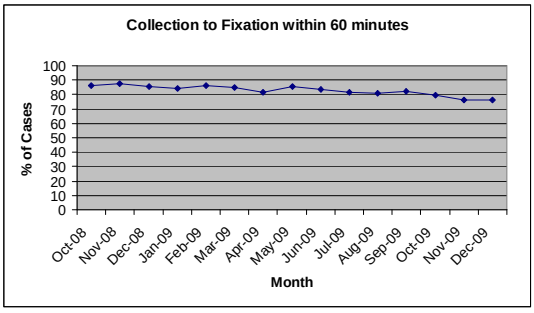
- **Step 3, lobby for change:** Met hospital administration to lobby for additional FTE for development of standardized rapid tissue acquisition
  - Improve quality of specimens for clinical diagnosis and translational research
  - Patient safety issue (OR runner could indentify error in specimen labeling and have them corrected real time before leaving OR)
  - Addition of 2 FTE to actively collect specimens from OR for transport to pathology
  - ***APPROVED!***

MONTH	DATES	AVERAGE TIME FROM COLLECTION TO RECEIVE	AVERAGE TIME FROM COLLECTION TO FORMALIN	AVERAGE TIME FROM RECEIVED TO FORMALIN
October	2008	0:24	0:39	0:14
November	2008	0:29	0:39	0:15
December	2008	0:22	0:39	0:17
January	2009	0:36	0:52	0:16
February	2009	0:27	0:48	0:21
March	2009	0:28	0:43	0:16
April	2009	0:31	0:46	0:16
May	2009	0:25	0:42	0:18
June	2009	0:24	0:41	0:17
July	2009	0:26	0:43	0:16
August	2009	0:25	0:43	0:17
Sept.	2009	0:28	0:47	0:18
October	2009	0:27	0:43	0:15
Live Average		0:27	0:43	0:16

Percentage of Specimens Received within 30 Minutes from Collection



Percentage of Samples Placed in Formalin within 60 minute from Collection



Patient Safety Component: Catching Error at the OR Door

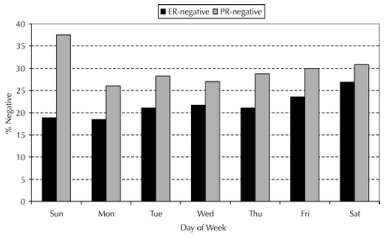
8 Point Patient Safety Check (Done at time of Pick-Up)		
1	√	Biopsy site on container and requisition correspond and are highlighted correctly
2	√	One requisition per specimen is provided
3	√	Specimen label matches requisition label
4	√	Ordering doctor is present
5	√	Clinical history is complete
6	√	Collection time is provided on requisition
7	√	OR number is listed on requisition
8	√	OR requisition has the Pathology watermark

How Important is Tissue Handling for Breast Cancer Testing?

- Delay to start of formalin fixation may adversely affect ER & PR and HER2 analyses
  - Test results determine how BC patients are treated
    - Only ER+ patient's receive hormonal therapy
    - Only HER2+ patients receive HER2-targeted therapy
  - Invalid results could significantly alter the type of adjuvant therapy a patient receives
- **Selecting the wrong adjuvant treatment has the potential to adversely impact patient outcome**

Elements of ASCO/CAP ER Guideline: Time to Fixation

- Why is the time to fixation (<1 hr) important?
  - To **prevent loss of ER activity**, stop the cellular process that destroys the ER during ischemia
    - ER more labile and sensitive to ischemia than HER2 protein
  - *"The test begins in OR when tissue is removed from the patient for processing"* (Dr. Elizabeth Hammond)
- **Higher ER-negative results on cases done late in week**
  - Tissue handling more variable
  - Time to fixation often delayed



5077 women with breast cancer among the study hospitals were tested for ER/PR between 1997 and 2003 in central lab

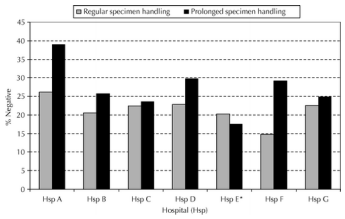
Frequency of ER and PR negative test results by day of surgery

Day	Cases	ER-Negative	PR-Negative
Sunday	16	3	6
Monday	1252	230	325
Tuesday	1176	248	332
Wednesday	784	170	212
Thursday	904	191	259
Friday	919	216	276
Saturday	26	7	8
System	**5077**	**1065**	**1418**

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

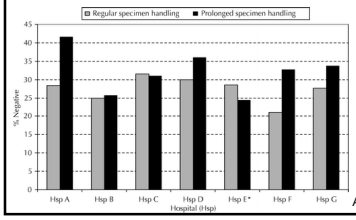
Frequency of ER/PR negativity significantly increased with each day of the week, both for ER ($P = 0.03$) and PR ($P = 0.059$ for trends).

Arch Pathol Lab Med. 2010;134:606-612



Frequency of estrogen receptor–negative test results by hospital and by specimen handling group

* Surgical specimens removed and tested in-house at the reference laboratory



Frequency of progesterone receptor–negative test results by hospital and by specimen handling group

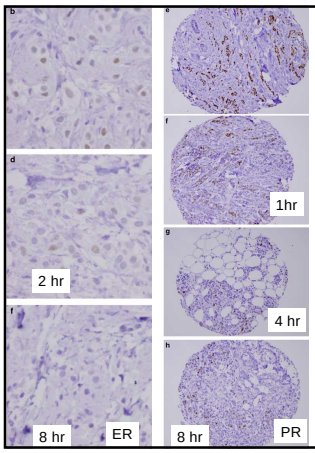
* Surgical specimens removed and tested in-house at the reference laboratory

Arch Pathol Lab Med. 2010;134:606-612

How Important is Tissue Handling for Breast Cancer Testing?

- Effects of progressive delay to formalin fixation, breast cancer biomarkers
  - 10 large invasive breast cancers excised, underwent immediate gross evaluation
  - Tumor tissue procured and divided into eight equal parts
    - Consecutively fixed after:
      - 0, 10, 30 min,
      - 1, 2, 4, and 8 h
      - One section was kept in saline and stored overnight at 4° C.

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67



ER, PR Testing by IHC

Mean Q score started to decline 2 h mark for estrogen receptor and 1 h for progesterone receptor

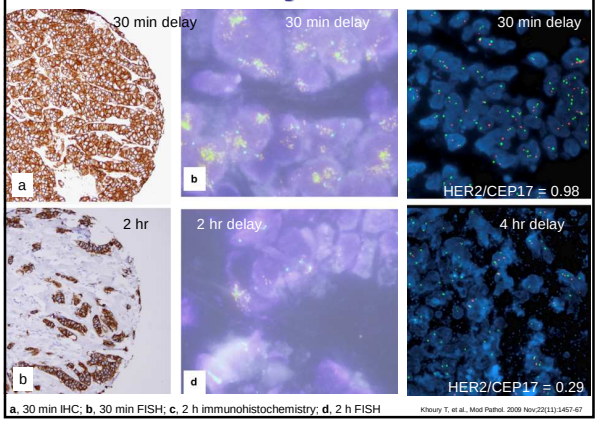
ER expression case # 1
b - 0 min, Q score 3
d - 2 h, Q score 2
f - 8 h, Q score 0

PR expression case # 8
e - 0 min
f - 1 h
g - 4 h
h - 8 h

Note decreased number of positive cells with the time of delayed fixation

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

HER2 Testing IHC and FISH



a, 30 min IHC; **b**, 30 min FISH; **c**, 2 h immunohistochemistry; **d**, 2 h FISH

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

How Important is Tissue Handling for Breast Cancer Testing?

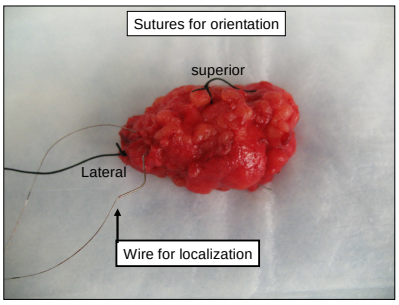
- Conclusion: delays to formalin fixation can negatively impact breast biomarker testing
  - Delays impacted both IHC and FISH
  - Effects on FISH > IHC for HER2 testing
  - Recommend start of formalin fixation within 1 hour from time of sample collection

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

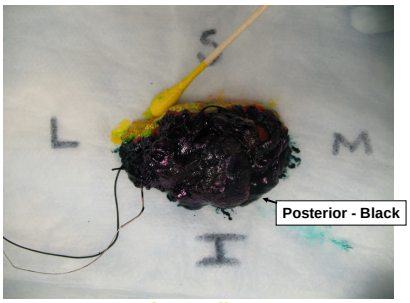
Standardized Breast Tissue Handling in the Pathology Laboratory

- Getting the tissue to the lab quickly is not enough
- Needed to change procedures in gross room to assure specimen promptly evaluated and placed into formalin as soon as it was available
- Requires immediate gross assessment for breast samples

Breast Lumpectomy: Specimen Orientation

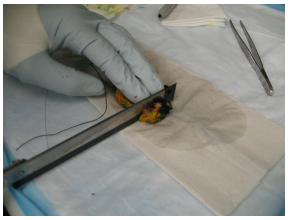
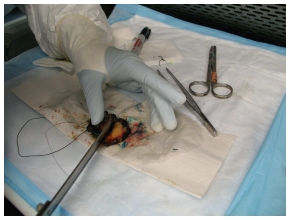


Different Colored Ink for Assessment of Surgical Margins



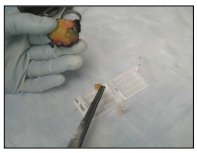
Superior - Yellow

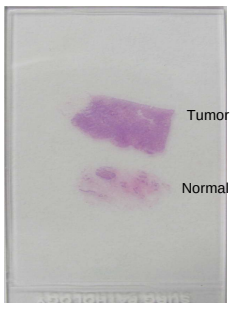
Specimen 'Bread-loafed': Long Axis (0.2 - 0.5 CM)

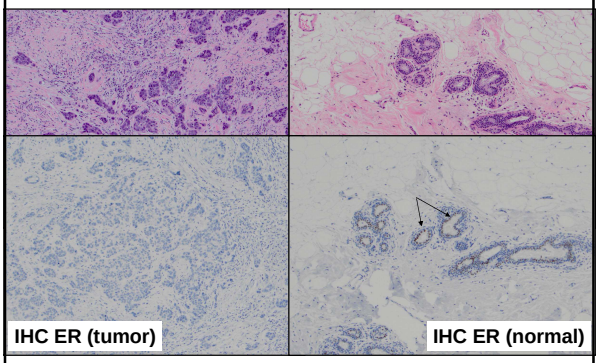
Tumor/Normal Block

(take at the time of immediate gross assessment)





Tumor/Normal Tissue Block: Collected at Time of initial Gross Assessment



Tumor & fibrous normal breast from same patient fixed & processed in identical fashion

New ER Guideline Require Recording Time Points for Tissue Handling

Required **3 time points be recorded for each specimen** and available when you sign out report so time to fixation will be known

1. Time tissue is removed (OR staff to record)
2. Time tissue is received in grossing room
3. Time tissue was placed in fixative

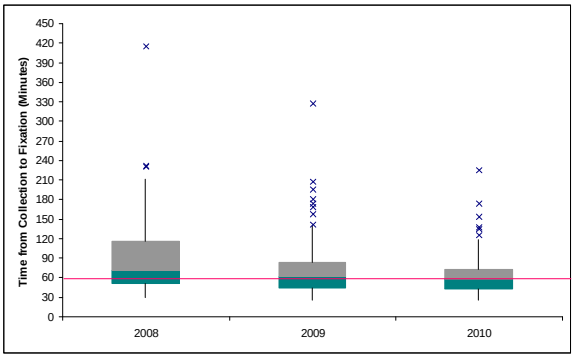
These time point will help in trouble shooting unexpected test results

*Standard procedure at URMC Medical Center for over one year for all OR specimens

Is the 1 Hour Time Window Feasible?

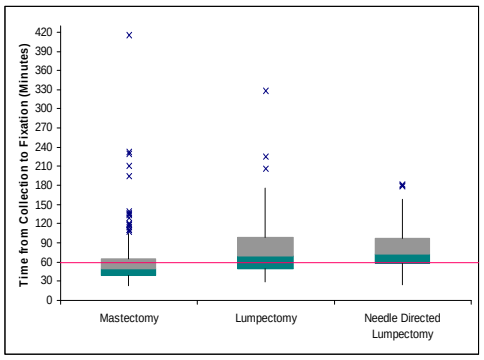
- 2008 to 2010 collection and fixation start times breast cancer specimens were analyzed (n=361)
- 3 Breast surgeons performed the majority of the procedures (343/361)
  - Mastectomies (n=139)
  - lumpectomies (n=91)
  - needle directed lumpectomies (n=131)
- Median collection to formalin time
  - 2008 was 72 minutes
  - 2009 was 62 minutes
  - 2010 was 58 minutes

Time to Fixation: All Specimens



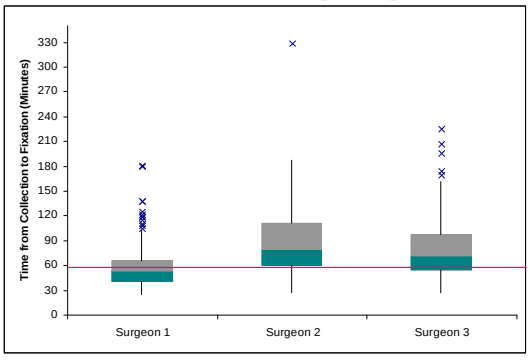
2008 and 2009 (p=.0094) and 2008 and 2010 (p=.0073)

Time to Fixation by Specimen Type



Mastectomies and Lumpectomies (p<.0001)
Mastectomies and Needle-Directed Lumpectomies (p<.0001)

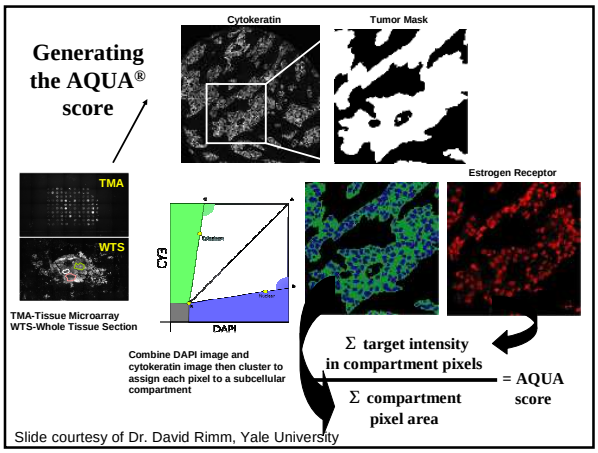
Time to Fixation by Surgeon



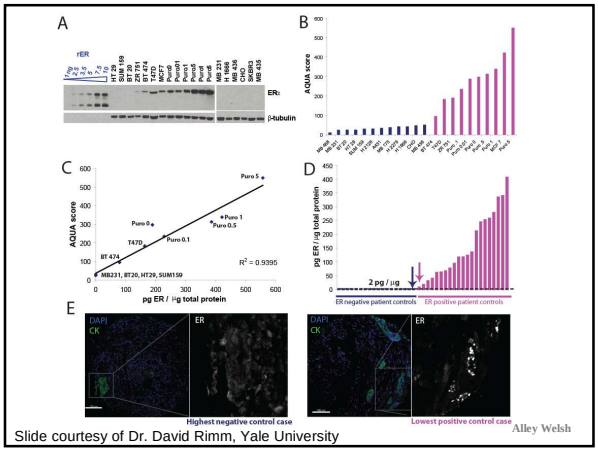
Surgeon 1 and Surgeon 2 (p<.0001)
Surgeon 1 and Surgeon 3 (p<.0001)

Collaboration With Yale University

- Collection time, lab receipt & fixation start time recorded for all breast resections at URMC (2 y)
  - Ischemic interval for each specimen can be easily calculated
  - Needle core biopsies for subset of patient also available (placed immediately in formalin)
    - Represent time zero
- Discussion with Dr. David Rimm lead to a collaboration to study these cases
  - Can we identify reference proteins that degrade in a predictable fashion?
  - Can these reference proteins be used to normalize potential target protein?
  - Assessment using automated quantitative immunofluorescences (AQUA Technology)



Slide courtesy of Dr. David Rimm, Yale University



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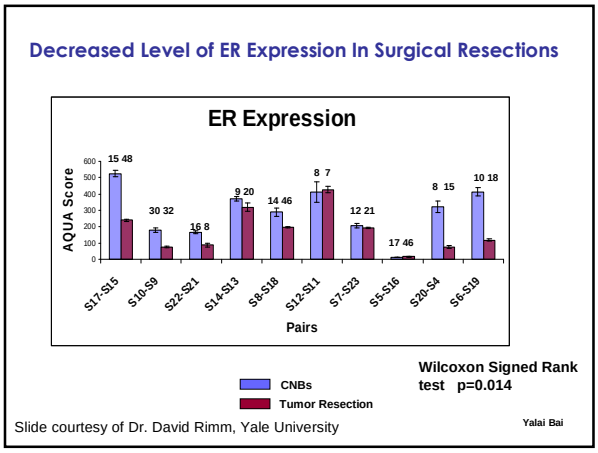

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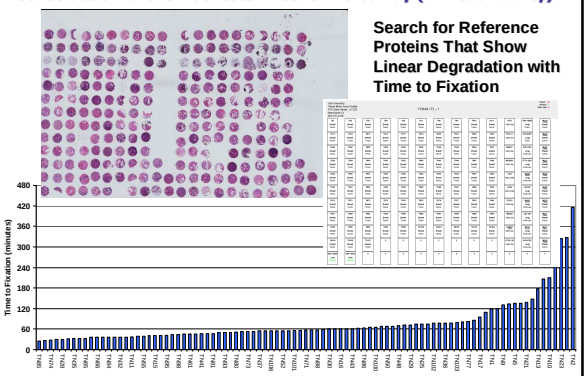
Construction of the Rochester Tissue Microarray (2x redundancy)



Search for Reference Proteins That Show Linear Degradation with Time to Fixation

Two fold redundancy
N=125 , tumor=93, normal=2, cell lines=10 control breast tumor=10 ,control lung tumor = 10

Diagnosis and Treatment Planning is Changing Challenge/Opportunity for all Pathologists:

Be an Agent of Change for Breast Cancer Testing

- Be interested in the whole test, not just your part
- Think about the possibilities for change and improvement as opportunities instead of obstacles
  - Be visible and plan interactions where insights and knowledge can be shared
  - Be observant and explore options to share with the patient care team
- Take the lead in suggesting ways to define and implement best practices

Standardizing Time to Fixation

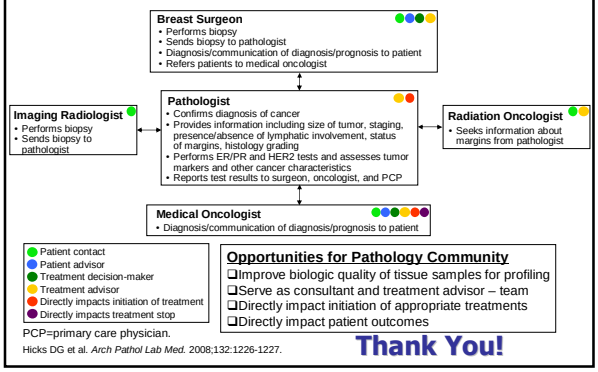
*Need to get tissue from OR to Lab as soon as it is available
Strategies for overcoming barriers:

Barrier	Strategy
Other staff not within my control	Involve them in the problem-solving process, give them co-ownership of the problem
I/Others don't see the importance	Educate others, show data that testing is affected by delayed fixation
Samples removed at odd times	Create an SOP, communicate its importance, and ensure it is followed
Samples removed at remote/other locations	Create an SOP, communicate its importance, and ensure it is followed
(All barriers)	Stress importance ER & HER2 testing for adjuvant treatment

•Requires good communication and collaboration between lab, OR and breast clinics
•Pathology needs to take the lead in helping to define best practices

•*Opportunity for new technology and innovation

Breast Cancer Treatment: Multidisciplinary Team Approach



- Patient contact
- Patient advisor
- Treatment decision-maker
- Treatment advisor
- Directly impacts initiation of treatment
- Directly impacts treatment stop

Opportunities for Pathology Community

- ❑Improve biologic quality of tissue samples for profiling
- ❑Serve as consultant and treatment advisor – team
- ❑Directly impact initiation of appropriate treatments
- ❑Directly impact patient outcomes

PCP=primary care physician.

Hicks DG et al. *Arch Pathol Lab Med.* 2008;132:1226-1227.

Thank You!