COLLEGE of AMERICAN PATHOLOGISTS

What's New in the 2020 Update to the CAP/ASCO ER/PR Testing Guidelines in Breast Cancer?

American Society of Clinical Oncology (ASCO) / CAP Guideline Update Webinar

Kimberly H Allison, MD, FCAP

February 26th, 2020

Webinar Host

- **This series is sponsored by the Personalized Healthcare Committee (PHC).**
- **Today's webinar host is Jason Rosenbaum, MD**

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- **This presentation will be recorded. The recording and PDF will go out to all registrants in one week**
- **All lines are muted during the presentation**
- **Please send in your questions as you think of them via the "Question Box" in your control panel**

Kimberly Allison, MD, FCAP

- **Director of Breast Pathology and Professor of Pathology at Stanford University of Medicine.**
- **Has a special interest in development of high-quality diagnostic standards and is active in setting practice guidelines and patient communication.**
- **Actively involved in resident/fellow training as Director of the Stanford Breast Pathology Fellowship and Residency Director for the Department of Pathology.**

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Learning Objectives

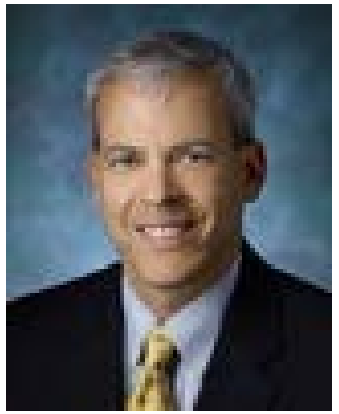
- **Identify new aspects of the CAP/ASCO ER/PR testing in breast cancer guideline updates that affect hormone receptor standard testing operating procedures, interpretation and reporting for invasive breast cancer and ductal carcinoma in situ.**
- **Review how to apply the new recommendations in specific ER/PR testing patient care scenarios.**

Also: To Answer your Frequently Asked Questions!

Why Update Now?

- **CAP and ASCO agreed to partner to develop guidelines starting with HER2 testing in breast cancer in 2007**
- **After this successful venture, the ER/PgR guideline was jointly published in 2010**
  - **2,800+ unique citations in publications from more than 99 different countries.**
  - **Now 1,400+ labs participate in CAP PT for ER/PgR**
- **Two updates to HER2 testing guidelines (updates in 2013 &2018)**
- **10 years since initial ER/PgR guideline (2010)**
  - **More recent data on 1-10%/"low positive" ER cases**
  - **Is 1% threshold for positive still the most clinically relevant?**

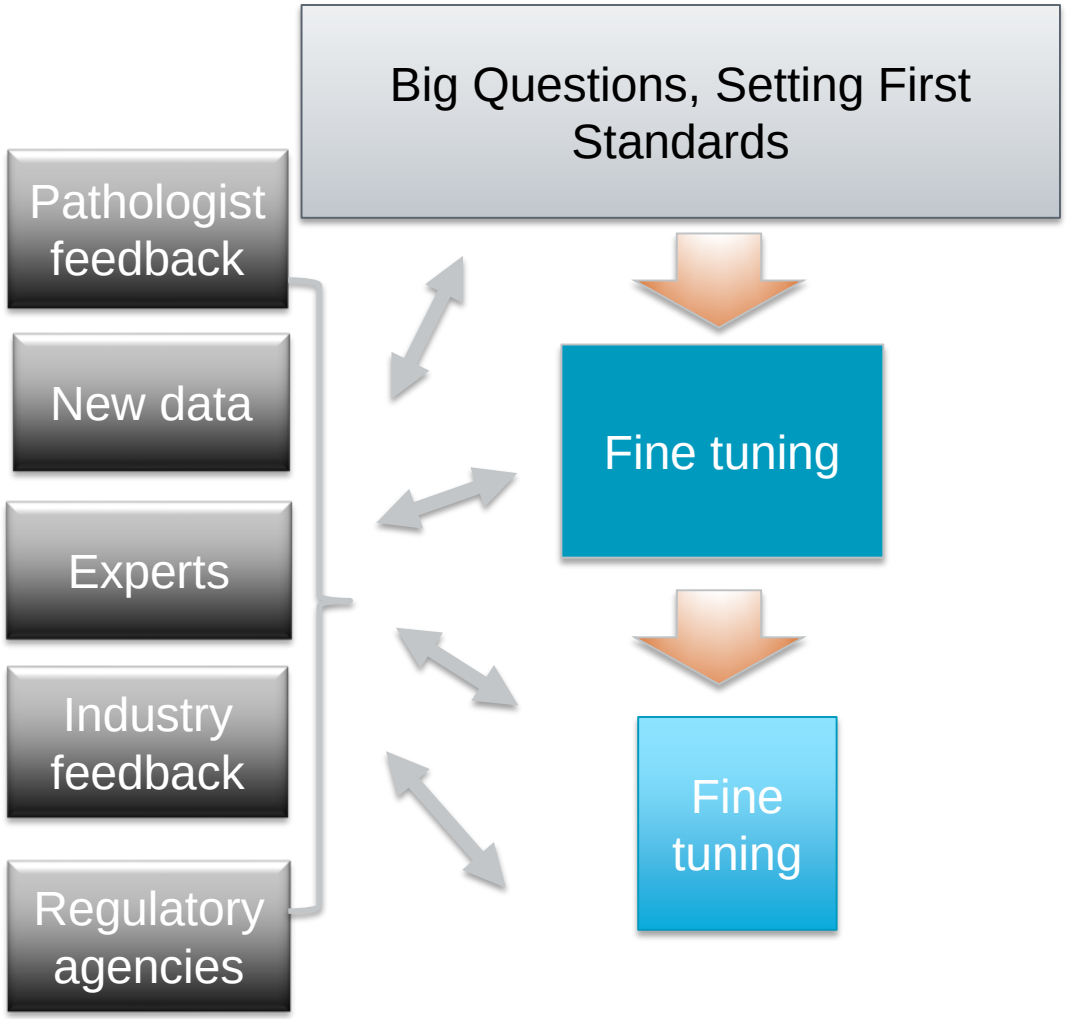
Dr. Elizabeth Hammond



Dr. Antonio Wolff

Guidelines in Breast Cancer are Living Documents

- **First focused on big questions and standards for all cases**
- **Subsequent updates based on new data, feedback**
- **Fine tuning, often focused on less common scenarios**



Has there been improvement in ER/PgR testing since the 2010 Guidelines?

Where and how can additional improvements be made?

ER/PgR Testing in 2010

- **No single national/international guideline**
- **Only some countries had QA/PT systems tracking data (UK-NEQAS, Australia, NordicQC)**
- **Canadian ER testing controversy (~40% false negative rate)**
- **Variability between local vs central labs: Tables 3-6 from 2010 guidelines**
- **Issues:**
  - **Long ischemic times, fixation variable**
  - **Variability in thresholds used for positive**
  - **Lack of appropriate internal or external positive controls**
  - **Labs without sufficient expertise in IHC methods, QA or validation**

Study of Interlaboratory Reliability and Reproducibility of Estrogen and Progesterone Receptor Assays in Europe

Documentation of Poor Reliability and Identification of Insufficient Microwave Antigen Retrieval Time as a Major Contributory Element of Unreliable Assays

Anthony Rhodes, PhD,[1] Bharat Jasani, PhD,[2] Andre J. Balaton, MD,[3] Diana M. Barnes, DSc,[4] Elizabeth Anderson, PhD,[5] Lynda G. Bobrow, FRCPath,[6] and Keith D. Miller, FIBMS[1]

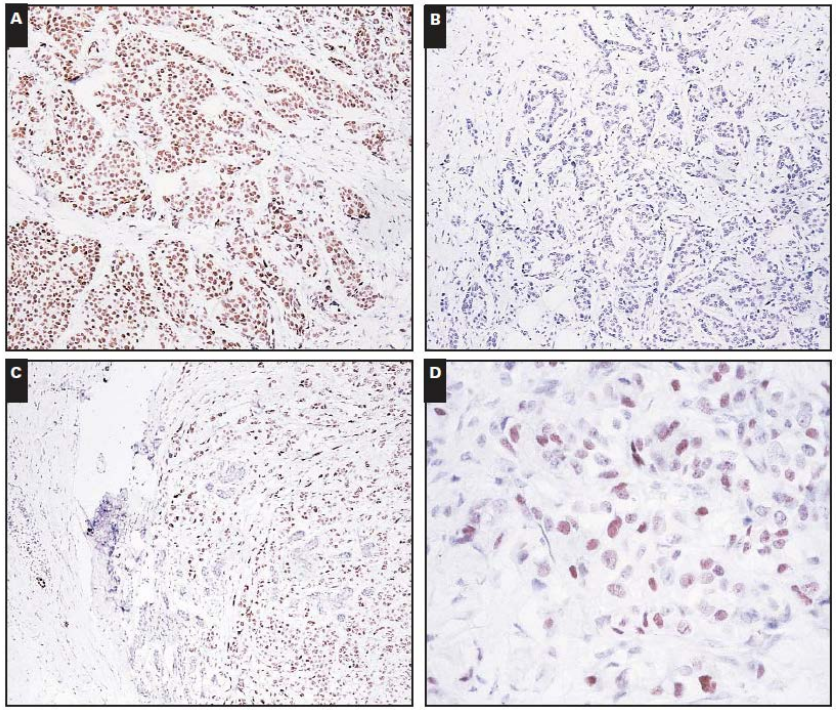
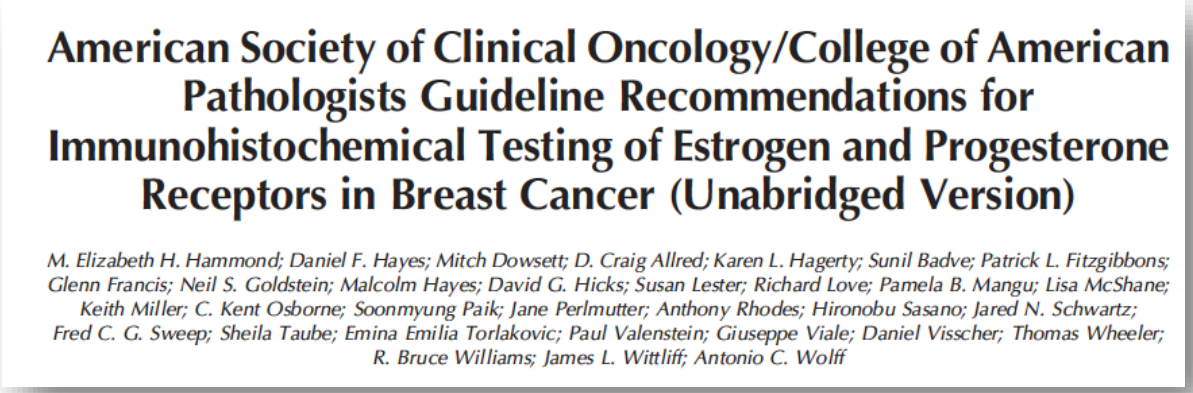


Image 1 Optimal demonstration by the organizing laboratory of the 3 tumors used in the microwave antigen retrieval study (clone 1D5 [Dako, Ely, England], pressure-cooker pretreatment, and *Streptomyces* avidin biotin complex detection). **A**, The relatively high estrogen receptor (ER)-expressing infiltrating ductal carcinoma (IDC) (×100). **B**, The ER-negative IDC (×100). **C**, The low ER-expressing IDC (×100). **D**, The low ER-expressing IDC (×400).

Am J Clin Pathol 2001;115:44-58

2010 Guidelines Set New Standards



American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer (Unabridged Version)

M. Elizabeth H. Hammond; Daniel F. Hayes; Mitch Dowsett; D. Craig Allred; Karen L. Hagerty; Sunil Badve; Patrick L. Fitzgibbons; Glenn Francis; Neil S. Goldstein; Malcolm Hayes; David G. Hicks; Susan Lester; Richard Love; Pamela B. Mangu; Lisa McShane; Keith Miller; C. Kent Osborne; Soonmyung Paik; Jane Perlmutter; Anthony Rhodes; Hironobu Sasano; Jared N. Schwartz; Fred C. G. Sweep; Sheila Taube; Emina Emilia Torlakovic; Paul Valenstein; Giuseppe Viale; Daniel Visscher; Thomas Wheeler; R. Bruce Williams; James L. Wittliff; Antonio C. Wolff

Arch Pathol Lab Med—Vol 134, July 2010

- **Issues Addressed:**
  - Long ischemic times, fixation variable → **recommendations made to standardize and track/report**
  - Variability in thresholds used for positive → **standard set at 1% for positive**
  - Lack of appropriate internal or external positive controls → **required reporting of and recommendations for rejection/retesting**
  - Labs without sufficient expertise in IHC methods, QA or validation → **CAP accreditation requirements set for Validation and Proficiency Testing, CanadianIQc, etc.**

ER/PgR Testing since 2010: How are we doing?

- **Look at Clinical Trial Data on Local vs Central Test Results:**
  - **Local testing was routine per local lab standards without anticipation of central re-testing (not a "test") and on whole sections**
  - **ER false negatives still a problem in "triple negative trials"**
  - **Drawbacks: Trial population dependent/case selection, blocks/sample may be different local vs central**
- **Look at Proficiency Testing and EQA Programs:**
  - **Can control for more pre-analytical variables and do deeper dive into frequency of analytical and post-analytical variability/errors and their causes**
    - **Some use consensus and some use reference standard**
  - **Dramatic increase in number of labs doing PT (ex. CAP now 1500+ labs)**
  - **Drawbacks: Case selection for high agreement (ex. Weak positives often thrown out due to failure to achieve 80% agreement for CAP PT) and known test environment**

NordiQC Trends Towards Higher Pass Rates

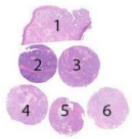
Assessment B25 2018

Estrogen receptor (ER)

Material

The slide to be stained for ER comprised:

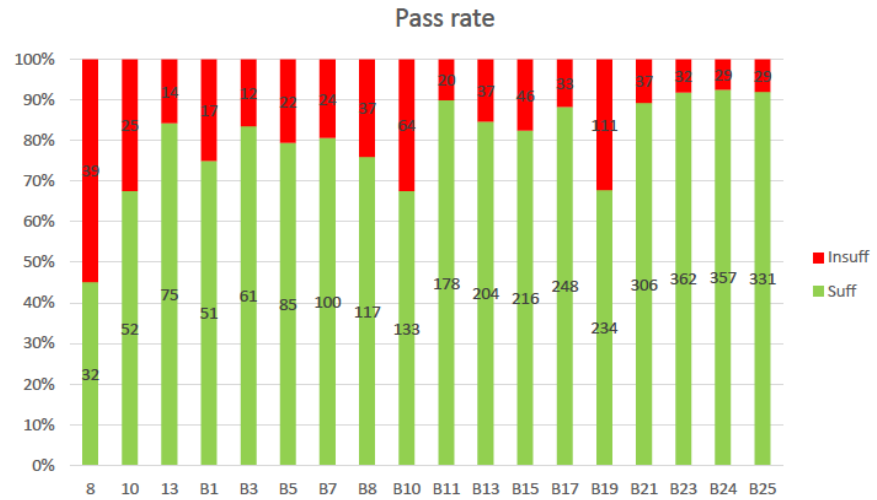
No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80- 90%	Moderate to strong
2.	Tonsil	< 2-5%	Weak to strong
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	90- 100%	Moderate to strong
5.	Breast carcinoma	60-80%	Weak to moderate
6.	Breast carcinoma	90-100%	Weak to moderate



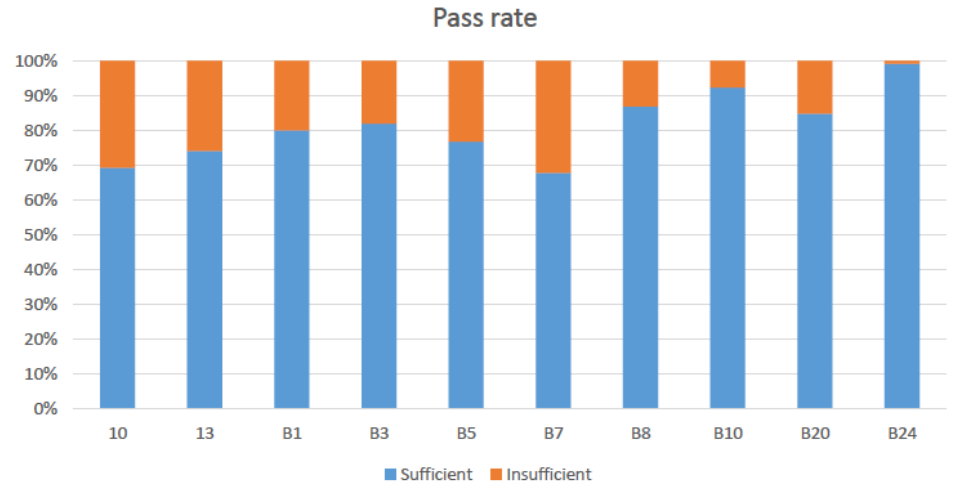
*ER-status and staining pattern as characterized by the NordiQC reference laboratories using the rmAb clones EP1 and SP1.

All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Yaziji et al. (1).

Graph 1. **Participant numbers and pass rates for ER during 18 runs**



Graph. **Pass rate in the NordiQC assessments for PR**



Causes of improvement: Harmonization of use of optimized protocol setting, Fewer labs using 1D5, HIER in alkaline buffer

Persistent issues: False negatives in cases with weaker staining but 10-80% positive

Recommended controls: cervix for strong +, tonsil for weak +, ER- breast cancer for negative control

Causes of improvement: Careful calibration of titer and HIER time

Persistent issues: False positives w 1E2 (strong staining of tonsil B cells)

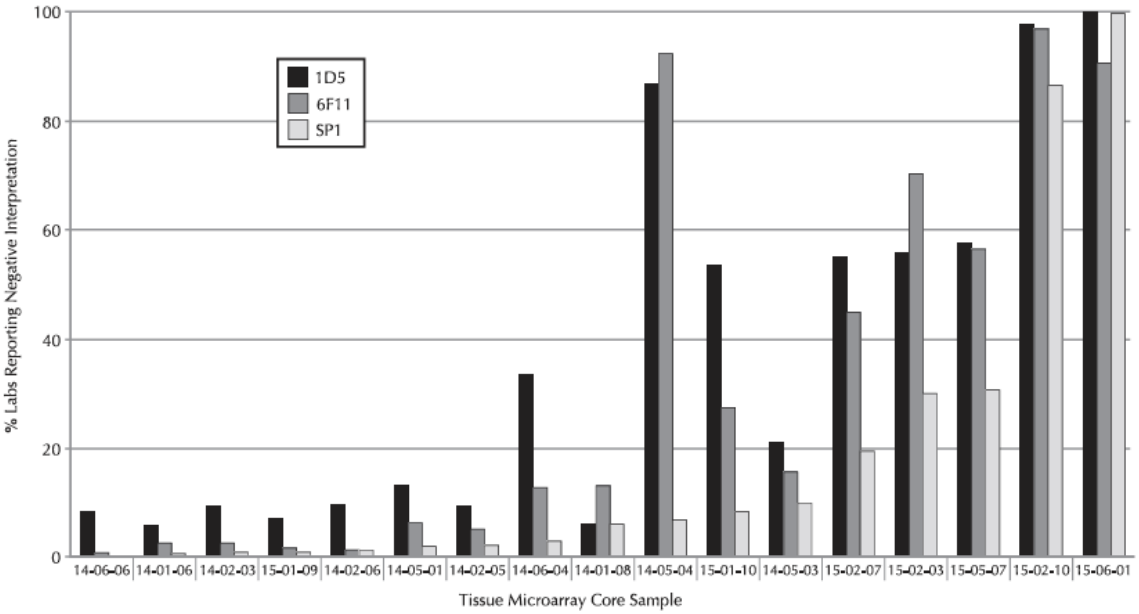
Recommended controls: cervix for + (not post-menopause), tonsil for negative

www.nordiqc.org

Comparison of Estrogen and Progesterone Receptor Antibody Reagents Using Proficiency Testing Data

Megan L. Troxell, MD, PhD; Thomas Long, MPH; Jason L. Hornick, MD, PhD; Abiy B. Ambaye, MD; Kristin C. Jensen, MD

- **CAP PT 80 cores: 56 were scored similarly by 3 most common antibodies (1D5, 6F11, SP1)**
- **ER: 92.5% graded, of intended positives with variability 17% were SP1+/1D5- or 6F11 – (more false neg ER for labs that don't use SP1)**
- **PR: 78.8% graded, of intended negatives with variability →often near 1% threshold, frequent 1E2+/- by other clones ("false +" PR with 1E2?)**



Arch Pathol Lab Med—Vol 141, October 2017

Current issues ("Fine-Tuning"):

- **Variability still exists but is improved → need for continued regulation and guideline recommendations, importance of publishing antibody-specific results + ideal methods**
  - **Need to focus on avoiding false negatives for ER (weak intensity cases particularly sensitive)**
  - **Need to focus on avoiding false positives for PgR (careful titration with appropriate controls)**
- **1-10% ER cases → uncommon (not in PT sets) but can cause variability between labs (especially false negs)**
  - **Is benefit of hormonal-Rx significant?**
  - **Should they be treated with ER negative treatment algorithms?**
  - **Do they have the prognosis of ER positive cancers?**
  - **Is variability in this range avoidable? What are ways to improve reproducibility?**
- **PR and heterogeneity of expression (challenging on proficiency testing)**

Key guideline questions:

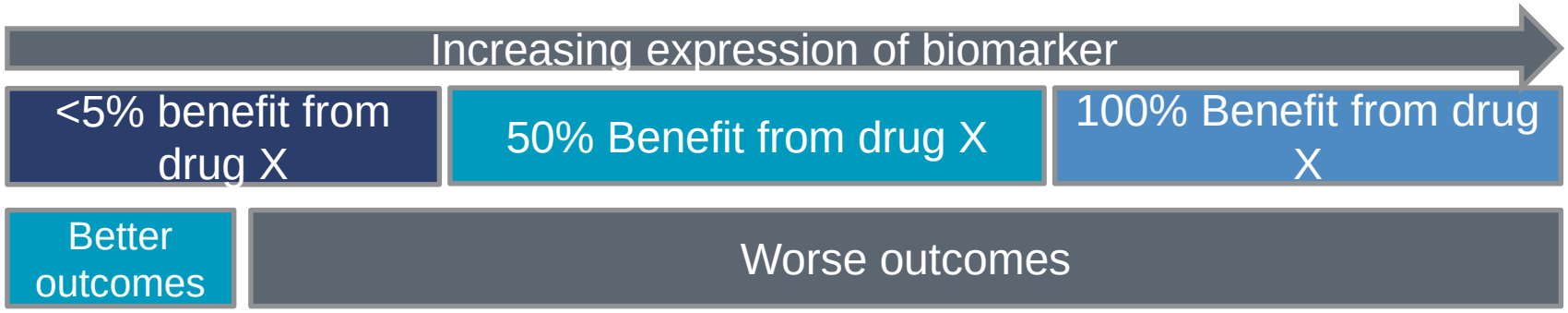
1. **What is the optimum quality assurance, tissue handling, *scoring system and reporting* for determining potential benefit from endocrine therapy?**
2. **What additional strategies can promote optimal performance, interpretation, and reporting of IHC assays, *particularly in cases with low ER expression*?**
3. **Are other ER expression assays acceptable for identifying patients likely to benefit from endocrine therapy?**
4. **Should DCIS be routinely tested for hormone receptors?**

Threshold question

How to avoid false negative/low

Setting Thresholds for Biomarkers

- **Dependent on what trying to prognosticate vs predict:**
  - **Best predictive threshold will depend on risk/benefit in giving drug**
- **There will usually be a grey zone near the threshold**
  - **More variability in test results**
  - **Less clear clinical implications**



Multiple current uses of ER/PR Testing

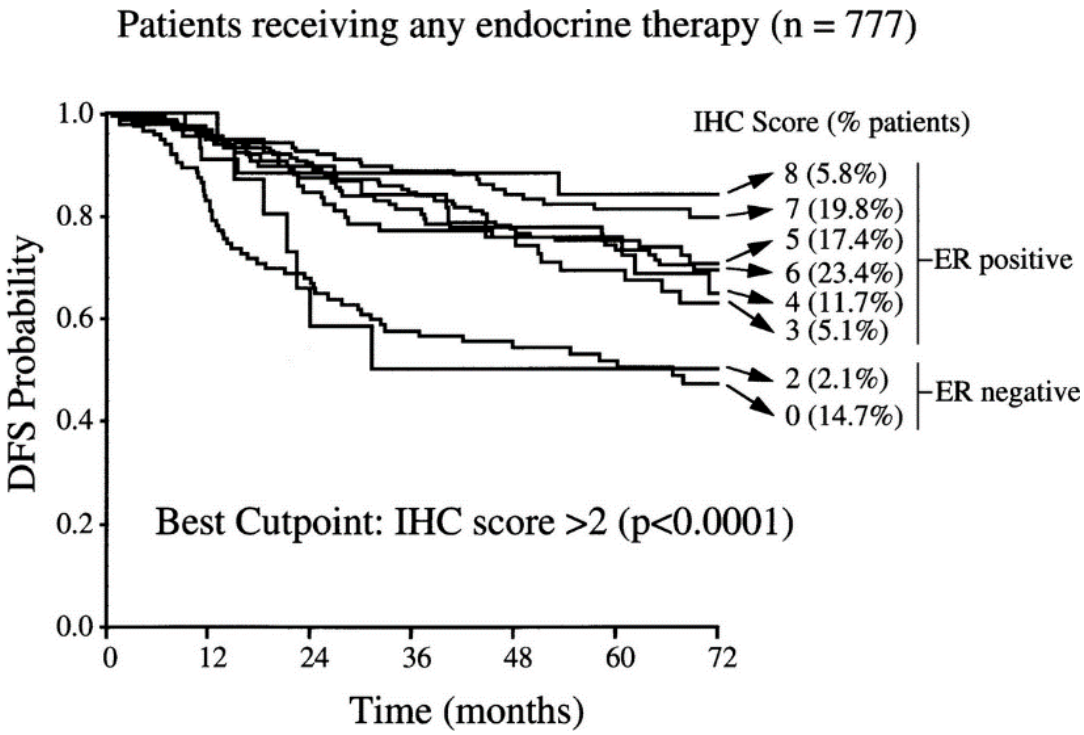
1. **Determining potential benefit from endocrine therapies**
2. **Overall treatment pathways determined by ER+ vs ER- (ex. NCCN guidelines)**
3. **Surrogates for intrinsic/molecular subtype determination (along with HER2)**
4. **Prognostic role (ex. AJCC prognostic subgroups)**
5. **Metastatic setting: ER+ vs ER- treatments**
6. **Diagnostic testing (is metastatic cancer breast?)**

Test validated for as a predictive biomarker = Guideline's focus

Is the 1% threshold valid for all uses?

Allred study: Showing best predictive threshold?

- **All patients received endocrine therapy**
- **Actually only Prognostic…**
- **Samples were not standard**



Harvey et al JCO 1999

Clinical Trial data: Best predictive threshold?

- Limited clinical data on threshold – mostly based on LBA data
- 20 trials with over 200,000 women-years of follow-up
- **Points to 10 fmol ER/mg at best threshold.**
  - 10-19 fmol ER/mg had recurrence reduced by 1/3 with 5 yrs Tam

Correlates best with 1% by IHC

Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials

*Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**

Lancet 2011; 378 771-84

Category	Events/woman-years (rate [% per year])		Tamoxifen events		Ratio of annual event rates
	Allocated tamoxifen	Allocated control	Log-rank O-E	Variance of O-E	Tamoxifen : control
(a) ER-poor					
ER=0	162/5060 (3·2)	163/5941 (2·7)	7·4	69·5	1·11 (SE 0·13)
ER 1–3	202/6645 (3·0)	192/6357 (3·0)	2·2	85·5	1·03 (SE 0·11)
ER 4–9	185/5490 (3·4)	188/5588 (3·4)	−6·6	77·5	0·92 (SE 0·11)
Other ER-poor	449/9528 (4·7)	451/8995 (5·0)	−14·9	195·5	0·93 (SE 0·07)
(a) Subtotal	**998/26723 (3·7% per year)**	**994/26881 (3·7% per year)**	**−12·0**	**428·0**	**0·97 (SE 0·05) 2p=0·6**
Test for trend χ^2_1=1·4; 2p=0·2					
(b) ER-positive by ER measurement					
ER 10–19	232/8173 (2·8)	316/7252 (4·4)	−47·4	120·6	0·67 (SE 0·08)
ER 20–29	158/5104 (3·1)	197/4630 (4·3)	−27·3	76·4	0·70 (SE 0·10)
ER 30–49	235/8107 (2·9)	260/6952 (3·7)	−29·0	112·1	0·77 (SE 0·08)
ER 50–99	293/10650 (2·8)	361/8973 (4·0)	−69·6	144·8	0·62 (SE 0·07)
ER 100–199	211/8429 (2·5)	344/7376 (4·7)	−80·4	122·8	0·52 (SE 0·07)
ER≥200	216/8279 (2·6)	325/6672 (4·9)	−78·2	119·0	0·52 (SE 0·07)
Other ER+	308/7868 (3·9)	415/6898 (6·0)	−72·9	161·3	0·64 (SE 0·06)
(b) Subtotal	**1653/56610 (2·9% per year)**	**2218/48753 (4·5% per year)**	**−404·8**	**856·9**	**0·62 (SE 0·03) 2p<0·00001**
Test for trend χ^2_1=9·5; 2p=0·002					

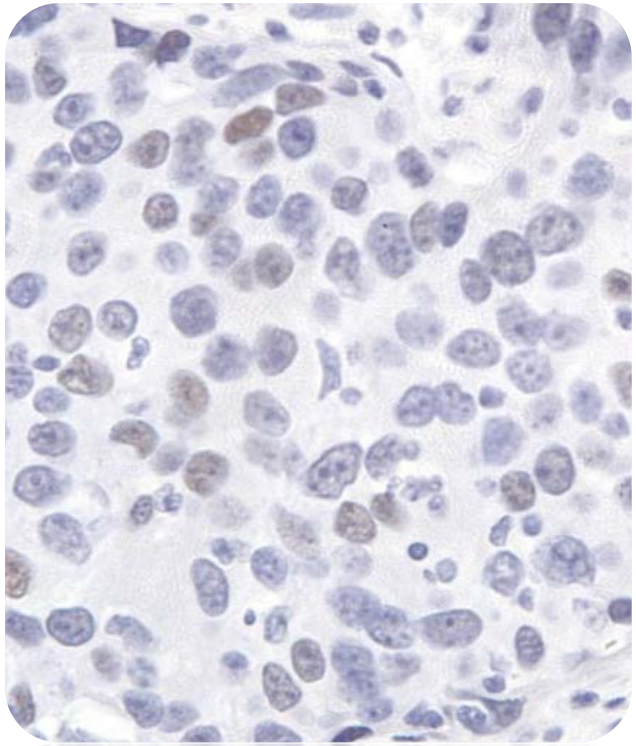
Imperfect Clinical Trials Data…but not likely to repeat clinical trials on endocrine therapy

Low risk drug with potentially high benefit…

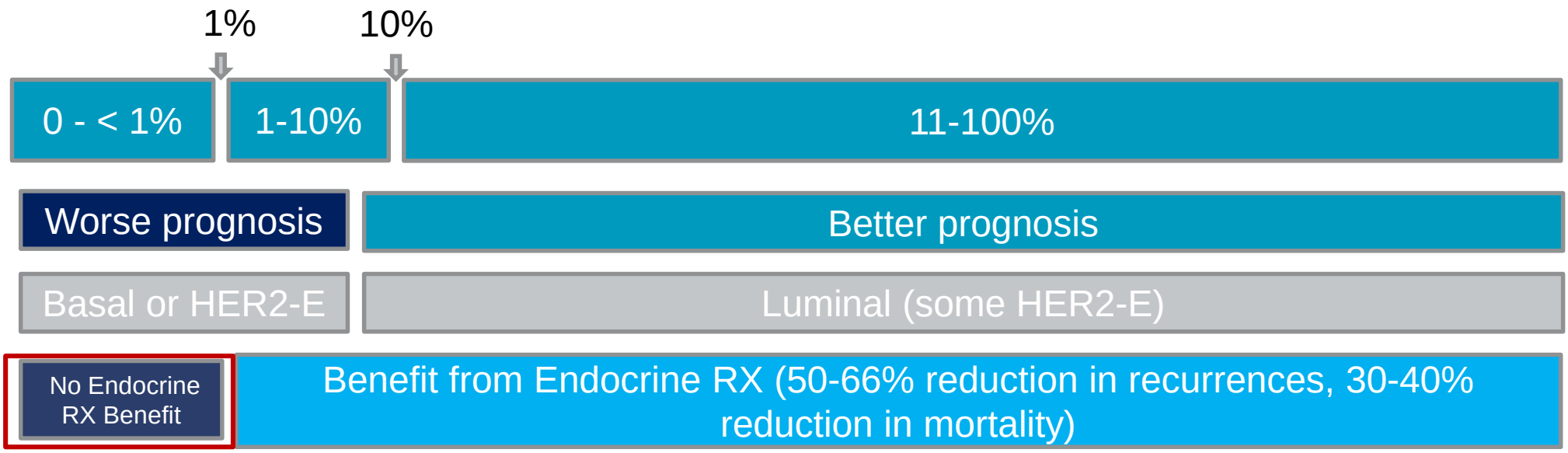
What do we know about ER Low Positive Cancers?

- **Heterogeneous group & rare (2-3%)**
- **Often "basal-like" features (histology, response to neoadjuvant chemotherapy and molecular profiles)**
  - **Don't want to exclude these patients from "triple negative" trials…?**
- **Potential benefit from endocrine therapy (although less than stronger positive):**

  May still need to be considered positive for at least at trial of endocrine therapy but intent not to be used to treat similar to other strong ER+ cancers…..



ER: What threshold for IHC+?



- **To segregate out who will definitely NOT benefit from endocrine therapy? ≥ 1% vs < 1% or 0%**
- **To select who is highly likely to benefit from endocrine therapy? Don't want to exclude pts from possible benefit in relatively low risk drug**
- **To determine overall treatment pathway? Use ~10%?**
- **To determine intrinsic/biologic subtype of breast cancer? Use ~10%?**
- **To determine overall prognostic groups? Use ~10%?**

EBCTCG (2015). https://doi.org/10.1016/s0140 - 6736(15)61074 -1

Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update

Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; and Antonio C. Wolff, MD[21]

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ASCO Guidelines

UPDATED JANUARY 2020

Recommendation 1.1. Optimal algorithm for ER/PgR testing

- **Samples with 1-100% of tumor nuclei positive for ER or PgR are interpreted as positive.**
- **For reporting of ER (not PgR), if 1-10% of tumor cell nuclei are immunoreactive, the sample should be reported as ER Low Positive with a recommended comment.**
- **A sample is considered negative for ER or PgR if <1% or 0% of tumor cell nuclei are immunoreactive.**

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

New Low Positive ER Category and Recommended Reporting Comment:

- **ER : LOW POSITIVE (1-10%), SEE COMMENT**

Result:	Additional recommended comment:
1-10% cells staining:	The cancer in this sample has a low level (1-10%) of ER expression by IHC. There are limited data on the overall benefit of endocrine therapies for patients with low level (1-10%) ER expression but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results are heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER negative cancers.

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Recommendation 1.1., continued

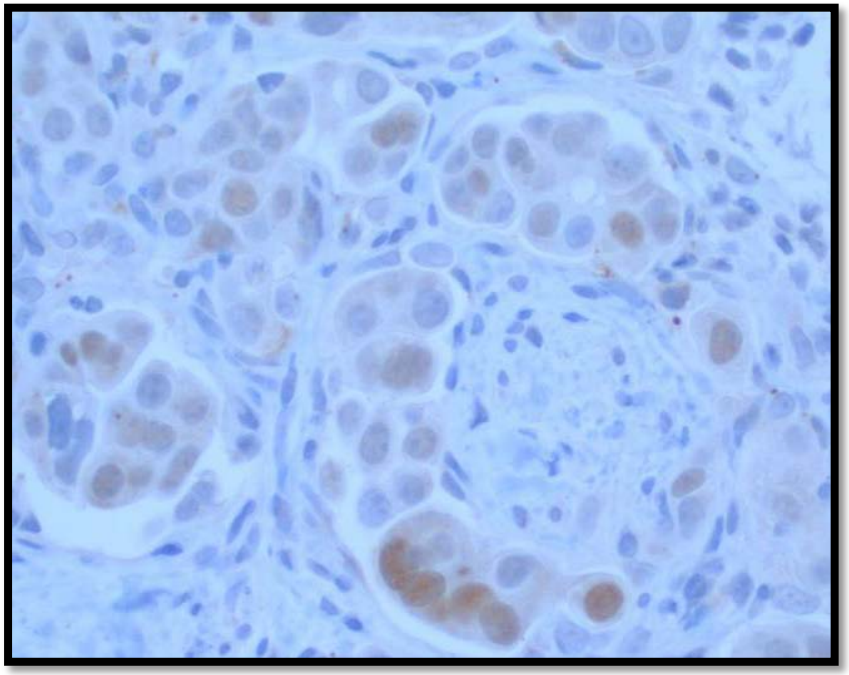
A sample may be deemed uninterpretable for ER or PgR if the sample is inadequate (insufficient cancer or severe artifacts present, as determined at the discretion of the pathologist), if external and internal controls (if present) do not stain appropriately, or if pre-analytical variables have interfered with the assay's accuracy (see manuscript Figures 1-4).

Clinicians should be aware of and able to discuss with patients the limited data on ER-low positive cases and issues with test results that are close to a positive threshold.

Strong Recommendation

Example Case:

- **Grade 3 invasive ductal carcinoma, LN neg**
- **Core Biopsy outside read by image analysis : ER 2%**
- **Core biopsy by our review: ER 10%, 1+**
- **Excision at Stanford: ER 20%, 1-2+**
- **Sent for Oncotype DX:**
  - **High RS (54; 34% recur)**



Quantitative Single Gene Report

The *Oncotype* DX assay uses RT-PCR to determine the RNA expression of the genes below. These results may differ from ER, PR, or HER2 results reported using other methods or reported by other laboratories.[1]

The ER, PR, and HER2 Scores are also included in the calculation of the Recurrence Score.

ER Score = **6.2** Negative

Range: Negative < 6.5 | Positive ≥ 6.5

Patient

<3.7 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5 12.0 ≥12.5

The ER Score positive/negative cut-off of 6.5 units was validated from a study of 761 samples using the 1D5 antibody (immunohistochemistry) and 607 samples using the SP1 antibody (immunohistochemistry). The standard deviation for the ER Score is less than 0.5 units.[2]

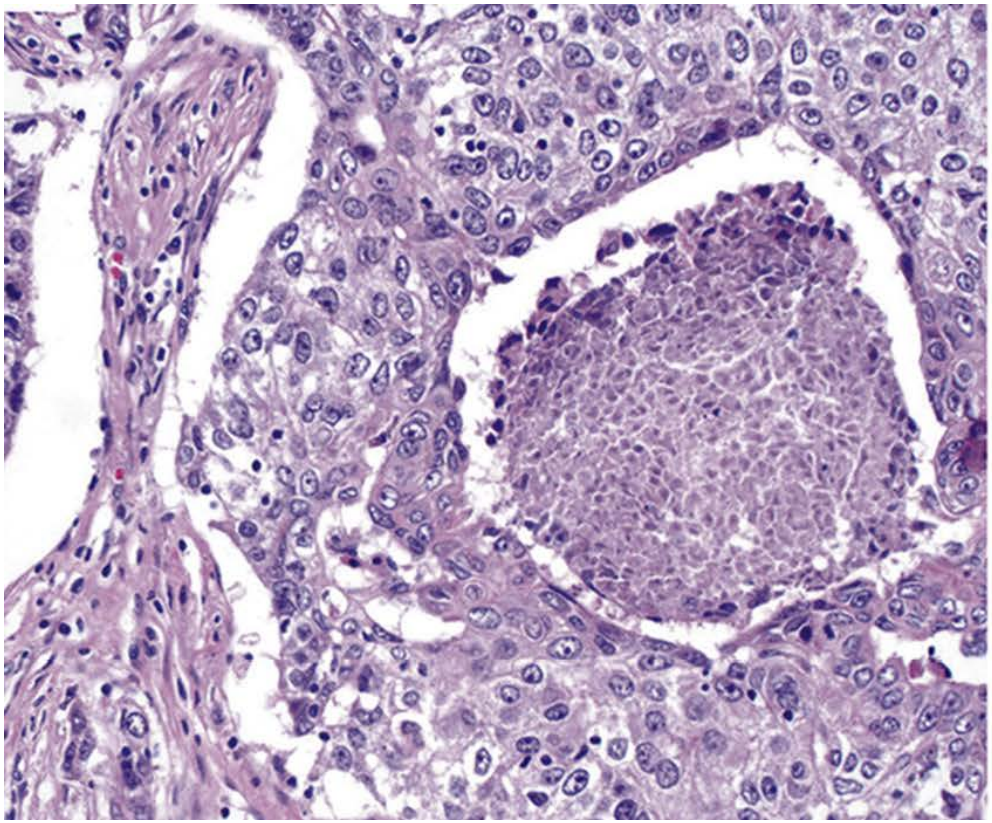
Clinical Experience:

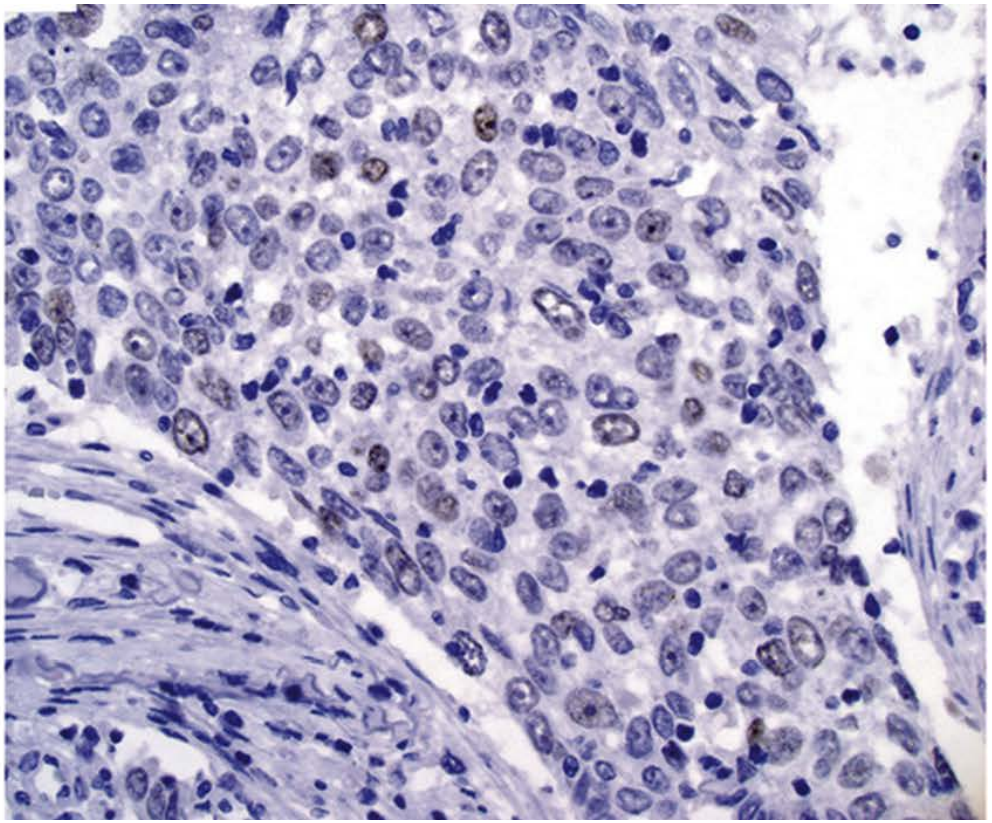
For ER positive breast cancer, the magnitude of tamoxifen benefit increases as the ER Score increases from 6.5 to ≥12.5.[3]

Please note: The Average Rate of Distant Recurrence reported on Page 1 based on the Recurrence Score was determined in patients who received 5 years of tamoxifen treatment and takes into account the magnitude of tamoxifen benefit indicated by the ER Score.

Cases close to threshold for positive are more likely to have different results by different assays, methods or samples.
Any positive result is treatable but need to acknowledge data limited.

Example Case: 35 y/o female with Grade 3 IDC and the following ER stain you estimate to be 1-10% positive (1+)



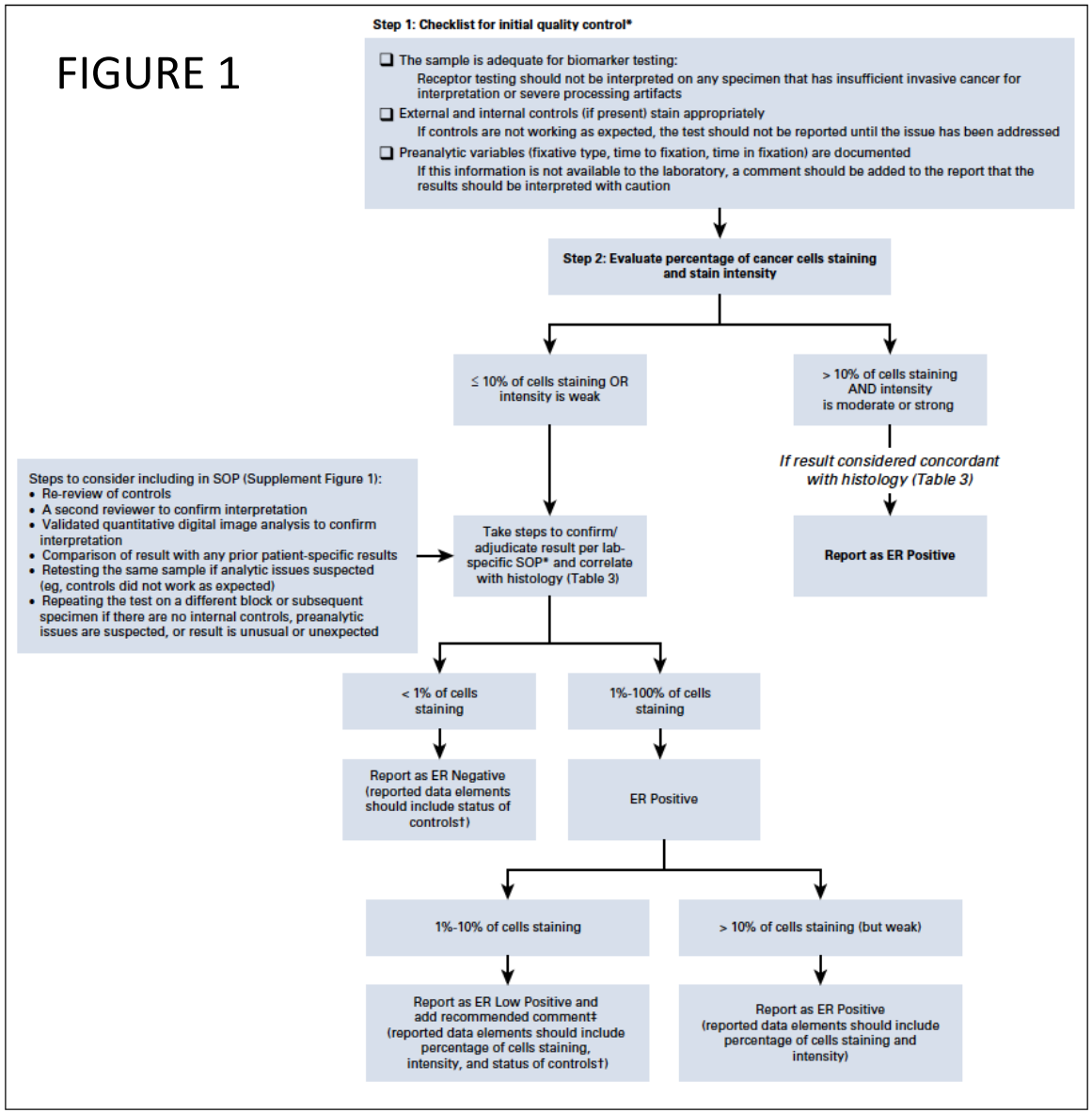


What do you do next?

Recommendation 2.3 (NEW)

Laboratories should establish and follow an SOP stating the steps the laboratory takes to confirm or adjudicate ER results for cases with weak stain intensity or ≤10% of cells staining (see Supplemental Digital Content Data Supplement 2, Figure 1 for an example SOP).

FIGURE 1

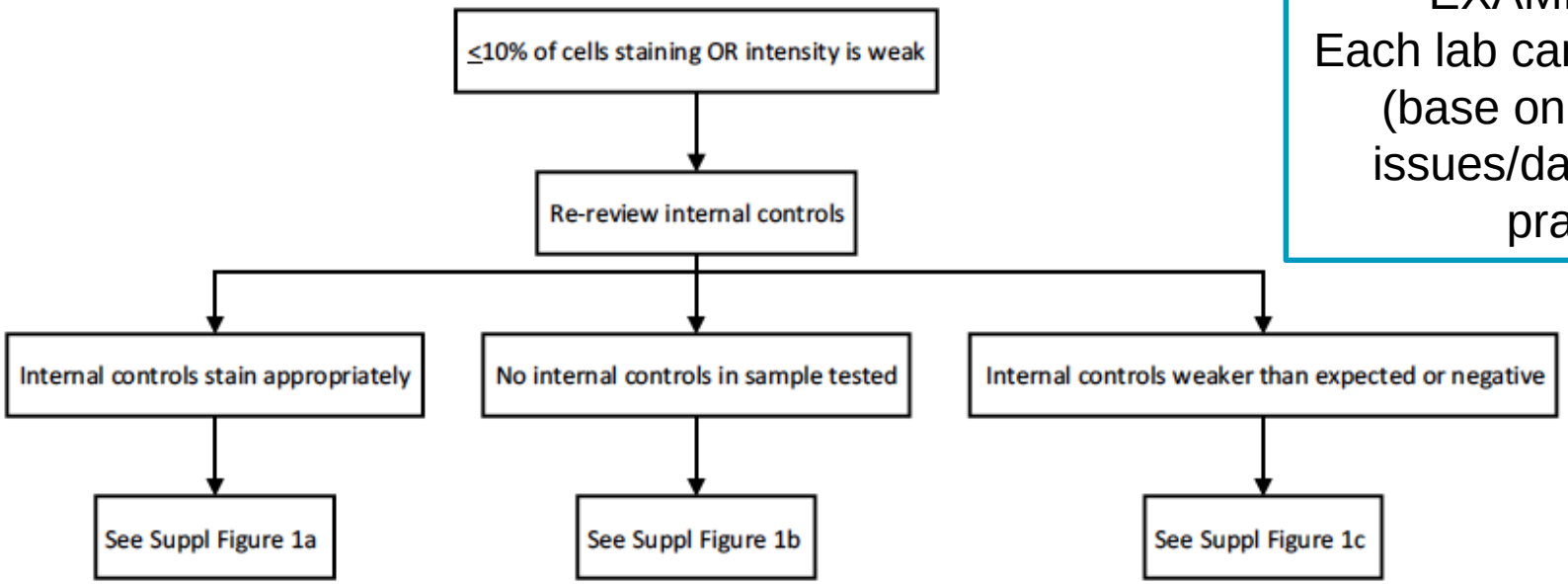


For cases with ≤ 10% or weak staining:

1. Take steps to confirm/adjudicate result per your lab's SOP.
2. Correlate with histology.

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

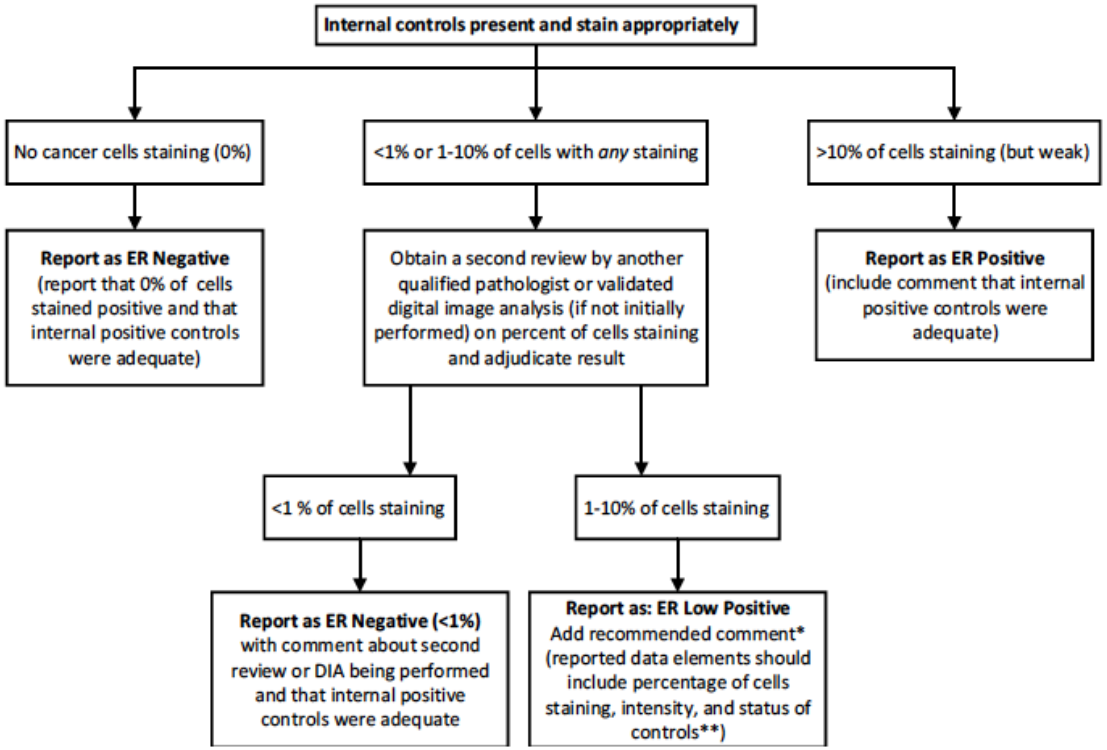
Data Supplement 2: Figure 1. Example of a Lab-Specific Standard Operating Procedure for cases with initial ER IHC result with ≤ 10% of cells staining or stain intensity is weak

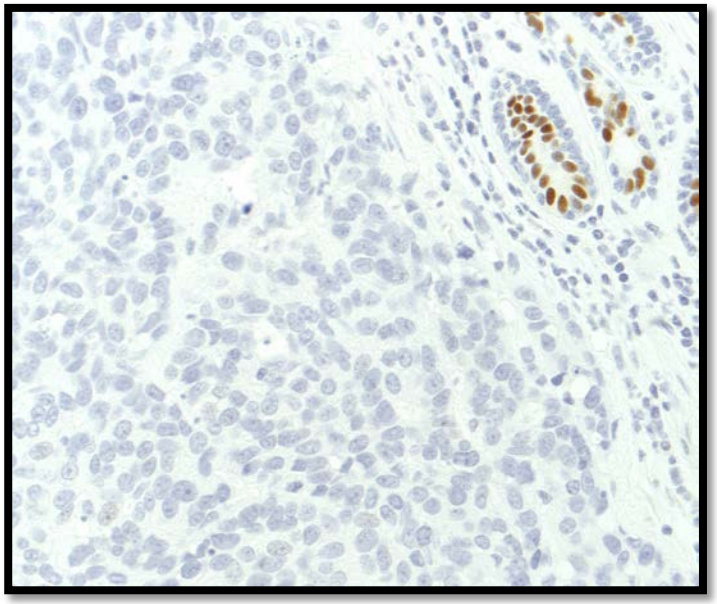


EXAMPLE SOP
Each lab can use their own (base on lab specific issues/data and good practice)

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Figure 1a. Internal controls present and stain appropriately



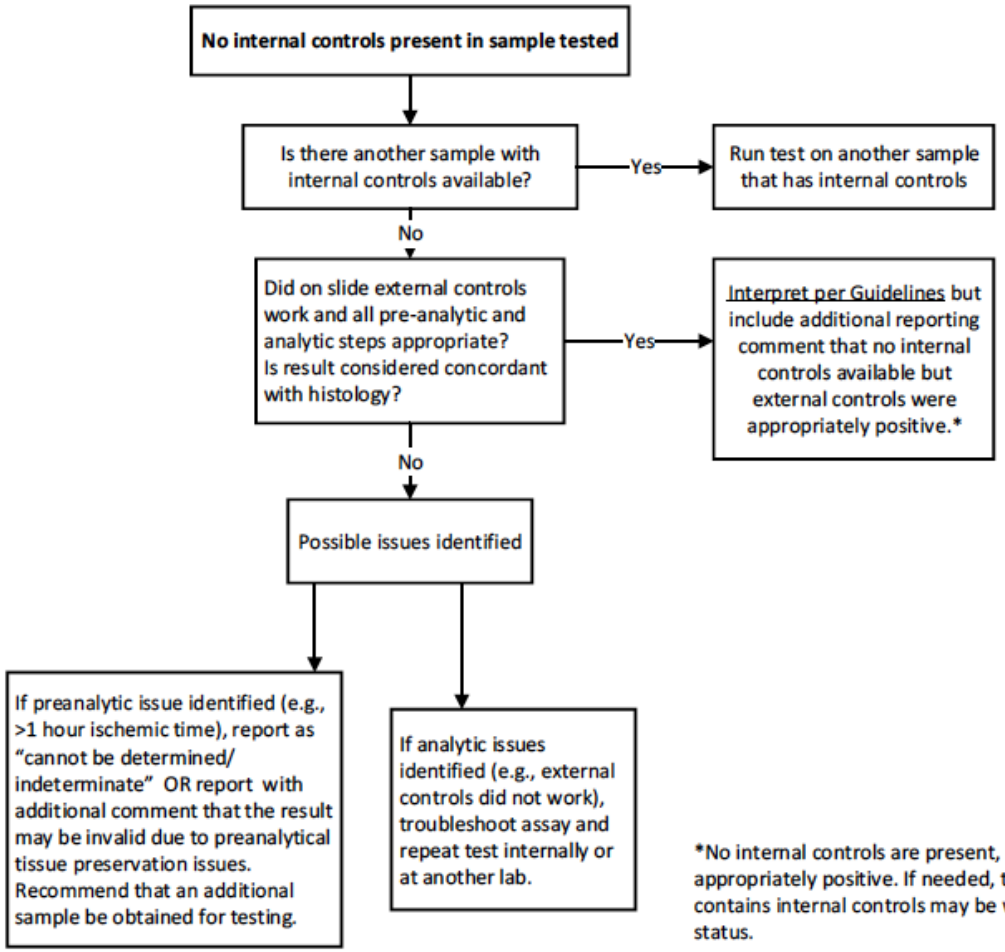


- ✓ Evidence stain worked on sample tested
- ✓ If close to threshold for positive → second review to make sure interpretation reproducible in your lab

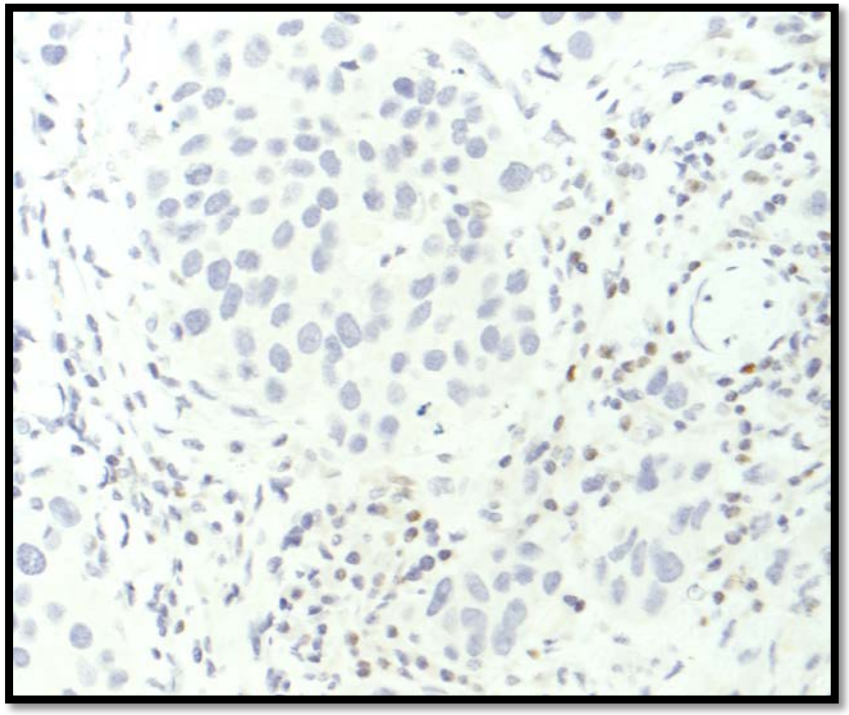
Report comments:
*Recommended comment for low positive results: The cancer in this sample has a low level (1-10%) of ER expression by IHC. There are limited data on the overall benefit of endocrine therapies for patients with these results, but they currently suggest possible benefit, so patients are considered eligible for endocrine treatment. There are data that suggest invasive cancers with these results are heterogeneous in both behavior and biology and often have gene expression profiles more similar to ER negative cancers.
**If the test results are either ER negative or low positive and no internal controls are present, the following comment should be included in the report: No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Figure 1b. No internal controls present in sample tested



*No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.



- ✓ Check if external controls appropriate.
- ✓ Check pre-analytic variables
- ✓ Report with additional comment about controls (recommended)

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Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

New Recommendations on Internal Control Reporting (Recommendation 2.4):

- **The status of internal controls should also be reported for cases with 0-10% staining (with a special comment for those lacking internal controls). See Table 2.**

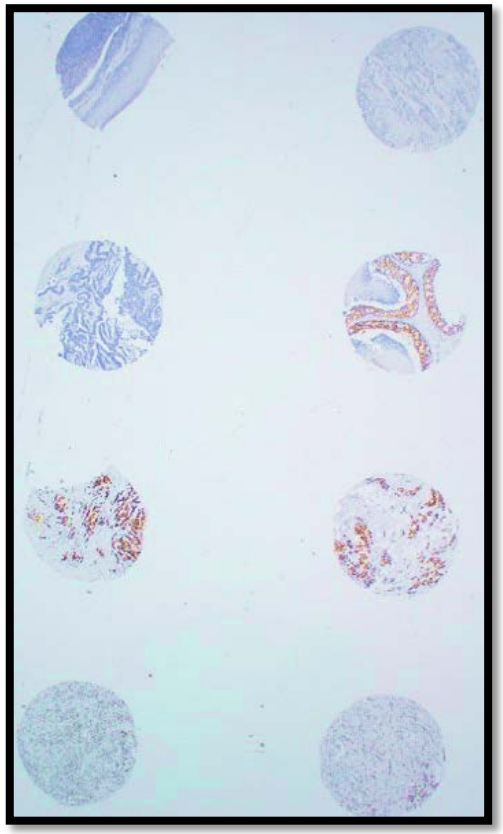
Result:	Additional recommended comment:
No internal controls and ER is 0-10%:	No internal controls are present, but external controls are appropriately positive. If needed, testing another specimen that contains internal controls may be warranted for confirmation of ER status.

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Recommendation 1.5.
Optimal internal QA procedures

Updated

Standardized operating procedures (SOPs) should be used that include routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections (or other appropriate control) on each tested slide, wherever possible. External controls should include negative and positive samples as well as samples with lower percentages of ER expression (such as tonsil). On-slide controls are recommended.



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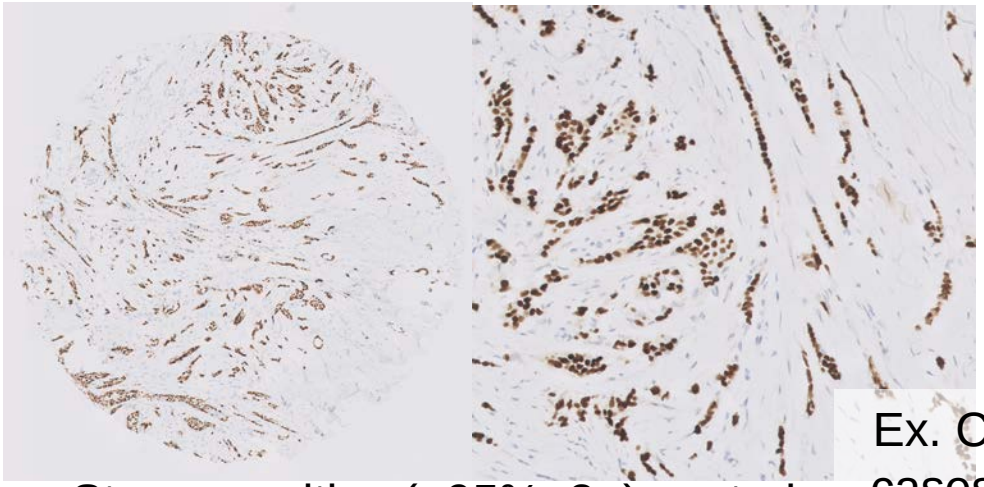
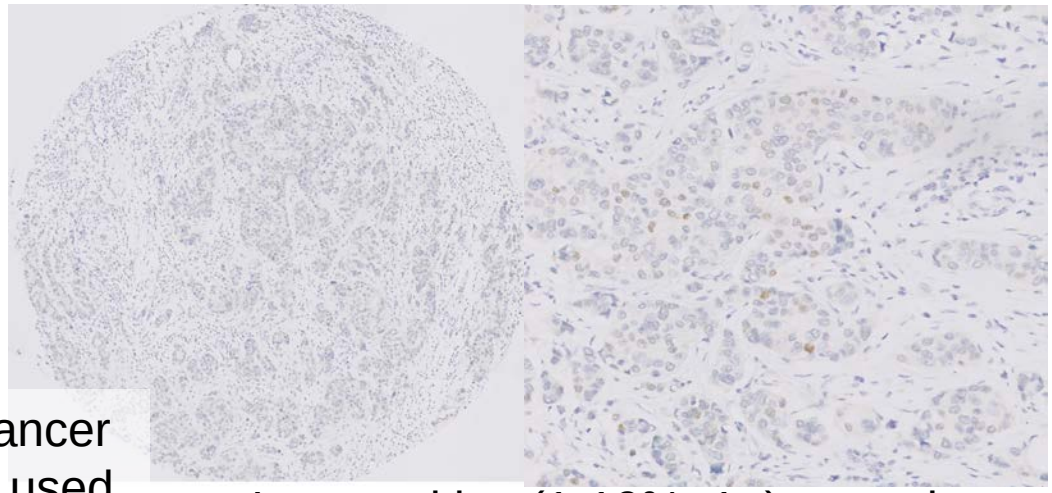
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

External Controls: Include a spectrum of ER expression, on-slide TMAs or similar preferred

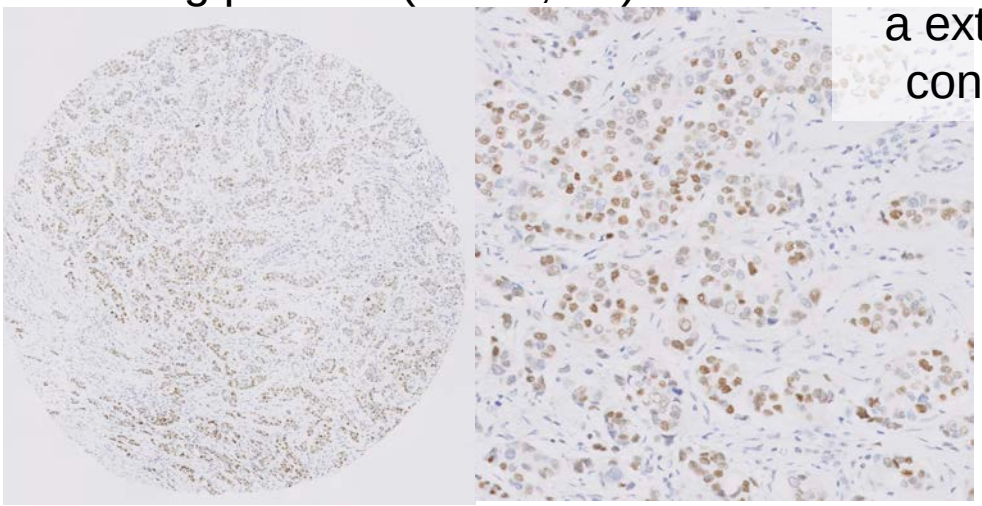


Strong positive (>95%, 3+) control.

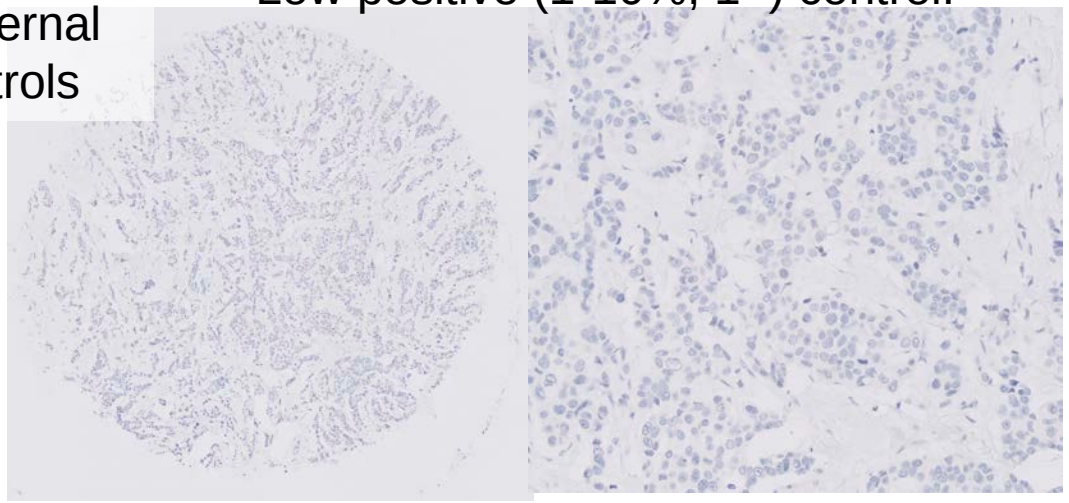


Low positive (1-10%, 1+) control.

Ex. Cancer cases used a external controls



Moderate intensity positive (80%, 2+) control.



Negative (0%) control.

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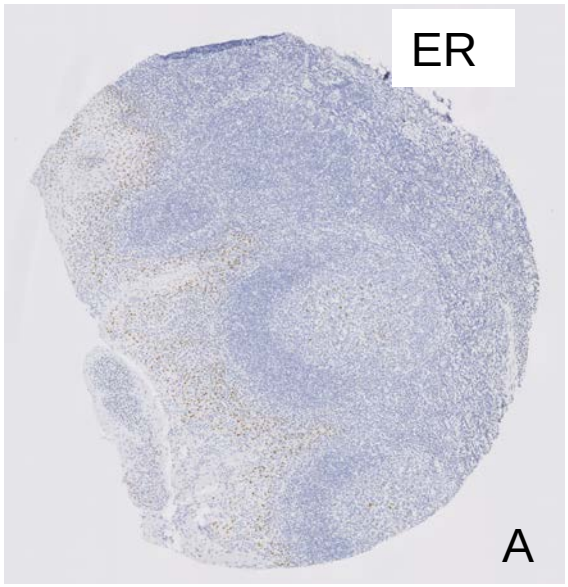
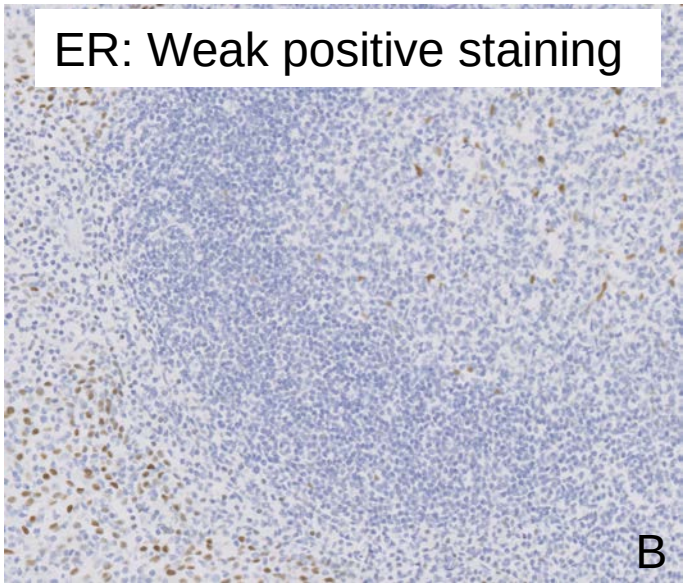
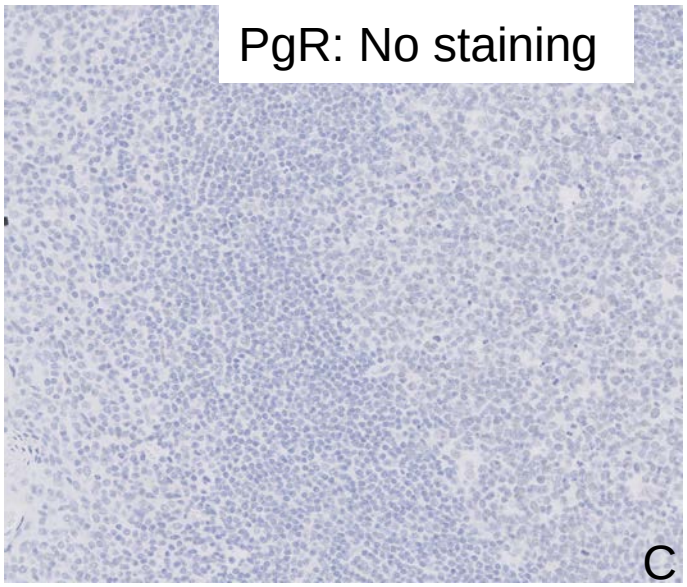
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

TONSIL: An Excellent External Control For Low ER Positive and PgR Negative







Tonsil is an excellent external control to monitor the analytical sensitivity for ER. Dispersed germinal center cells and the squamous epithelium should be ER positive but the B-cells in the mantle zones should be ER negative (as shown in panels A at 5x and panel B at 20x). Tonsil is an appropriate negative control for PgR. In contrast to ER, no nuclear PgR staining should be seen. Weak positive PgR staining in tonsil should result in work-up to determine if assay drift has occurred.

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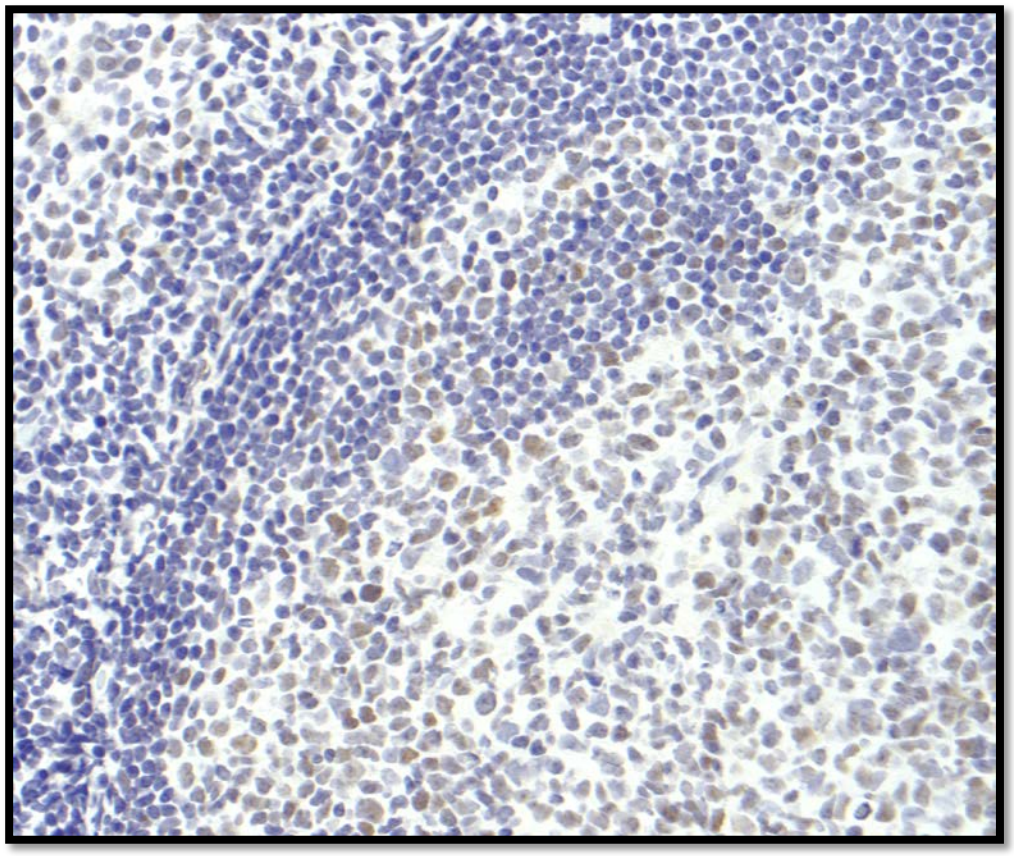
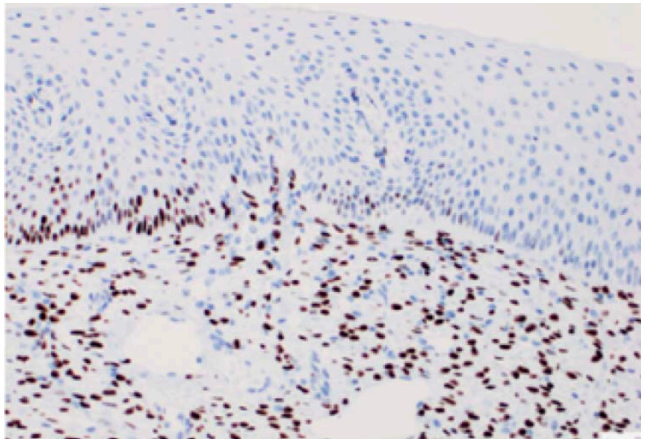
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

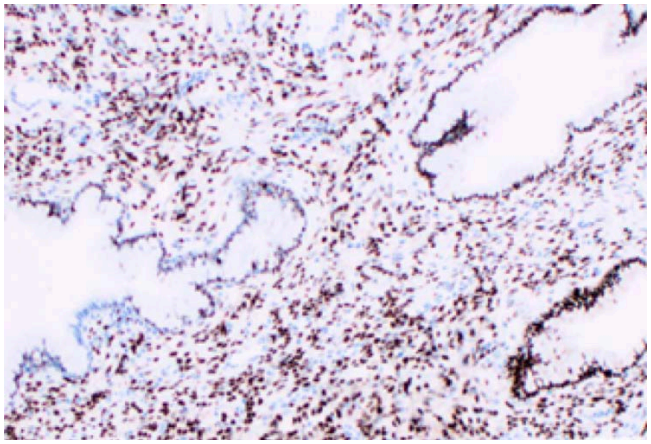
Example case: External tonsil control for PgR stain reviewed

Tonsil staining for PgR when should be negative….
Need to re-titer assay?
Drift occurring?



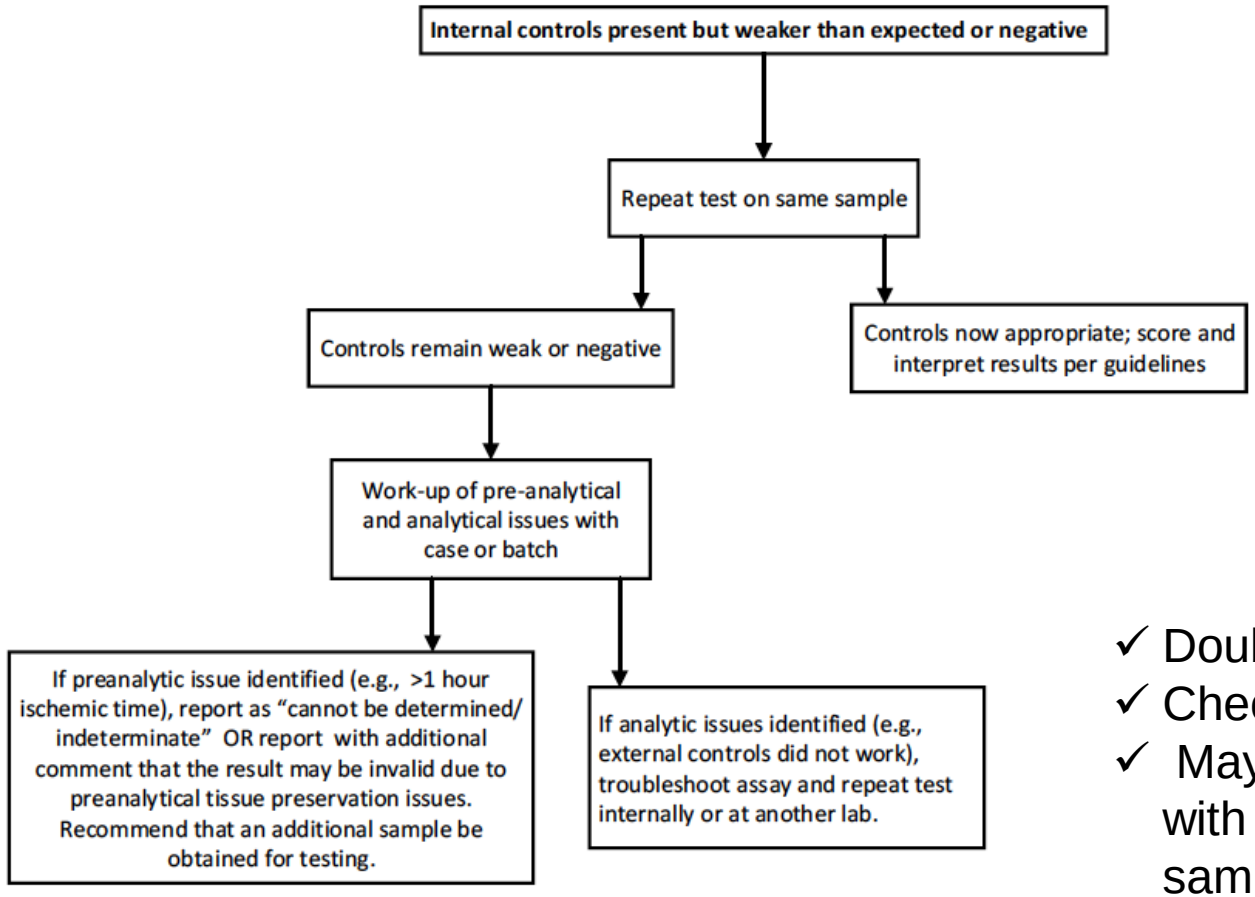
Cervix as an external control

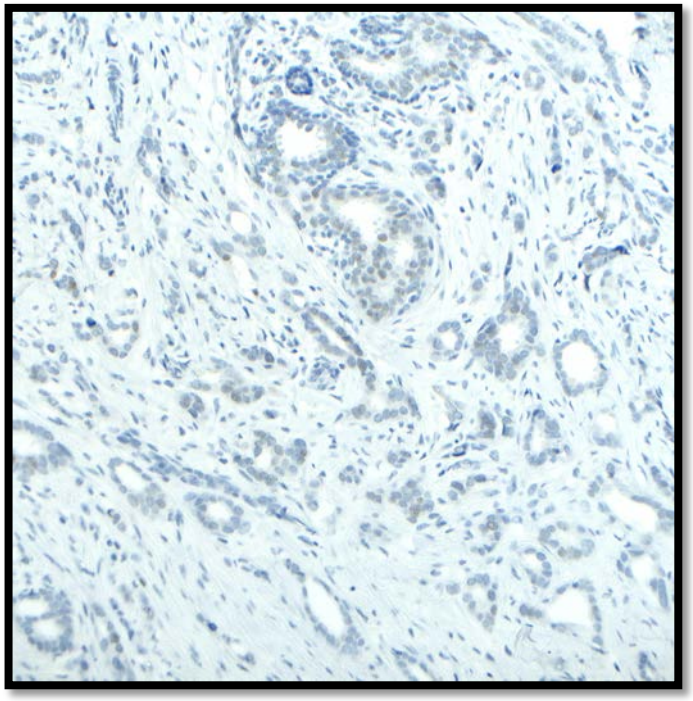




- **PgR should variably stain the basal layer of the squamous mucosa (good for low limit of detection control)**
- **PgR should also stain endocervical columnar epithelium (with some variability)**
- **ER should stain almost all endocervical columnar epithelial cells**
- **Note: May be less robust staining in cervical tissue from postmenopausal women**

Figure 1c. Internal controls present but weaker than expected or negative





- ✓ Double check stain worked (repeat test)
- ✓ Check pre-analytic variables
- ✓ May need to report as "indeterminate" with recommendations for additional samples if pre-analytic issues identified

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Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

FAQ: What if my case was decalcified?

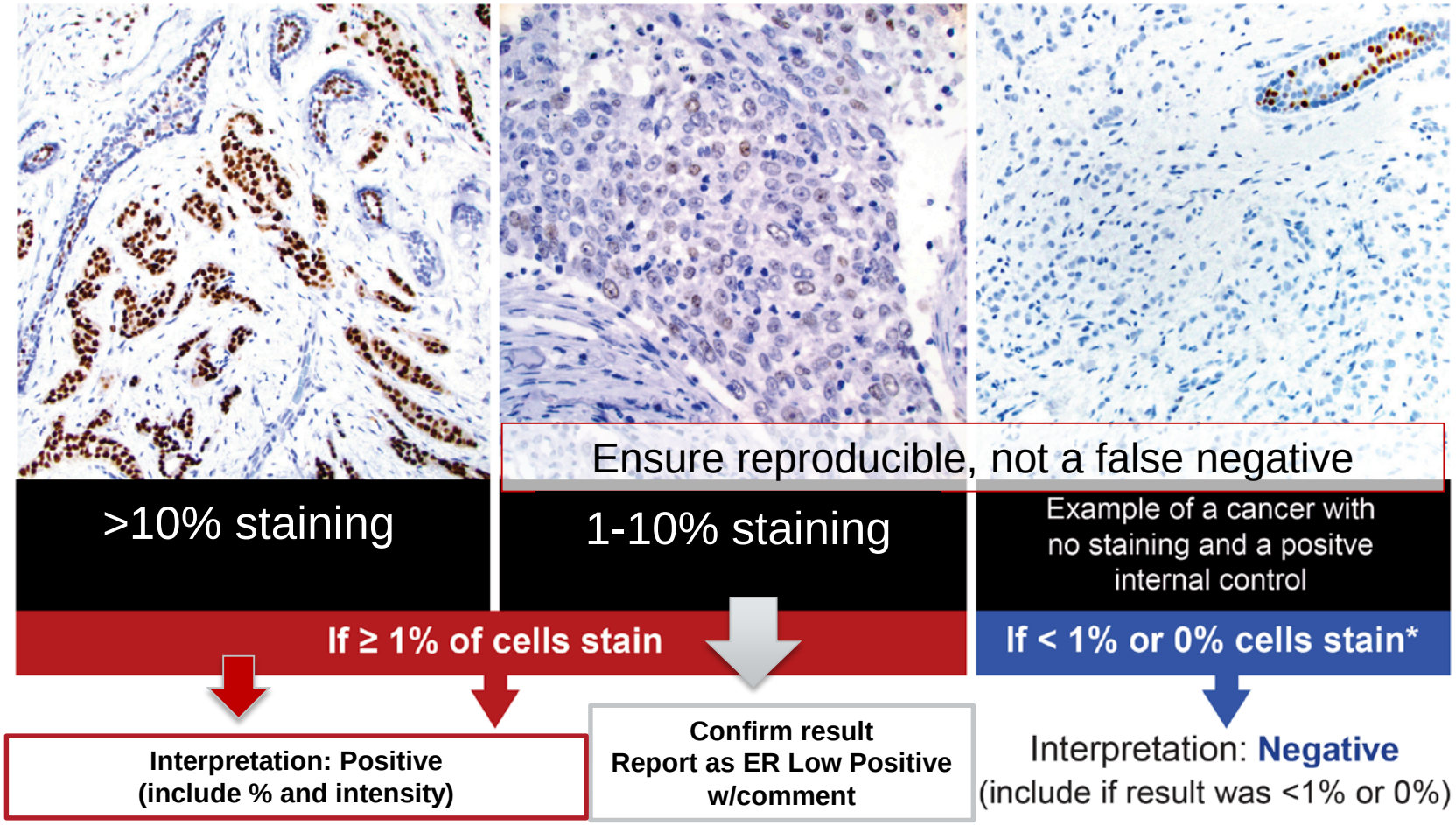
- **Recommend separating grossly:**
  - **bony fragments→ decal**
  - **non-bony fragments →NO DECAL**
  - **Helpful for FISH & molecular**
- **Validate your lab's decal/FFPE/Ab, or**
  - **CAP disclaimer "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."**
- **Also: Most cyto fixatives alcohol based**
  - **Many labs use formalin-only for suspected breast metastasis, or**
  - **Validate your lab's cyto fix/cell block/Ab**

Van Es. AJSP. 2019;43:1355–60	EDTA	Acetic	HCl/Formic
ER % change	-0.5%	-2.5%	-21%
ER false neg	0	0	42%
PR % change	-1.5%	-0.5%	-14.5%
PR false neg	0	0	33%
HER2 change	-0.3	-0.3	-0.8
ISH failure	1/16	15/16	all

See also: Clark. AIMM. 2019;27:223-30
Schrivjer. Mod Pathol. 2016;29:1460-70
Gertych. Diagn Pathol. 2014;9:213
Maclary. AIMM. 2017;25:144–149

Hormone Receptor Stain Interpretation (ER and PgR)

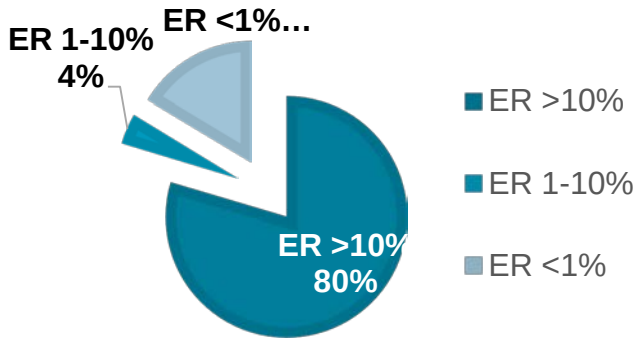
Evaluate overall percentage of cancer in sample with nuclear staining and intensity of stain



Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Stanford Practice: Data used to establish an SOP

Interpretation Category (Based on Majority)	Cases in Category	Cases with 100% (6 of 6) agreement	Cases with >80% (5 of 6) agreement
Negative (<1%)	16	67%	87%
Low Positive (1-10%)	6	0%	17%
Positive (>10%)	8	75%	100%



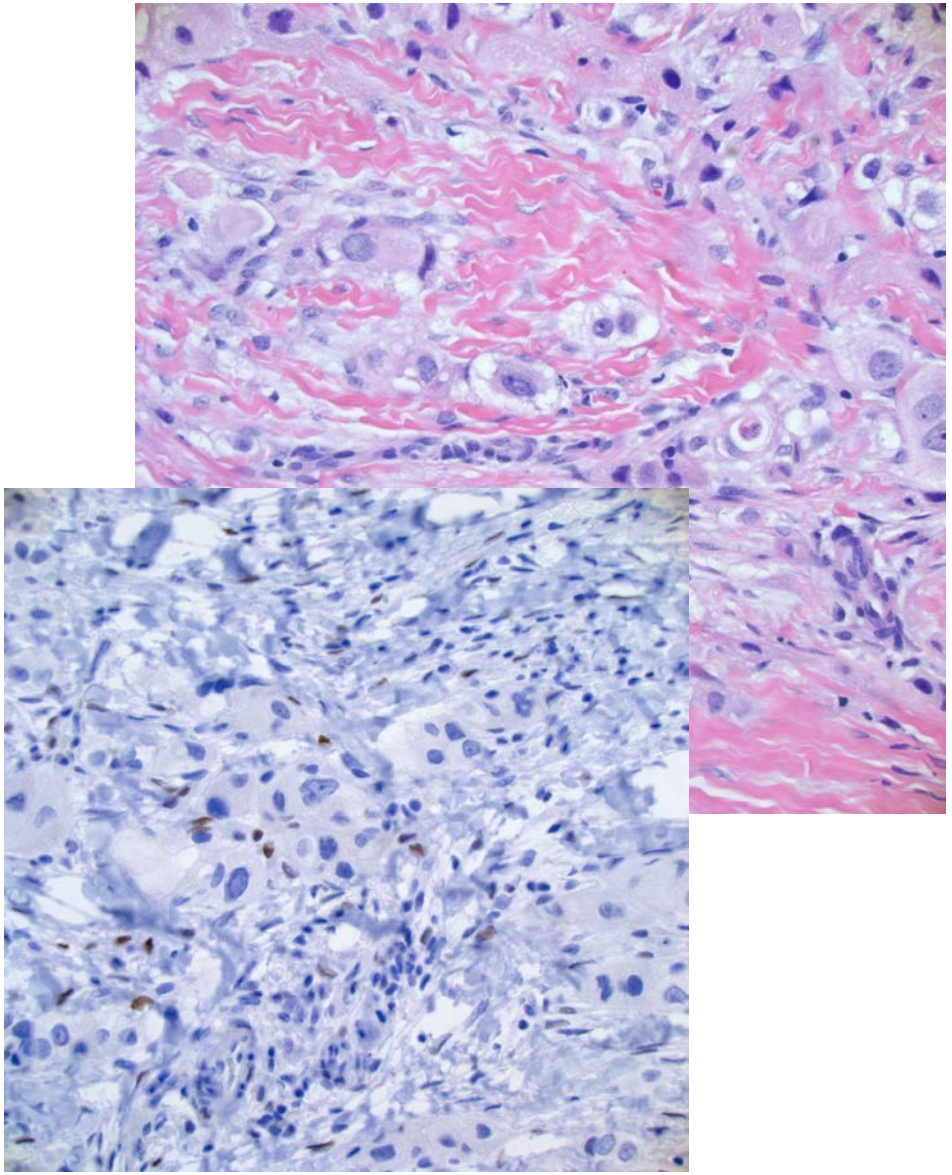
- Test set of 30 cases reported as ER Negative (0 or <1%), Low Positive (1-10%) or Positive (>10%) were identified.
- 5 breast pathologists who perform ER interpretations scored/interpreted each case
- Agreement was very high for > 10%
- Agreement was high for < 1% (best for 0%)
- Agreement was very low for Low Positive (1-10%)
- Decided our SOP should include second pathologist review for cases with 1-10% staining or close to the 1% threshold for positive
  - Would result in second review of approximately 4% of our cases

Example case:

You are reviewing as a second opinion a case with the following diagnosis from the original lab:

DIAGNOSIS: INVASIVE LOBULAR CARCINOMA

- **ER negative (0%) with positive internal controls**
- **PR negative (0%) with positive internal controls**
- **HER2 positive (3+) by IHC**



Revised Diagnosis: Invasive Pleomorphic Lobular Carcinoma

Recommendation 2.2.

Interpretation of any ER result should include evaluation of the concordance with the histologic findings of each case. Clinicians should also be aware of when results are highly unusual/discordant and work with pathologists to attempt to resolve or explain atypical reported findings (see manuscript Table 3 as an aid in this process).

Strong Recommendation

Invasive Breast Cancer Histopathologic Concordance with Estrogen Receptor Staining

HIGHLY UNUSUAL ER NEGATIVE RESULTS	HIGHLY UNUSUAL ER POSITIVE RESULTS
Low grade invasive carcinomas of no special type (also known as invasive ductal carcinoma)	Metaplastic carcinomas of all subtypes
Lobular carcinomas (classic type)	Adenoid cystic carcinomas and other salivary gland-like carcinomas of the breast
Pure tubular, cribriform, or mucinous carcinomas	Secretory carcinoma
Encapsulated papillary and solid papillary carcinomas	Carcinomas with apocrine differentiation

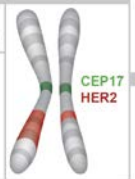
Note: If a result is considered highly unusual/discordant additional steps should be taken to check the accuracy of the histologic type or grade as well as the pre-analytic and analytic testing factors. This work-up may include second reviews and repeat testing. If all results appear valid the result can be reported with a comment noting that the findings are highly unusual and testing of additional samples may be of value to confirm the findings.

Also these should be HER2 Negative

Allison KH, Hammond MEH, Dowsett M, et al: J Clin Oncol doi: 10.1200/JCO.19.02309
Arch Pathol Lab Med doi: 10.5858/arpa.2019-0904-SA

Grey Zones in Dual Probe HER2 ISH Test Interpretation: 2018 Update Summary

Most cases

Group 1 Result		
Ratio	HER2 sign/cell	
≥ 2.0	≥ 6.0	

→ HER2 Positive

Group 5 Result		
Ratio	HER2 sign/cell	
< 2.0	< 4.0	

→ HER2 Negative

Grey Zones and Borderline Results: Confirmation, correlation and explanation

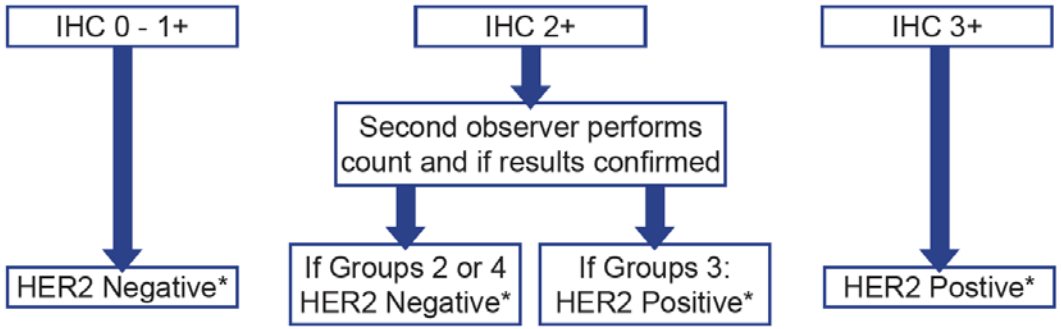
Unusual HER2 ISH Result Categories Requiring Additional Work-Up

Group 2 Result		
Ratio	HER2 sign/cell	
≥ 2.0	< 4.0	

Group 3 Result		
Ratio	HER2 sign/cell	
< 2.0	≥ 6.0	

Group 4 Result		
Ratio	HER2 sign/cell	
< 2.0	4.0 - 6.0	

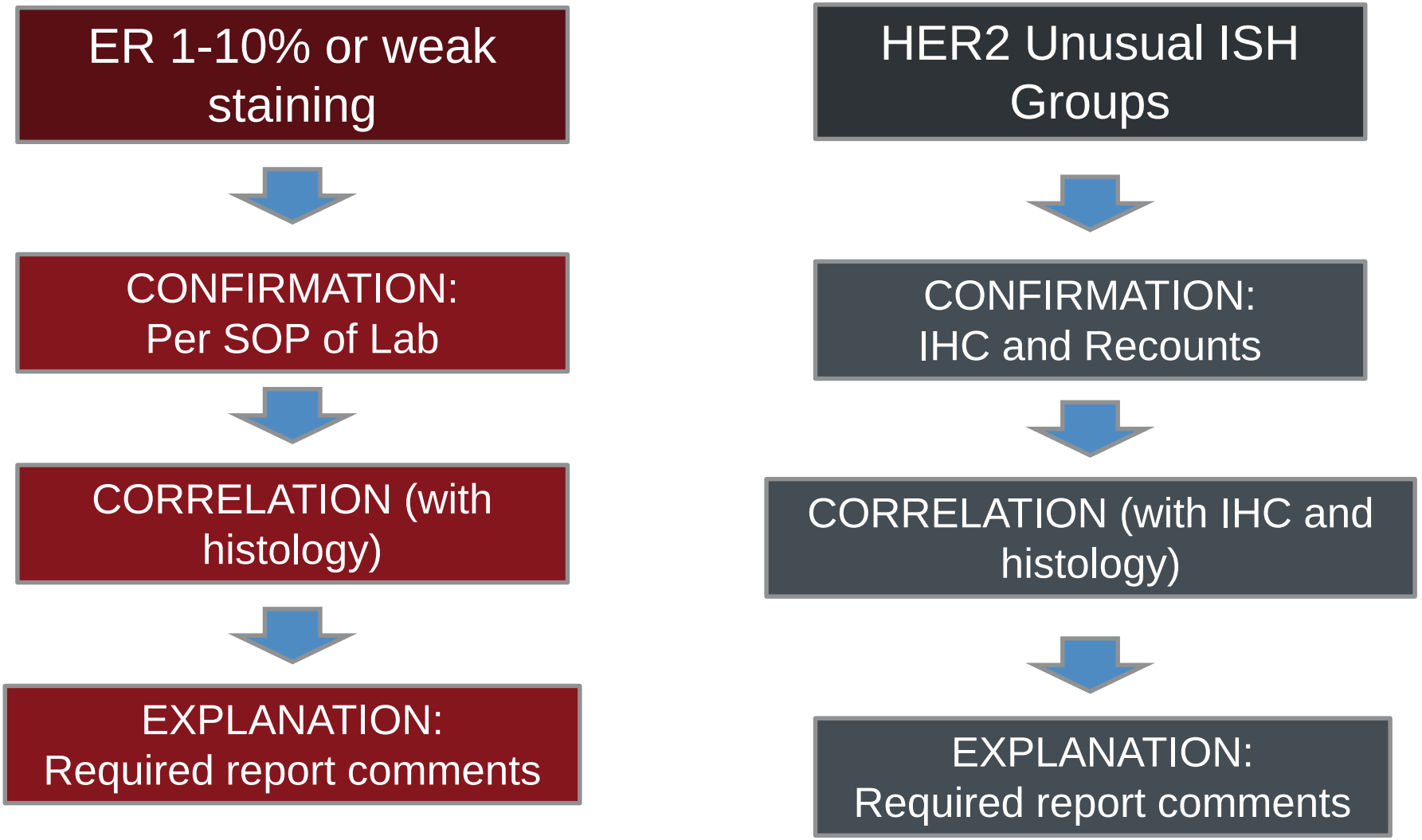
Review concurrent IHC from the same sample



Required comments

REFERENCE:
Wolff AC, e. J Clin Oncol 2018; 36: 2105–22.
WHO 5th edition Tumours of the Breast 2019

Borderline or Unusual ER or HER2 Results: Summary



Should we still do PgR Testing??

- **Yes, PR testing is still useful**
  - **Biology suggests that it is important in modulating ER**
  - **Marker of prognosis in multiple settings**
  - **IHC subtyping / defining TNBC for many clinical trials; new AJCC staging**
  - **To identify possible false-negative ER tests, as quality measure**
- **The utility of PgR testing continues to be largely prognostic in the ER positive invasive cancer population, but testing using similar principles to ER testing is still recommended for invasive cancers.**
- **No new evidence that PR+ vs PR- (however defined) is predictive marker for ET vs no or choice of ET; consistent that higher ER+/PR+ (eg ≥50%) is more ET-responsive**
- **No data for ER-/PR-/HER2- vs ER-/HER2- to define TNBC**
- **No new data on utility in DCIS**

Polling questions will now show up on your screen

FAQ: Do you have to report “Low Positive” for PgR 1-10%?

A. Yes

B. No

C. I don’t know

FAQ: Do you have to report “Low Positive” for PgR 1-10%?

A. **Yes**

B. **No (Optional to do so - but if you do – do <u>not</u> include the comment intended for Low Positive ER)**

C. **I don’t know**

Recommendation 4: DCIS

ER testing in cases of newly diagnosed DCIS (without associated invasion) is recommended to determine potential benefit of endocrine therapies to reduce risk of future breast cancer. PgR testing is considered optional.

Moderate Recommendation

FAQ: Do you have to report “Low Positive” for DCIS with ER between 1-10%?

A. Yes

B. No

C. I don’t know

FAQ: Do you have to report “Low Positive” for DCIS with ER between 1-10%?

A. **Yes**

B. **No (Optional to do so - but if you do – do <u>not</u> include the comment intended for Low Positive ER)**

C. **I don’t know**

FAQ: Do we still need to report stain intensity for ER and PgR?

A. **No**

B. **Yes**

C. **I don’t know**

FAQ: Do we still need to report stain intensity?

A. No

B. **Yes (Stain intensity helps determine how well the assay worked and may be important biologically, but only the % of cells staining determines if the result is positive or negative)**

C. I don’t know

FAQ: What if I use the Allred Score or H-score?

That is fine. But….

- ✓ **Report needs to include both percent and intensity overall raw results**
- ✓ **Low positive ER needs to be defined as 1-10% positive for consistency across labs.**

FAQ: Does the actual formalin time and cold ischemia time need to be included in the template/original pathology report?

Need to have documented:

- ✓ **Time the tissue is removed from the patient**
- ✓ **The time it is placed in fixative**
- ✓ **The cold ischemia time**
- ✓ **The duration of fixation**
- ✓ **The fixative type**

These can be recorded in the pathology report or in another suitable location that is available for review.

Including the specific times in the pathology report is at the laboratory's discretion (note: the CAP Laboratory Accreditation Program requires accredited laboratories to specify the type of fixative used and the cold ischemia time in all ER, PgR and HER2 reports).

The laboratory is also responsible for determining if the cold ischemia and fixation times meet the requirements specified in the latest version of the ASCO/CAP ER/PgR testing guidelines.

Recommendation 1.6. Optimal external proficiency assessment

The laboratory performing ER and PgR testing must participate in external proficiency testing or alternative performance assessment as required by its accrediting organization.

Strong Recommendation

Recommendation was updated to remove information about what constitutes satisfactory proficiency assessment. Laboratories are instructed to follow the requirements of their accrediting organization.

Recommendation 1.7. Optimal laboratory accreditation

- **On-site inspection every other year should be undertaken with annual requirement for self-inspection.**

- **Moderate Recommendation**
- **Statement Reaffirmed**

Initial test validation

- **There will be an upcoming CAP guideline on principles of predictive IHC test initial validation**
  - Recommendation 1.5., continued
  - Regular, ongoing assay reassessment should be done at least semiannually (as described in Fitzgibbons et al). Revalidation is needed whenever there is a significant change to the test system (Torlakovic et al).
  - Ongoing competency assessment and education of pathologists is required.
  - Strong Recommendation

Recommendation 3.

Validated IHC is the recommended standard test for predicting benefit from endocrine therapy. No other assay types are recommended as the primary screening test for this purpose.

Strong Recommendation

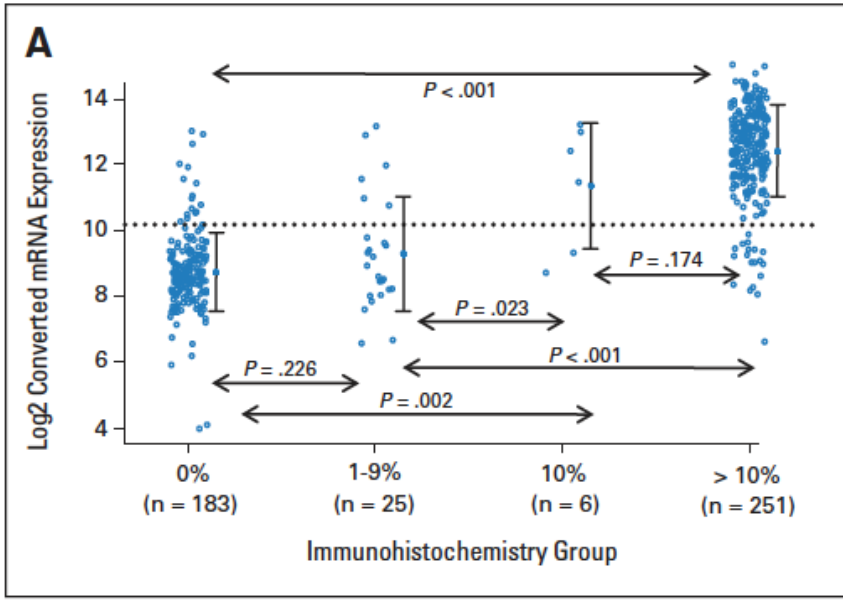
No study has included treatment in + vs - to examine if predictive of endocrine benefit

mRNA methods may be be less sensitive than IHC in detecting low level ER expression

Estrogen Receptor (ER) mRNA and ER-Related Gene Expression in Breast Cancers That Are 1% to 10% ER-Positive by Immunohistochemistry

Takayuki Iwamoto, Daniel Booser, Vicente Valero, James L. Murray, Kimberly Koenig, Francisco J. Esteva, Naoto T. Ueno, Jie Zhang, Weiwei Shi, Yuan Qi, Junji Matsuoka, Elliana J. Yang, Gabriel N. Hortobagyi, Christos Hatzis, W. Fraser Symmans, and Lajos Pusztai

- **Cancers with 1-9% ER staining by IHC had features overlapping with ER <1% cases (basal-like PAM-50, worse survival)**
- **Were often below threshold of positive for mRNA assay….**



J Clin Oncol 30:729-734. © 2012 by American Society of Clinical Oncology

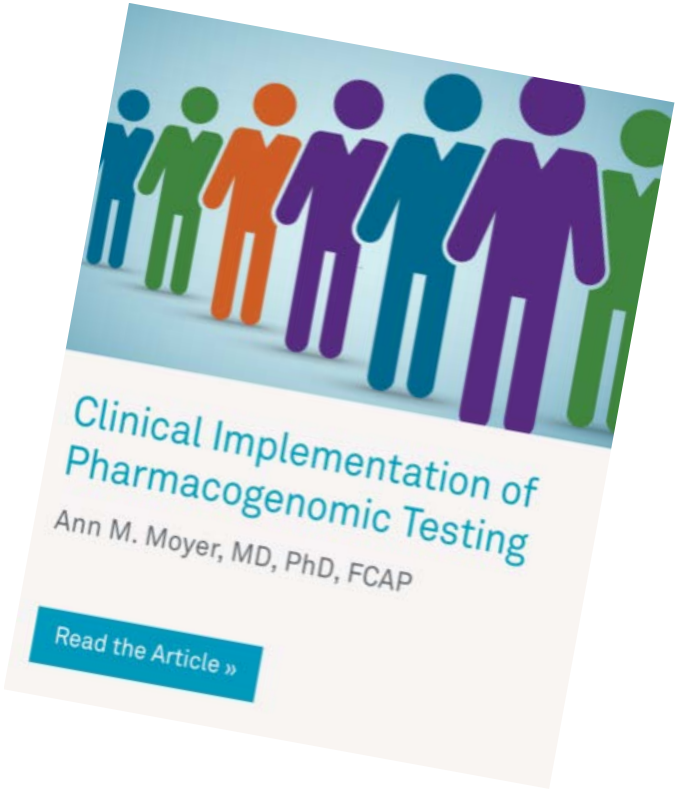
Summary of Major Impact of 2020 Updates to ER/PgR IHC Guidelines:

- **New ER Low Positive reporting category for invasive cases with 1-10% staining**
- **Need for lab specific SOP to ensure reproducibility/validity of invasive cases with <10% or weak staining and report status of controls**
- **Increased focus on appropriate internal and external controls (new comment for cases with no internal controls)**
- **Test DCIS for ER (PgR optional)**

CAP's Precision Medicine Webpage

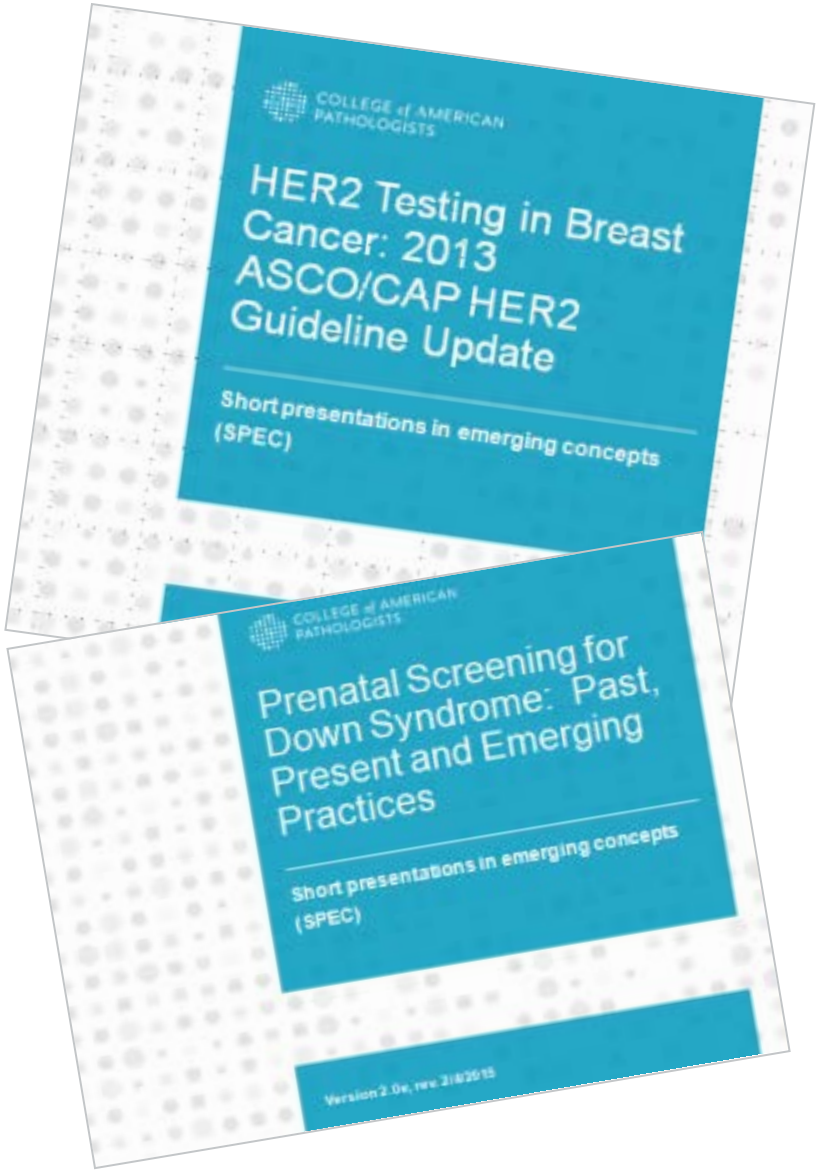
- **The webpage includes brief, relevant articles by CAP members that enable the reader to gain a better understanding of a particular area of precision medicine.**
  - **Examples include pharmacogenetics, immune response genes, and the latest in the molecular drivers of cancer.**
  - **Access them www.cap.org >**

    Member Resources > Precision Medicine



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **Short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **Recent topics include:**
  - **Microbiome**
  - **Biomarkers in Lung Cancer**
  - **MDS**
  - **Other emerging topics**
- **Access them at www.cap.org > Resources and Publications**



CAP's Pathology Resource Guide: Precision Medicine

- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**

THANK YOU!

Thank you for attending our webinar,

"What's New in the 2020 Update to the CAP/ASCO ER/PgR Testing Guidelines in Breast Cancer?

by Kimberly Allison, MD, FCAP

For comments about this webinar or suggestions for upcoming webinars, please contact phcwebinars@cap.org.

NOTE: There is no CME/CE credit available for today's free webinar. The PDF of the presentation will be sent out in a week.

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