COLLEGE of AMERICAN PATHOLOGISTS

Quantitative Image Analysis of HER2 Immunohistochemistry for Breast Cancer

CAP Guideline Update and Review of Draft Recommendations

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Disclosures

- There is no financial disclosure or conflict of interest.
- The presentation represents my personal and professional opinion only.
- Member of Digital Pathology Association Board of Directors & Executive Committee, Editorial Board member of Journal of Pathology Informatics, Member of Association of Pathology Informatics, CAP Digital Pathology Committee and contributing editor of CAP Digital Pathology Resource Guide 2014-2017, Chair of the CAP Pathology and Laboratory Quality Center Expert Panel of the HER2 IHC Quantitative Image Analysis guideline.

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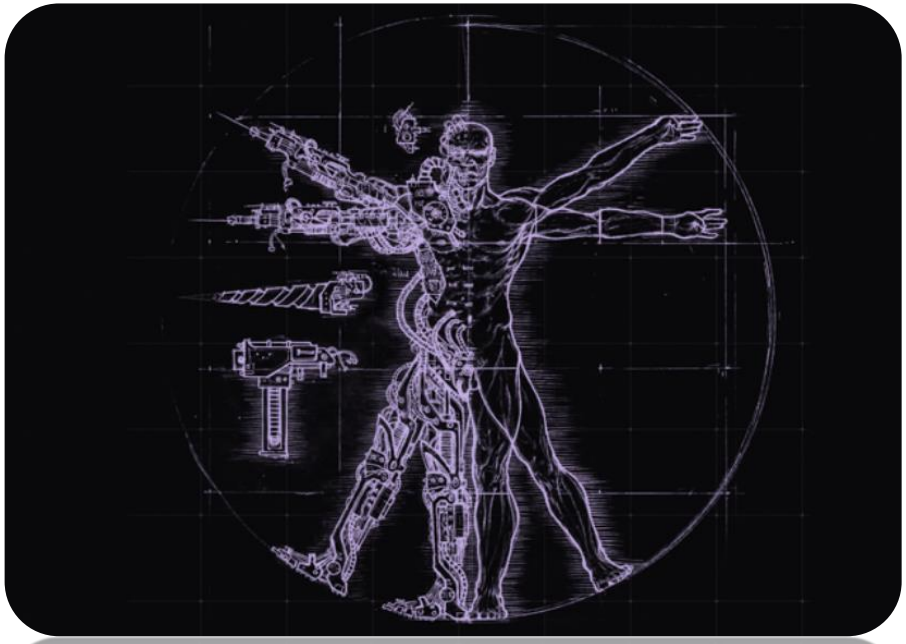
Outline

- **Introduce quantitative image analysis (QIA)**
- **Discuss some of the challenges of QIA and HER2 IHC for breast cancer interpretation and reporting**
- **Review draft recommendations from CAP guideline on HER2 IHC QIA in progress**



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Introduction

Quantitative image analysis (QIA) = Quantitative extraction of meaningful information from images

QIA is a powerful advantage of digital pathology

- **When the slides are digitalized, they can be numerically analyzed using computer algorithms.**
- **Algorithms can be used to automate the manual counting of structures, or for classifying the condition of tissue, like algorithms used in grading tumors.**
- **This could reduce human error and improve accuracy of diagnoses.**

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WIKIPEDIA
The Free Encyclopedia

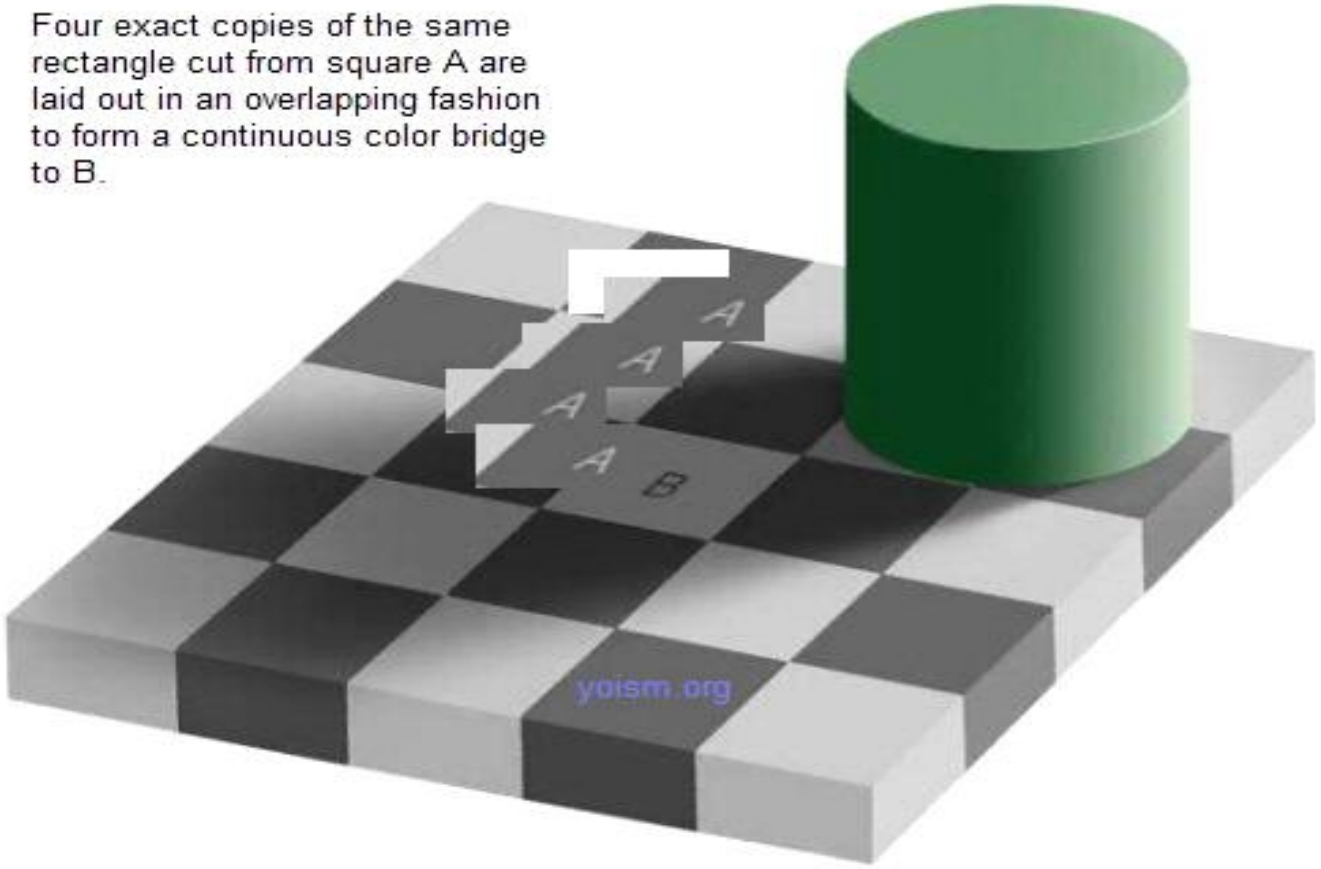
The power of image analysis

Reliable

Measurable

Repeatable

Quantifiable



Courtesy of Dr. Mark Lloyd

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Benefits of image analysis

- Better accuracy (more precise quantitative measurements)
- Standardization (more reproducible results, especially for intermediate categories & complex scoring systems)
- Automation (reduce time consumption for pathologists, especially for performing mundane tasks like counting)
- Enhanced efficiency (triage cases – eg, weed out negative cases)
- CAD (eg, help pathologists find, diagnose & grade disease like cancer)
- Enable big data projects (eg, image analysis for biomarker discovery)

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Current state of QIA

- Advancements in genomics, computing and imaging technology have spurred new opportunities to use QIA in diagnostic medicine
- Current shift from research to clinical applications, especially in diagnostic testing
- Diagnostic pathology transition from qualitative (descriptive, analog) to quantitative (automated) science
- Precision medicine currently demands precision diagnostics
- Most widely employed clinical diagnostic algorithms are for breast cancer biomarkers (ER, PR, HER2, Ki-67 and p53)

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Image analysis tools

Software Type	Image Format Compatibility	Technical Knowledge Level	Customization Level	Features	Examples
Basic Science Image Analysis	Most Image Formats	Moderate	High	Variety of measurement tools Access to image processing tools Some automation	Image Pro Premier Metamorph ImageJ/FIJI Cell Profiler
Slide Scanner Based	Limited Image Formats	Low	Low-Moderate	Direct access to images Access to common algorithms US IVD for HER2/ER Pattern recognition Batch processing Designed for Digital Pathology	Roche/Ventana Leica/Aperio 3D Histech HALO PathXL TissueMark
Digital Pathology Inspired	Most Image Formats	Moderate	Moderate	Workflow based Easily adjustable parameters Batch processing Pattern recognition Access more feature data Designed for Digital Pathology	InForm, Visiopharm Definiens Tissue Studio
Algorithm Based	Most Image Formats	High	High	Fully customizable Unique algorithms Even more feature data Batch processing	MatLab Visiopharm Definiens Developer

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Image analysis tools

- **Examples of whole slide image analysis:**
  - **Positive pixel count**

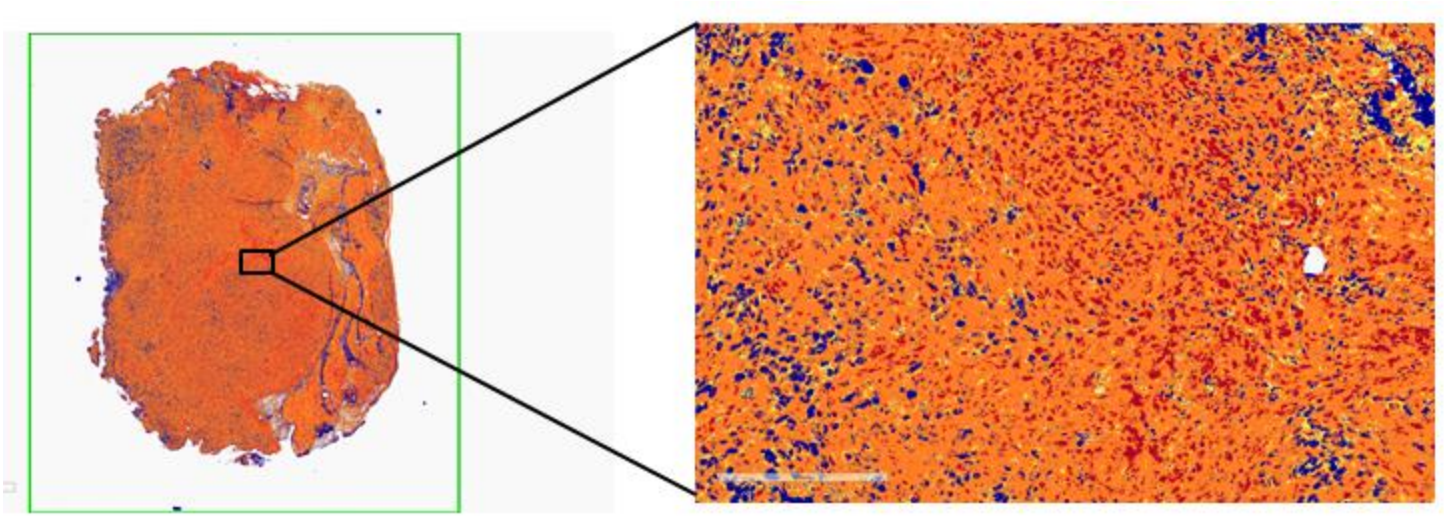

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Image analysis tools

- **Examples of whole slide image analysis:**
  - **Nucleus analysis**

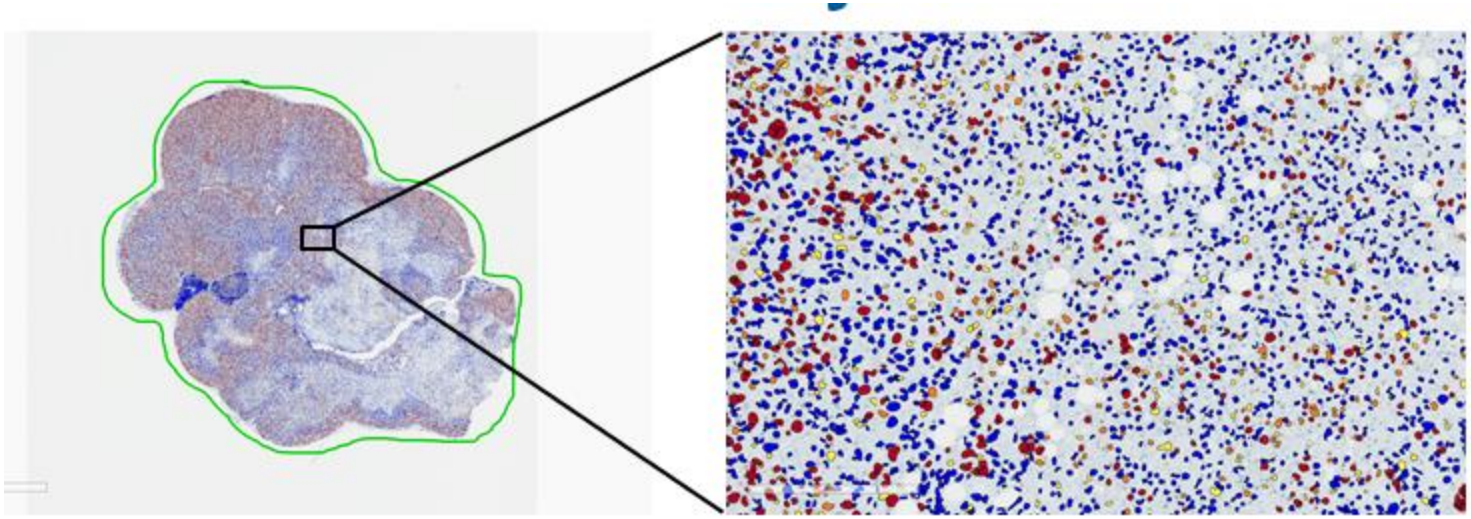

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Image analysis tools

- **Examples of whole slide image analysis:**
  - **Cytoplasm analysis**

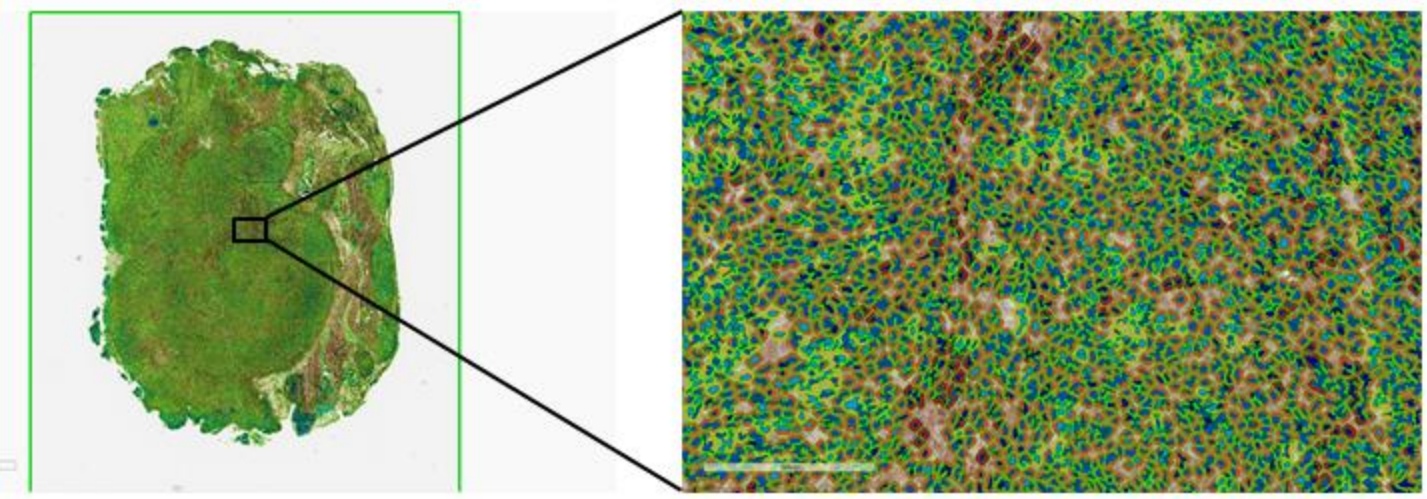
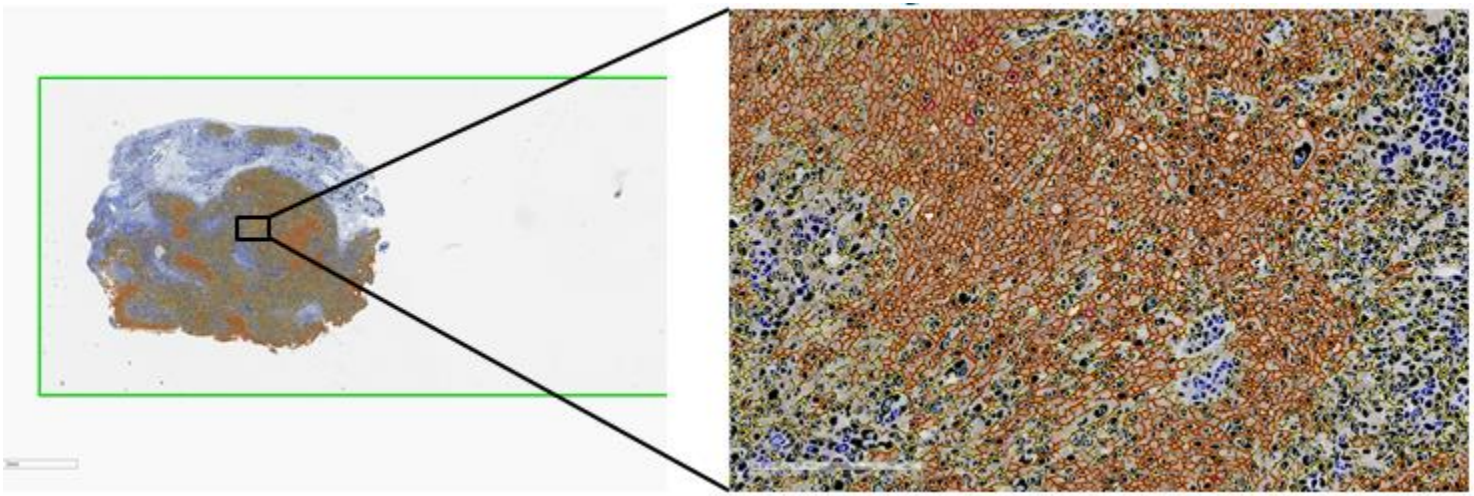


Image analysis tools

- **Examples of whole slide image analysis:**
  - **Membrane**



Algorithms for QIA

RESEARCH	CLINICAL
Many apps	Limited algorithms
Modifiable parameters	Locked down apps
Lab developed tests	Approved (FDA)
Research environment	Regulated lab (CLIA)
Continuous data	Discrete results
Variable output	Match manual scores
Researchers	Pathologist oversight
Financial benefit	Questionable ROI (CPT code)
Stand-alone system	Integrated workflow
Widespread use	Slow adoption

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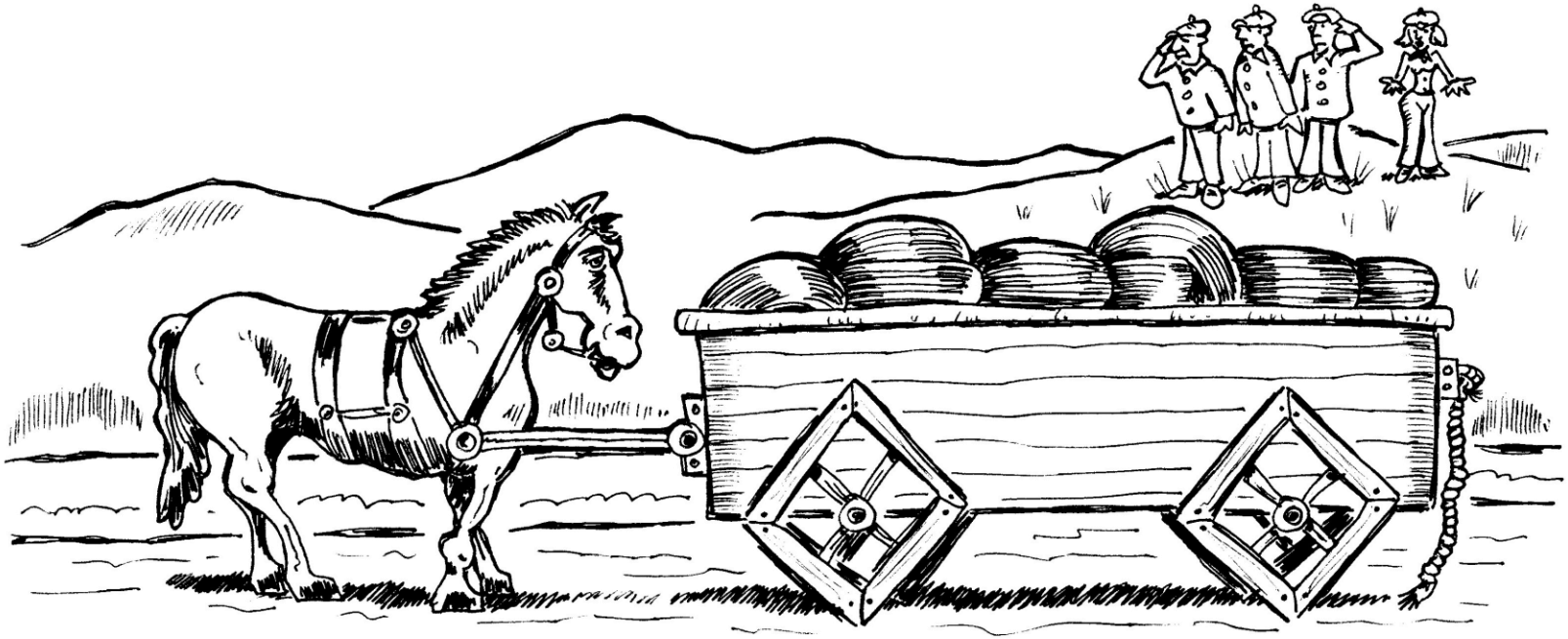
Challenges



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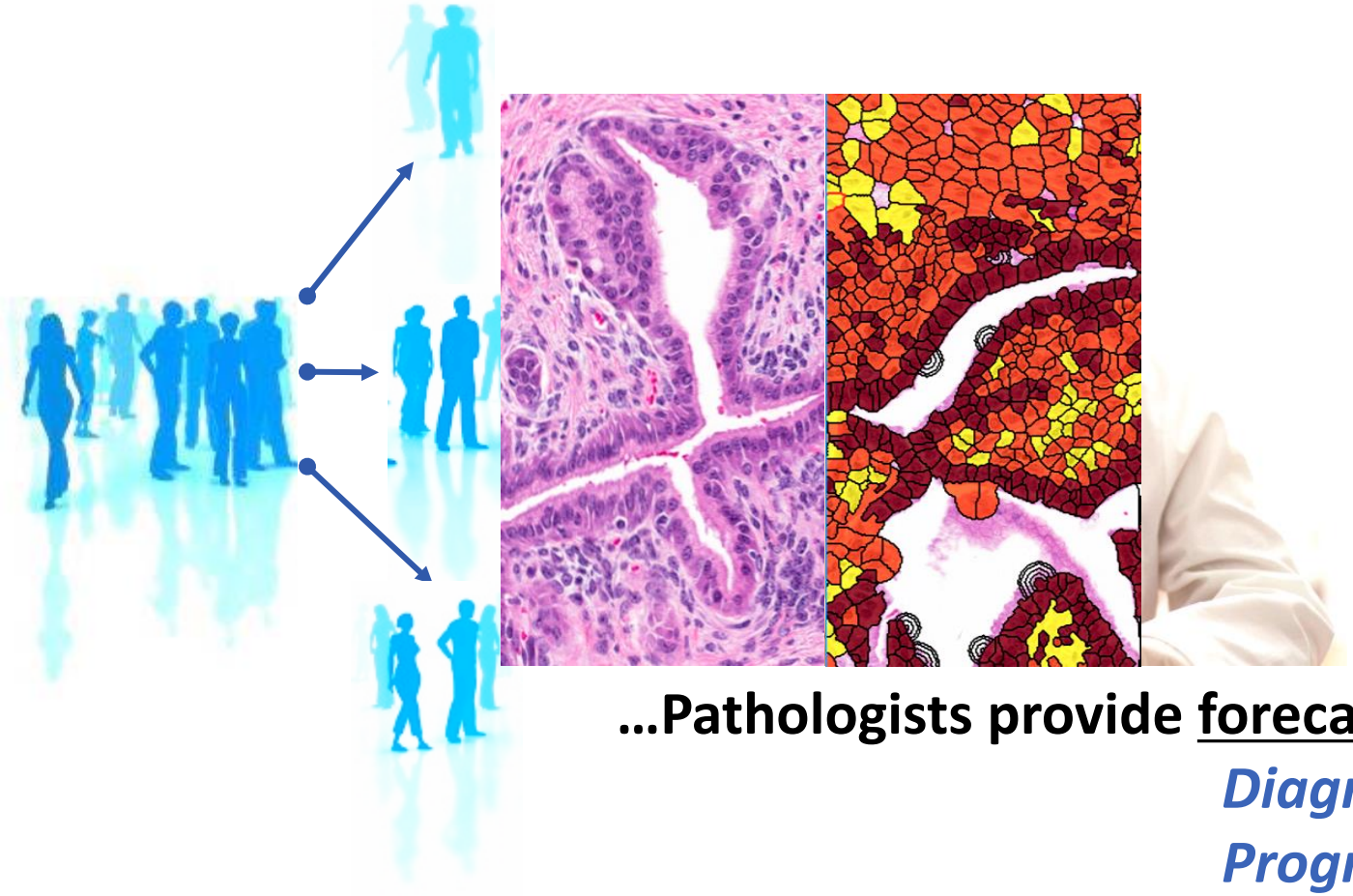
A patient's medical journey begins with their diagnosis…



…Pathologists provide <u>forecast of</u>

Diagnosis

Prognosis

Therapeutic selection &

Prediction of response

Courtesy of Dr. Mark Lloyd

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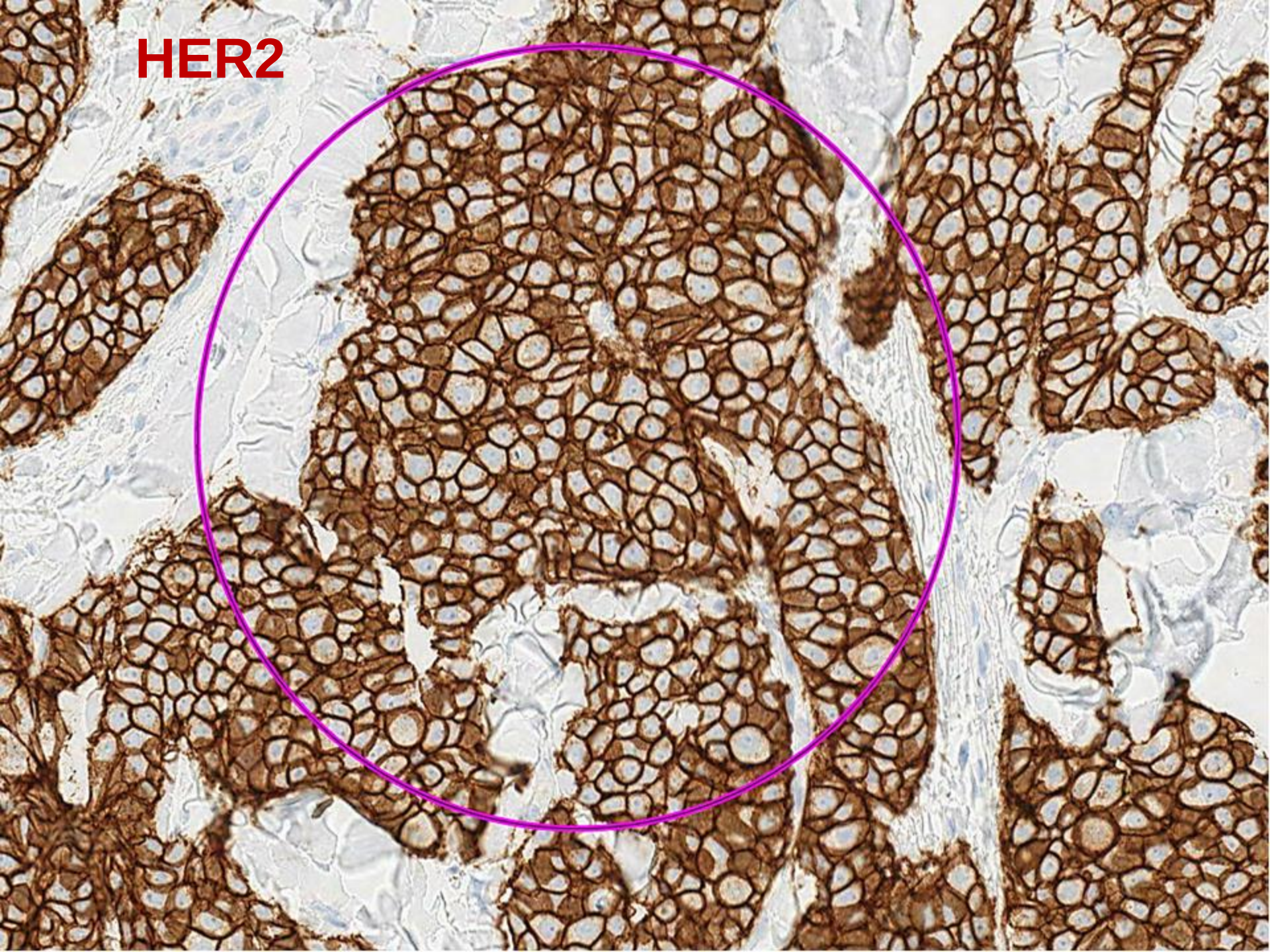
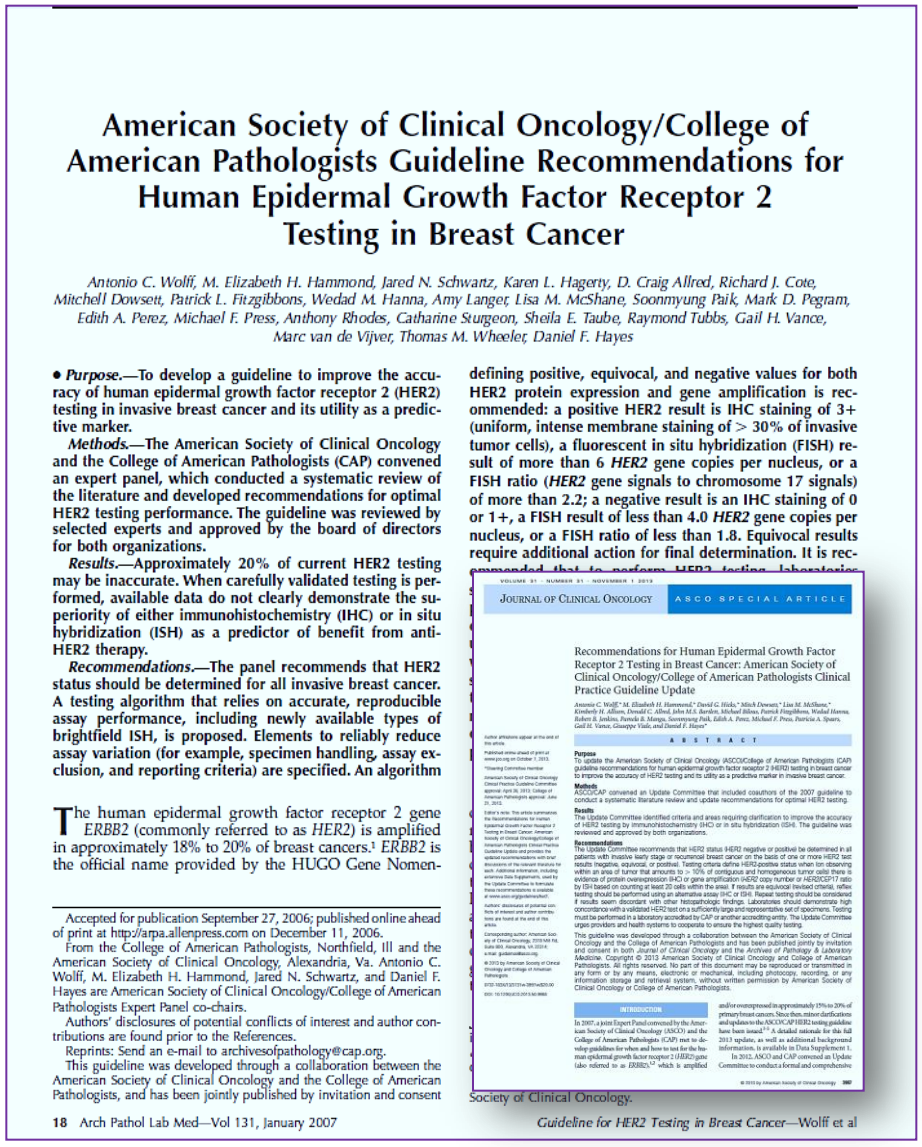

HER2

ASCO/CAP HER2 guideline



American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, Daniel F. Hayes

• ***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 rec-

The human epidermal growth factor receptor 2 gene *ERBB2* (commonly referred to as *HER2*) is amplified in approximately 18% to 20% of breast cancers.[1] *ERBB2* is the official name provided by the HUGO Gene Nomen-

Accepted for publication September 27, 2006; published online ahead of print at http://arpa.allenpress.com on December 11, 2006.

From the College of American Pathologists, Northfield, Ill and the American Society of Clinical Oncology, Alexandria, Va. Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, and Daniel F. Hayes are American Society of Clinical Oncology/College of American Pathologists Expert Panel co-chairs.

Authors' disclosures of potential conflicts of interest and author contributions are found prior to the References.

Reprints: Send an e-mail to archivesofpathology@cap.org.

This guideline was developed through a collaboration between the American Society of Clinical Oncology and the College of American Pathologists, and has been jointly published by invitation and consent

JOURNAL OF CLINICAL ONCOLOGY — ASCO SPECIAL ARTICLE

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update

ABSTRACT

Purpose

Methods

Results

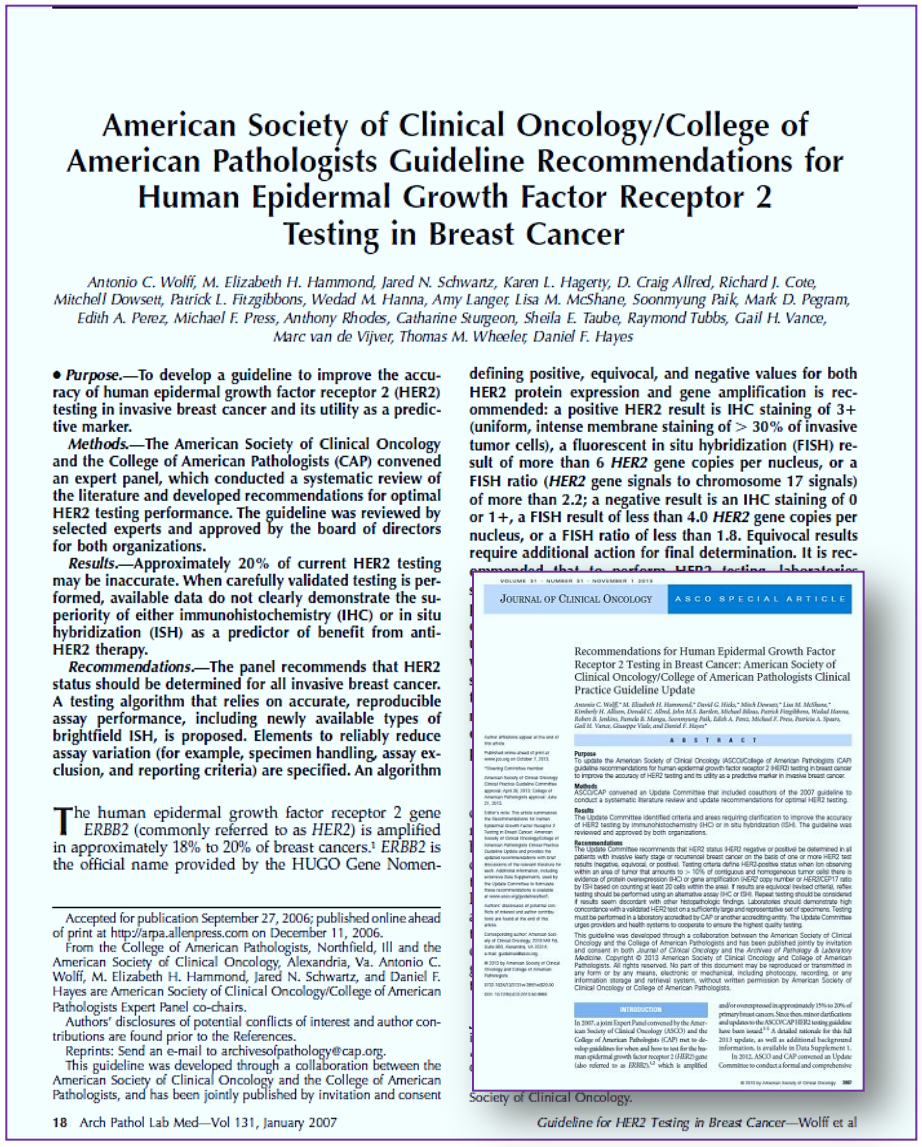
Recommendations

INTRODUCTION

Society of Clinical Oncology.

- **HER2 status must be determined in all patients with invasive breast cancer.**
- **In the US, recommend using an assay that has received FDA approval, although a CLIA-certified laboratory may choose instead to use a LDT.**
- **If results are equivocal (revised criteria), reflex testing should be performed using an alternative assay (IHC or ISH).**
- **Must ensure that interpretation and reporting guidelines for HER2 testing are followed.**

ASCO/CAP HER2 guideline



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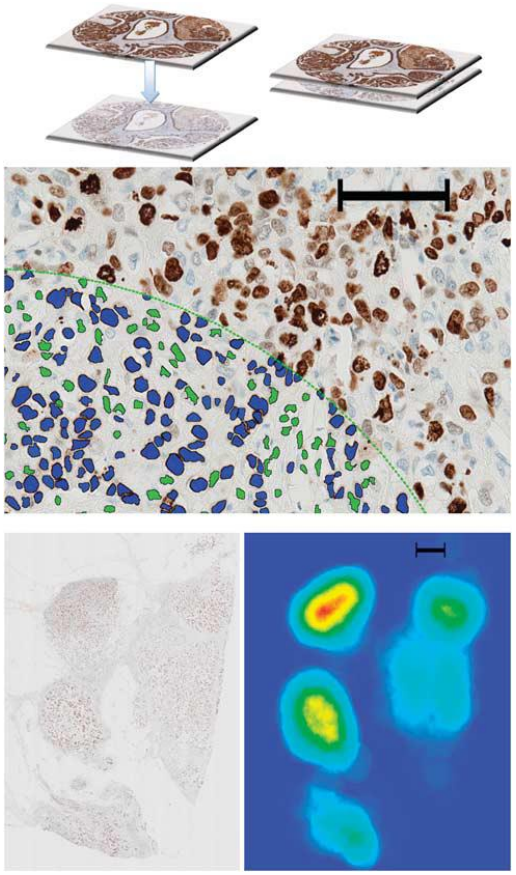
- If results are equivocal (revised criteria), reflex testing should be performed using an alternative assay (IHC or ISH).
- Must ensure that interpretation and reporting guidelines for HER2 testing are followed.
- Image analysis can be used to achieve consistent interpretation.
- However, a pathologist must confirm the image analysis result.
- Image analysis procedures must be validated before implementation.
- Image analysis equipment, just as other laboratory equipment, must be calibrated and subjected to regular maintenance and internal quality control evaluation.

Modern Pathology (2016) 29, 318–329

Digital image analysis outperforms manual biomarker assessment in breast cancer

Gustav Stålhammar[1,2], Nelson Fuentes Martinez[1,3], Michael Lippert[4], Nicholas P Tobin[5], Ida Mølholm[4,6], Lorand Kis[7], Gustaf Rosin[1], Mattias Rantalainen[8], Lars Pedersen[4], Jonas Bergh[1,5,9], Michael Grunkin[4] and Johan Hartman[1,5,7]

[1]Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden; [2]St Erik Eye Hospital, Stockholm, Sweden; [3]Södersjukhuset, Stockholm, Sweden; [4]Visiopharm A/S, Hoersholm, Denmark; [5]Cancer Center Karolinska, Stockholm, Sweden; [6]Department of Applied Mathematics and Computer Science, Technical University of Denmark, Kongens Lyngby, Denmark; [7]Department of Clinical Pathology, Karolinska University Hospital, Stockholm, Sweden; [8]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden and [9]Department of Oncology, Karolinska University Hospital, Stockholm, Sweden



- In conclusion, the system for DIA evaluated here was in most aspects a superior alternative to manual biomarker scoring.
- It also has the potential to reduce time consumption for pathologists, as many of the steps in the workflow are either automatic or feasible to manage without pathological expertise.

Table 2 Molecular 'intrinsic' breast cancer subtypes and surrogate definitions by immunohistochemical profile

Intrinsic subtype	*Surrogate IHC classification*
Luminal A	ER ≥ 1% and/or PR ≥ 20% and HER2 'negative' and Ki67 'low'
Luminal B	1. ER ≥ 1% and/or PR ≥ 20% and HER2 'negative' and Ki67 'high' or 2. ER ≥ 1% and PR < 20% and HER2 'negative.' Any Ki67 or 3. ER ≥ 1% and/or PR ≥ 1% and HER2 'positive.' Any Ki67
HER2-enriched	ER < 1% and PR < 1%. HER2 'positive.' Any Ki67
Basal-like	ER < 1% and PR < 1%. HER2 'negative.' Any Ki67

% = Proportion of tumor cells stained with the respective biomarker. 'Positive' and 'negative' = as defined by the American Society of Clinical Oncology and College of American Pathologists recommendations for human epidermal growth factor receptor 2-testing in breast cancer.[30] 'High' and 'low' = as defined by each laboratory's own reference data,[3,6,17] with threshold generally in the range of 14–29%.[4,5,19–21]

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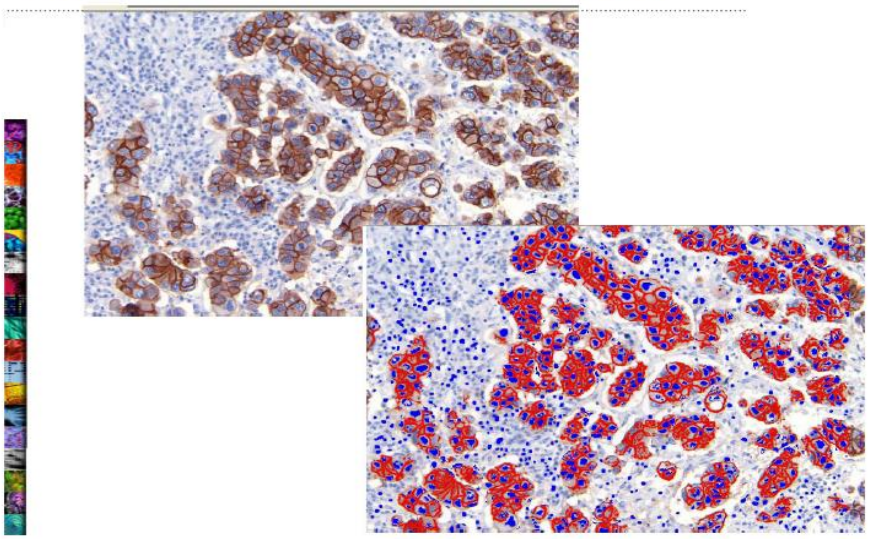
HER2 image algorithms



FDA-approved

Date	K-Number	Tissue - Stain	Reagent	Application	
PATHIAM (BioImagene, Sunnyvale, CA)					
2010/10	K092333	Breast - P53/Ki-67	Dako		Image Analysis
2009/02	K080910	Breast - *HER2/neu*	Dako	Image Analysis	
2007/02	K062756	Breast - *HER2/neu*	Dako	Image Analysis (SW only)	
ScanScope XT System (Aperio Technologies, Vista, CA)					
2009/08	K080564	Breast - *HER2/neu*	Dako	*Tunable Image Analysis*	
2008/10	K080254	Breast - PR	Dako	*Reading on Monitor*	
2008/08	K073667	Breast - ER/PR	Dako Image Analysis		
2007/12	K071671	Breast - *HER2/neu*	Dako *Reading on Monitor*		
2007/10	K071128	Breast - *HER2/neu*	Dako	Image Analysis	
VIAS (Tripath Imaging, Burlington, NC)					
2006/09	K062428	Breast - *P53*	Ventana	Image Analysis	
2006/04	K053520	Breast - *Ki-67*	Ventana	Image Analysis	
2005/08	K051282	Breast - *HER2/neu*	*Ventana*	Image Analysis	
2005/05	K050012	Breast - ER/PR	Ventana	Image Analysis	
ARIOL (Applied Imaging, Santa Clara, CA)					
2004/03	K033200	Breast - ER/PR	Dako	Image Analysis	
2004/01	K031715	Breast - *HER2/neu*	Dako	Image Analysis	
ACIS (Clarient, Aliso Viejo, CA/Chroma Vision, San Juan Capistrano, CA)					
2004/02	K012138	Breast - ER/*PR*	Dako	Image Analysis	
2003/12	K032113	Breast - *HER2/neu*	Dako	*Image Analysis (system)*	
QCA (Cell Analysis, Highland Park, IL)					
2003/12	K031363	Breast - ER	Dako	Image Analysis (SW only)	

Lange H. Lab Med. 2011;42(10):587-591.

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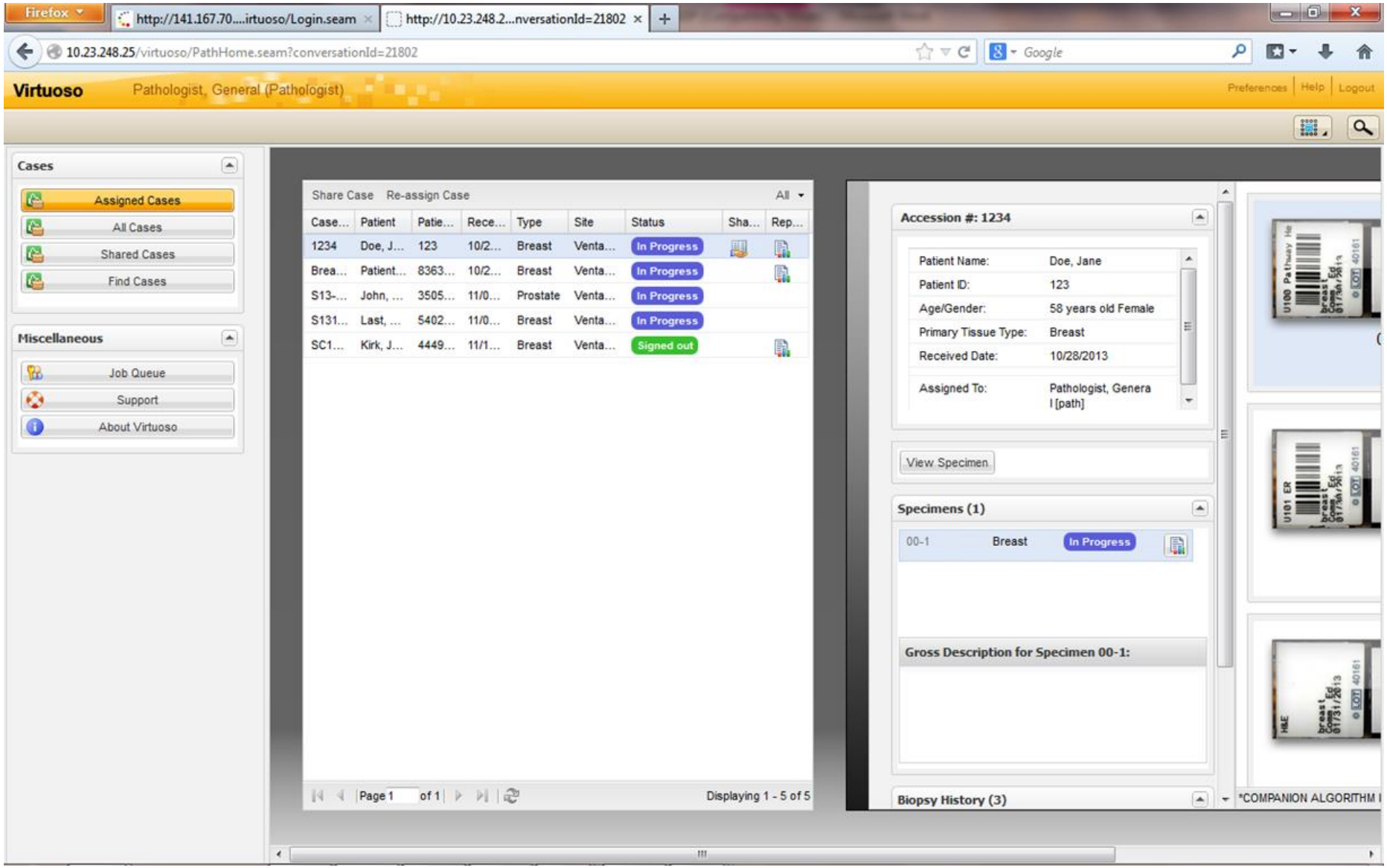

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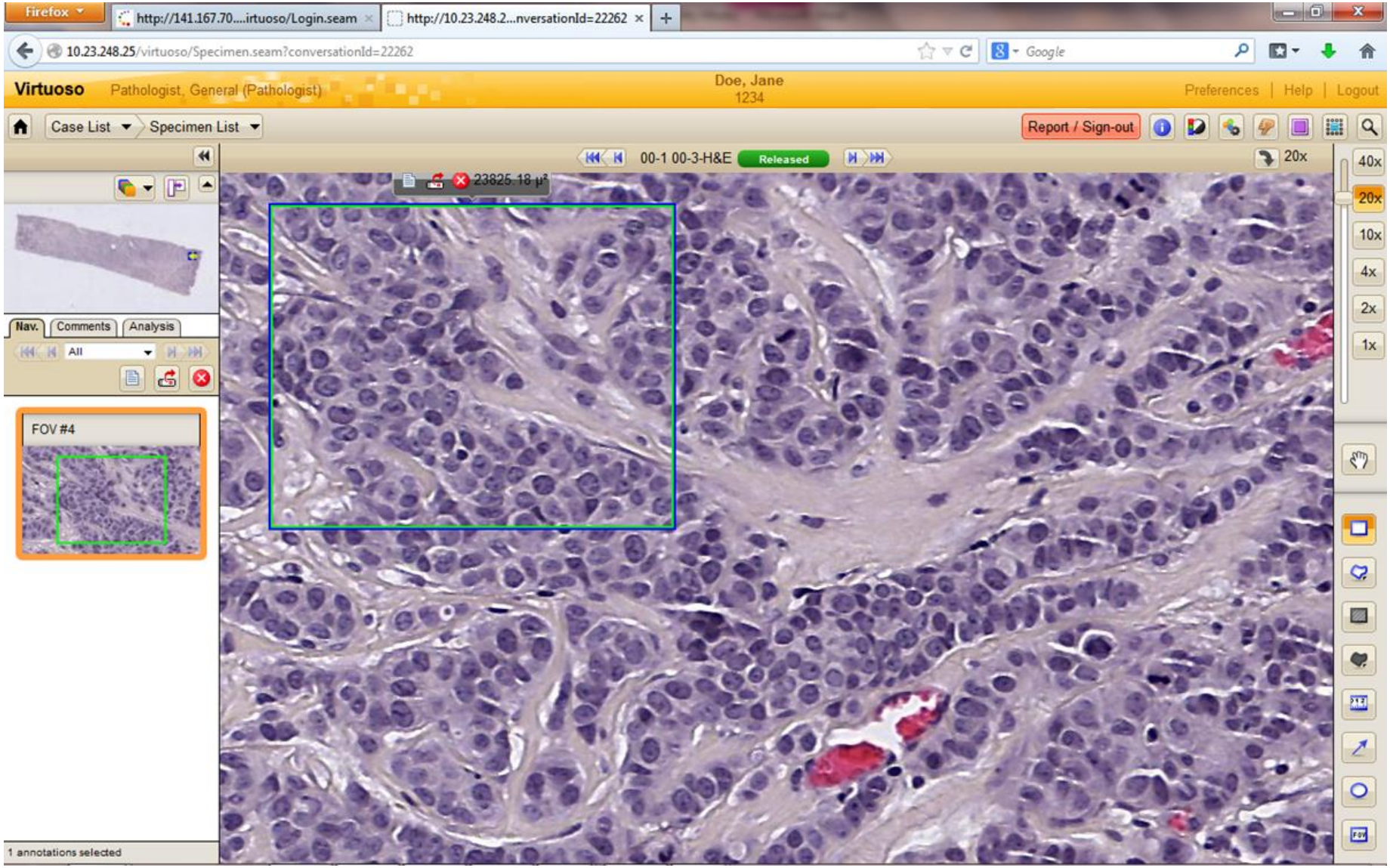
Courtesy of Roche Diagnostic Corporation



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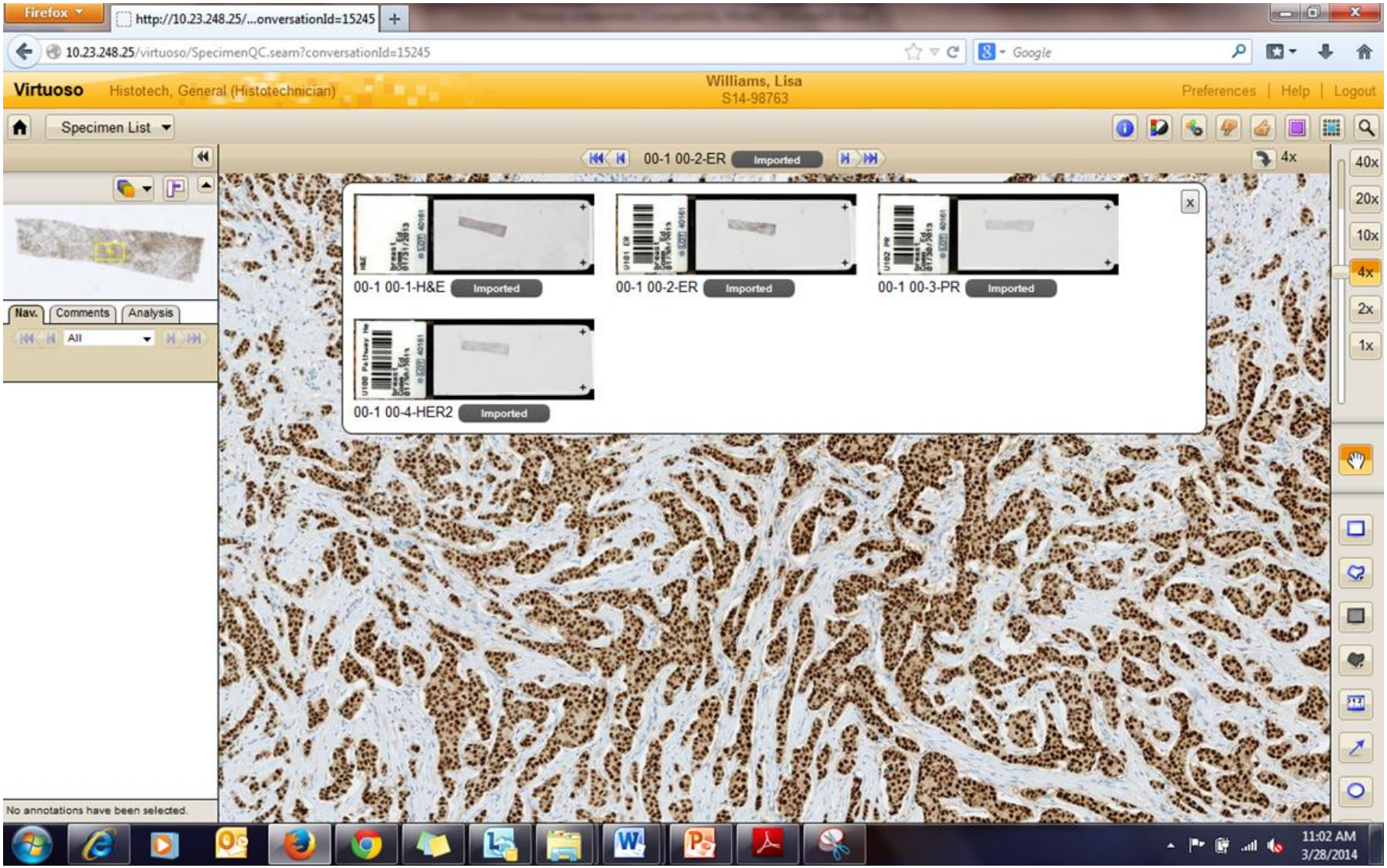

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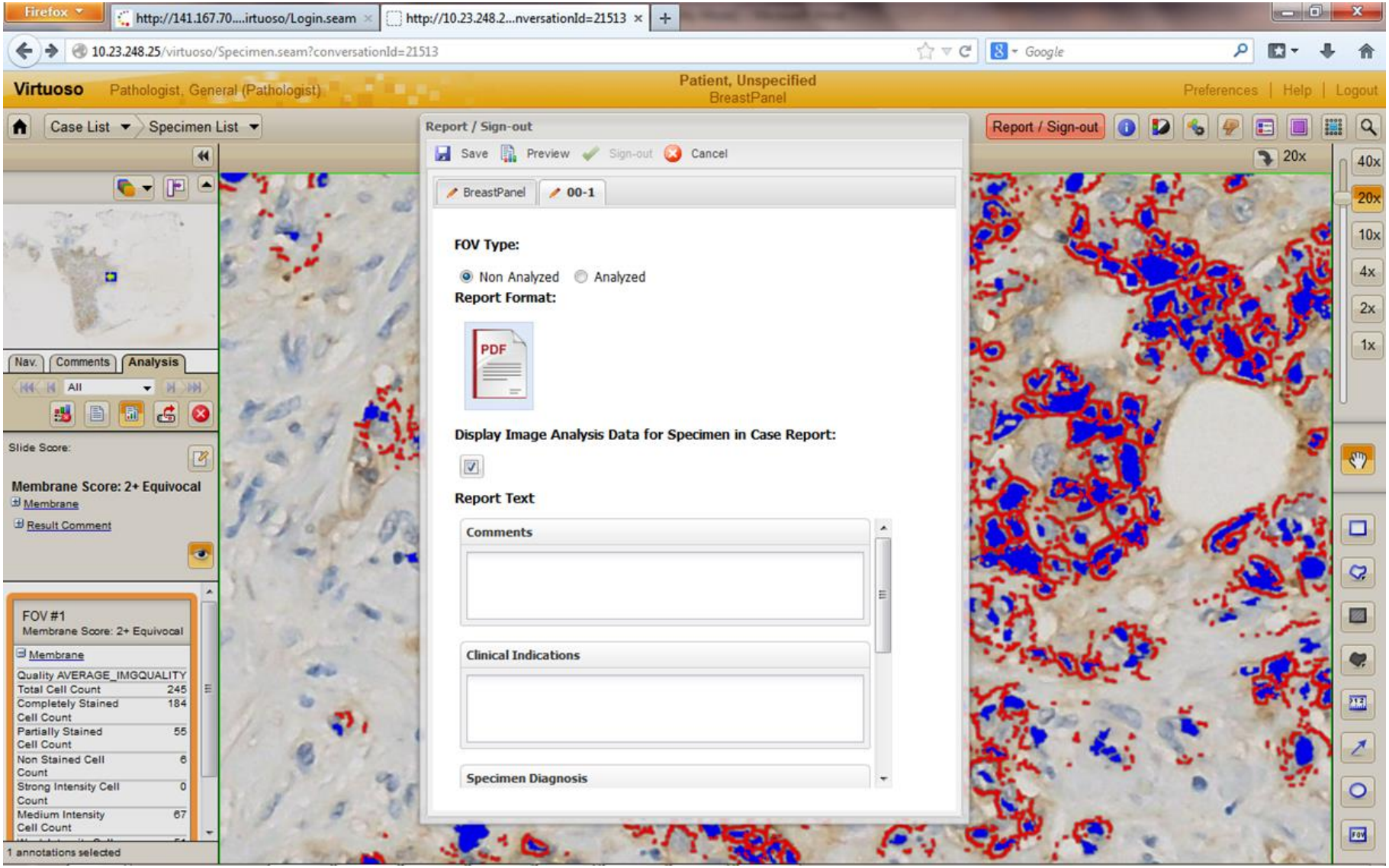

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Courtesy of Roche Diagnostic Corporation

Validation

- **Parameters to consider validating:**
  - **System** (software, etc.)
  - **Test** (IHC platform)
  - **Pathologist** (reader)
  - **Result** (comparison)
- **Gold standard = alternative, validated method**
  - **eg, FISH, another algorithm?**
  - **Accuracy** (concordance/correct)

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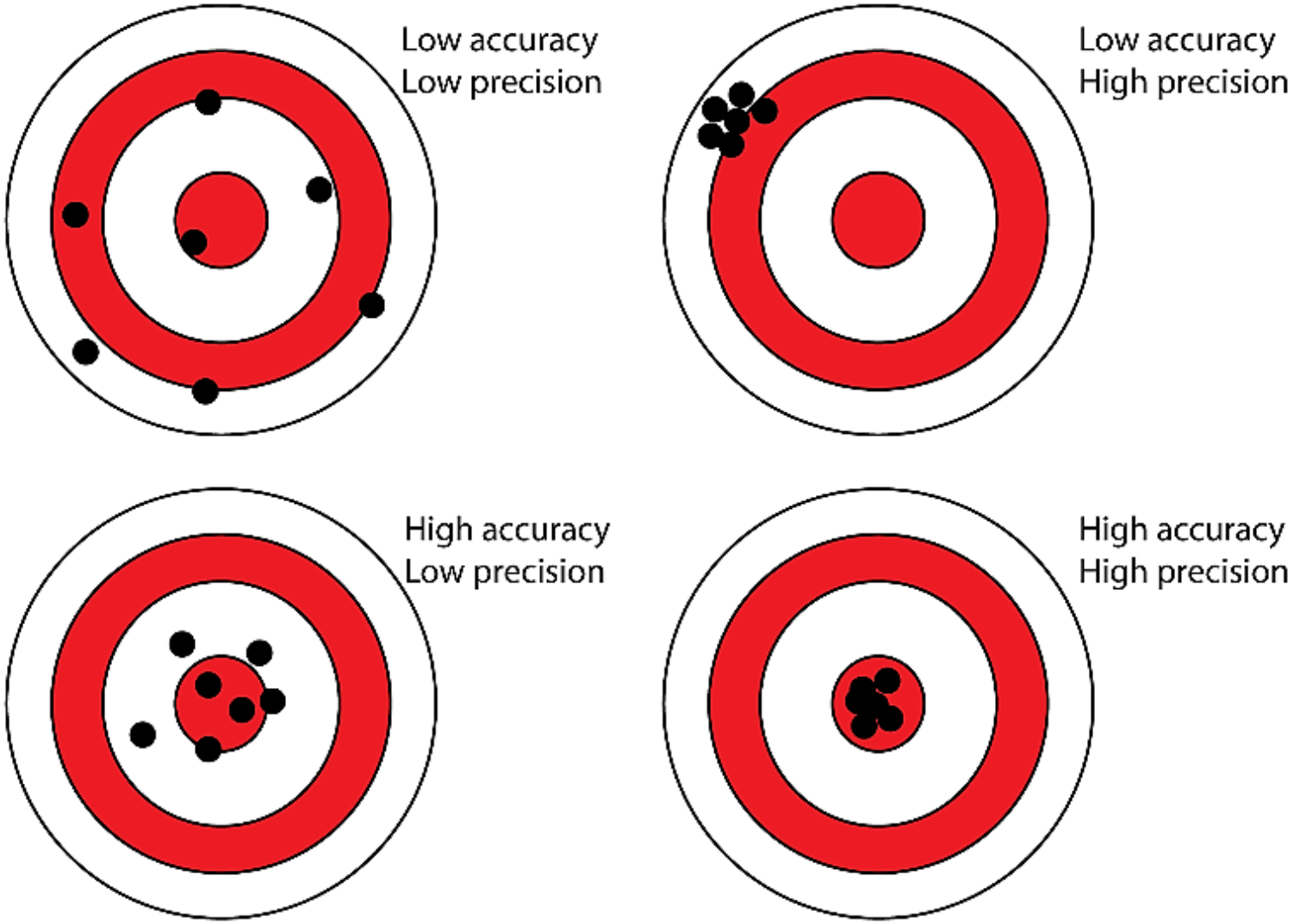

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Accuracy: the closeness of a measured value to a standard or know value
Precision: the closeness of two or more measurements to each other; precision is independent of accuracy.

Precision

- **Repeatability or reproducibility**
  - **Assay variations (batches/runs)**
  - **Technical variations (image acquisition)**
  - **Operator variability (ROI selection)**

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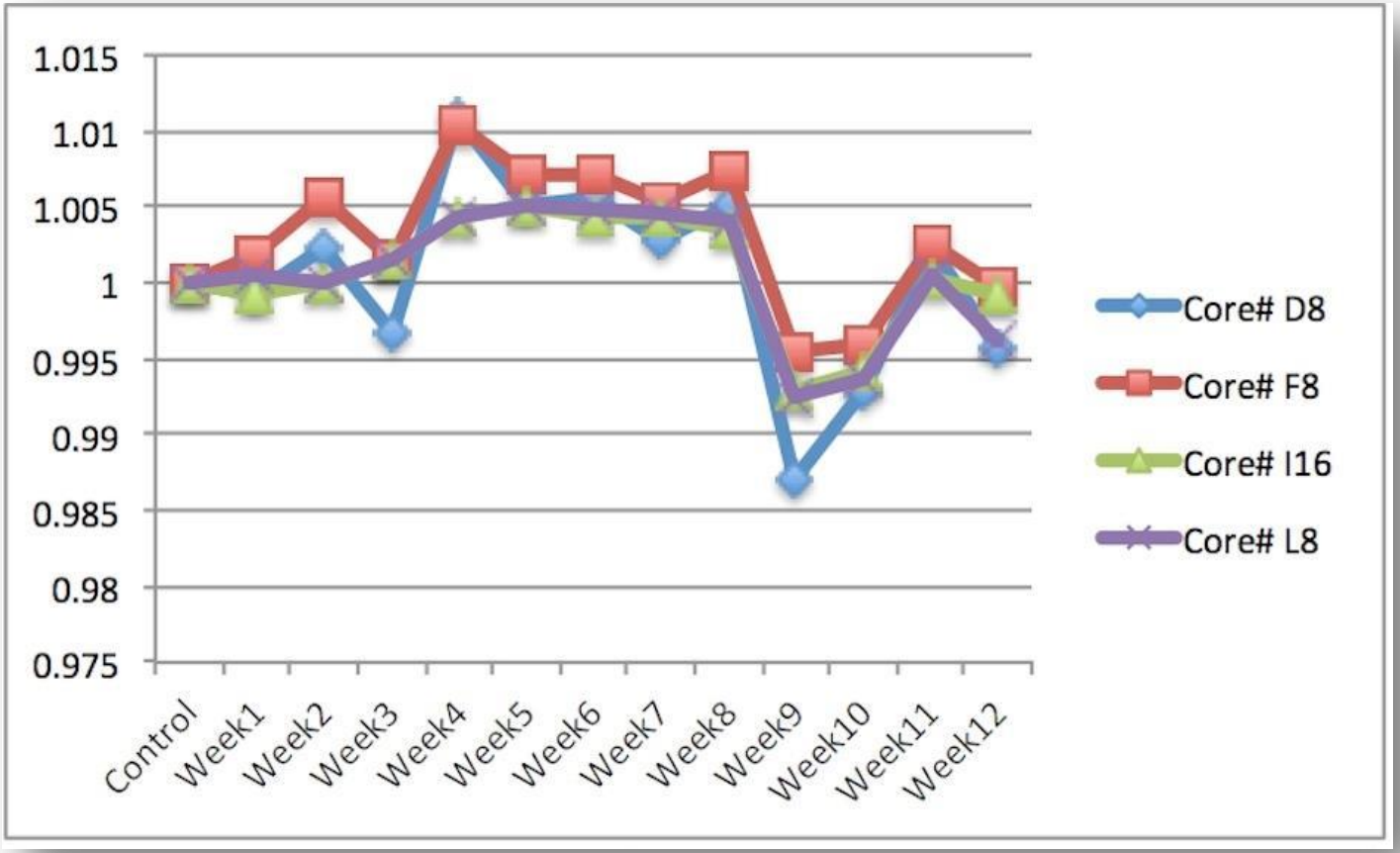
Same scanner variability



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WSI scanner reproducibility

- HER2/neu algorithms
  - Commercial algorithm
  - Preset parameters
- WSI from 3 scanners
- Inter-scanner variability
  - Different image properties
- Reducing discrepancies
  - Re-training (calibration)

Classifier	1+	2+	3+
Pathologist panel	21	137	83
Algorithm 1 on Aperio-CS	46	120	75
Algorithm 1 on Aperio-T2	65	101	75
Algorithm 1 on Hamamatsu	24	119	98
Algorithm 2 on Aperio-CS	13	145	83
Algorithm 2 on Aperio-T2	13	146	82
Algorithm 2 on Hamamatsu	14	149	78

HER2/neu: (Human epidermal growth factor receptor 2)

CAP

Keay et al. J Pathol Inform 2013, 4:19

The gap in practice

- QIA has been shown to improve consistency and accuracy of interpretation than manual scoring by pathologists, but has not gained widespread acceptance
    - In 2016, of the 826 laboratories enrolled in the CAP HQIP-A mailing, 183 (22.1%) reported using QIA
- While the ASCO/CAP HER2 testing guidelines addressing the key pre-analytical and IHC related issues, there is a need of guideline for HER2 IHC QIA

CAP

CAP QIA guideline

Scope:

- to provide recommendations for improving reproducibility, precision, and accuracy in the interpretation of HER2 IHC where QIA is employed

Methods:

- process follows the National Academy of Science (formerly IOM) standards for developing clinical practice guidelines
  - Built on systematic literature review
  - Draft recommendations by an expert panel with the input of an advisory panel
  - Public comment period
  - Grades provided for strength of evidence and strength of recommendation

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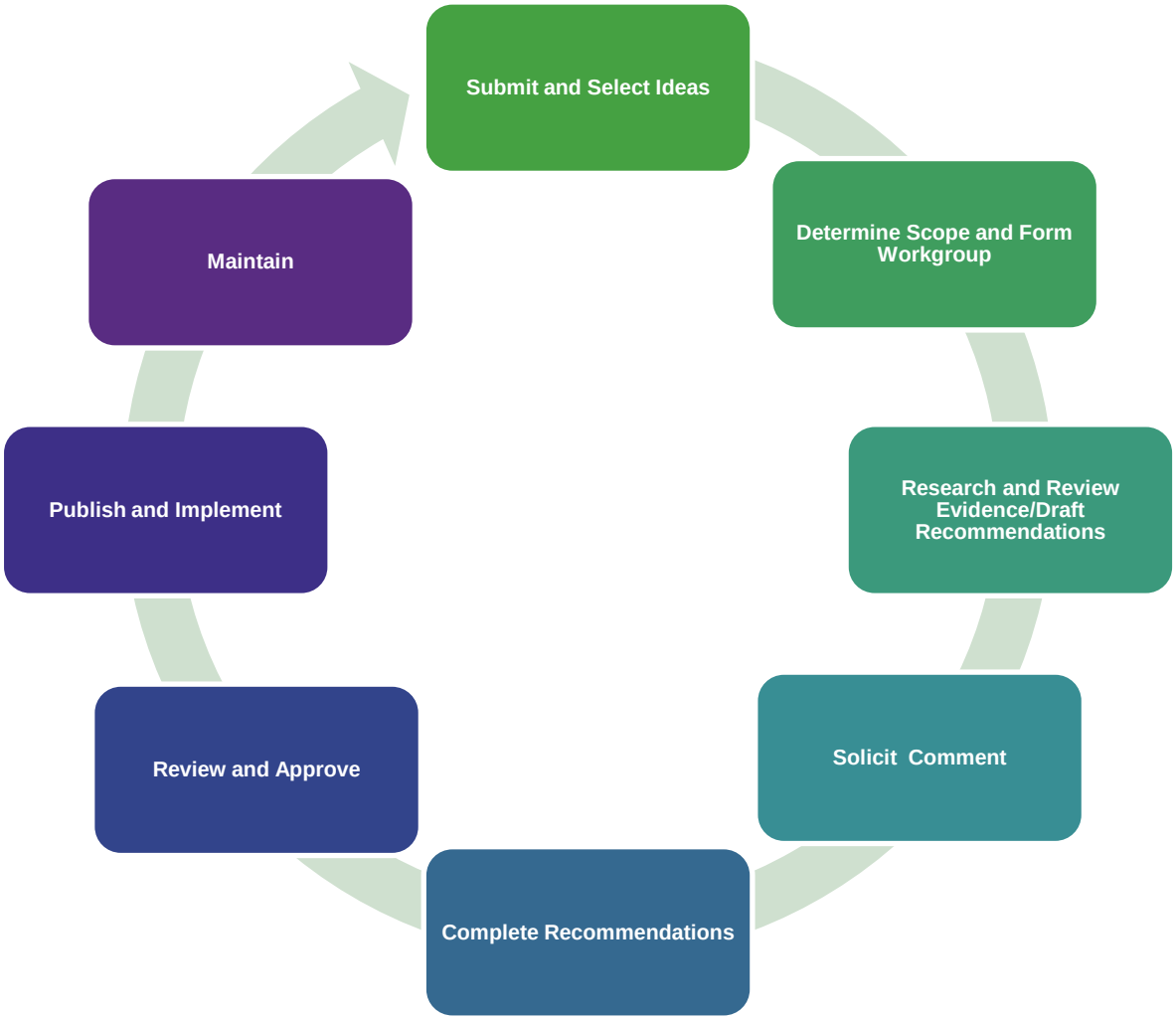
CAP Center guideline life cycle



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Key Questions

1. What equipment validation and daily performance monitoring is needed?
2. What training of staff and pathologists is required? What are the competency assessments needs over time?
3. How does one select or develop an appropriate algorithm for interpretation?
4. How does one determine the performance of the image analysis?
5. How should image analysis be reported?

CAP

Guideline Panel Members

Advisory panel

Kenneth J. Bloom, MD

M. Elizabeth Hammond, MD

Stephen Hewitt, MD, PhD

Richard Levenson, MD

David Rimm, MD, PhD

Mogens Vyberg, MD

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Christina Lacchetti, MHSc, methodology consultant

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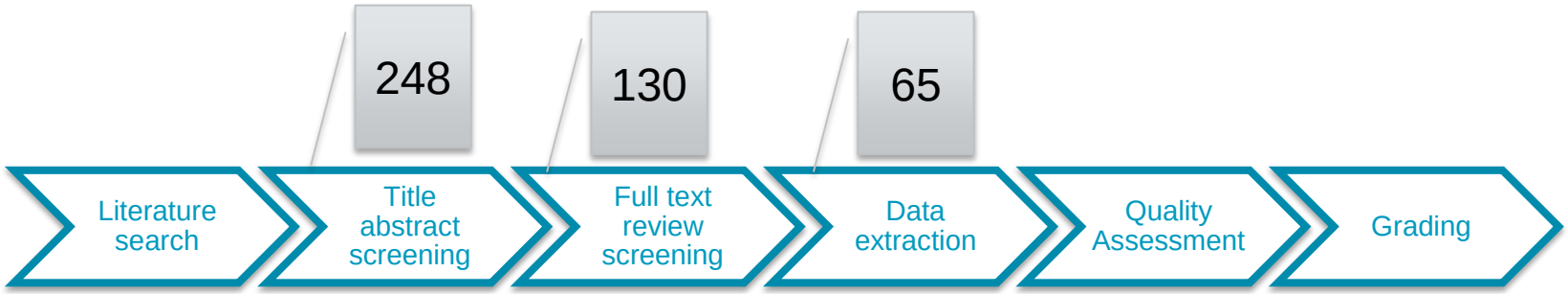
Results of the systematic review



- 248 references for title/abstract screening
- 52% (130) included in full text screening
- 64% (65) included in data extraction

Total number of included studies to be determined after the literature refresh

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Draft recommendations

- **11 draft recommendations**
  - 7 recommendations (based on laboratory accreditation requirements)
  - 4 expert consensus opinions
- **Data was difficult to synthesize**
  - Various imaging systems reported in the literature
  - Data not reported for many of the outcomes of interest

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Results of comment period

- **CAP hosted a three week comment period in March 2017 for any stakeholder to provide feedback to the draft recommendations**

- **More than 150 participants and more than 180 comments received**

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Draft guideline statements

1. **Laboratories should select a quantitative image analysis system for HER2 immunohistochemistry that is capable of meeting the standards for reporting as set forth by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in the guideline "Recommendations for Human Epidermal Growth Factor 2 Testing in Breast Cancer." – Expert Consensus Opinion**

85. 27% agree

14.73% disagree

Final statement will be revised

CAP

Draft guideline statements, continued

2. Laboratories should validate their quantitative image analysis results for clinical use by comparing them to an alternative, validated method. – Recommendation

93.64% agree

6.36% disagree

Final statement will be revised

CAP

Draft guideline statements, continued

3. Laboratories should ensure that the results produced by a quantitative image analysis system are reproducible within and between different batch analyses. – Recommendation

95.10% agree

4.90% disagree

No revision

CAP

Draft guideline statements, continued

4. Laboratories should ensure that the results produced by a quantitative image analysis system are reproducible between operators when they select regions of interest for analysis and/or perform annotation. – Expert Consensus Opinion

89.90% agree

10.10% disagree

No revision

CAP

Draft guideline statements, continued

5. Laboratories should continuously monitor and document the performance of their quantitative image analysis system. – Recommendation

89.8% agree

10.2% disagree

Final statement will be revised

CAP

Draft guideline statements, continued

6. Laboratories should have procedures in place to address changes to the quantitative image analysis system that could impact clinical results. – Recommendation

93.88 % agree

6.12% disagree

No revision

CAP

Draft guideline statements, continued

7. Laboratories should report that quantification was obtained by image analysis, the image analysis methods used, and at minimum, utilize the scoring schema recommended by the ASCO/CAP "Recommendations for Human Epidermal Growth Factor 2 Testing in Breast Cancer" guideline. – Expert Consensus Opinion

95.88% agree

4.12% disagree

Final statement will be revised

CAP

Draft guideline statements, continued

8. Personnel involved in the quantitative image analysis process should be trained specifically in the use of the technology. – Recommendation

92.78% agree

7.22% disagree

No revision

CAP

Draft guideline statements, continued

9. Laboratories should retain at minimum, the regions of an image that were analyzed and the metadata generated in adherence to local requirements and applicable regulations. –Expert Consensus Opinion

83.16% agree

16.84% disagree

No revision

CAP

Draft guideline statements, continued

10. A pathologist trained in QIA should oversee the entire process of quantitative image analysis used for clinical practice. – Recommendation

74.47% agree

25.53% disagree

Final statement will be revised

CAP

Draft guideline statements, continued

11. A pathologist trained in QIA must visually verify the image, the annotated image analysis output, and the algorithm results prior to finalizing the report. – Recommendation

78.72% agree

21.28% disagree

Final statement will be revised

CAP

Next steps

- **Manuscript and Methods Supplement**
  - **Expected submission in August, 2017**
  - **Publication (early online release) estimated in October, 2017**

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Summary

- QIA has been shown to improve consistency and accuracy of interpretation than manual scoring by pathologists, but has not gained widespread acceptance.
- Lack of a guideline is a practical gap.
- This guideline is to provide recommendations for improving reproducibility, precision, and accuracy in the interpretation of HER2 IHC where QIA is employed.
- This is an evidence-based guideline with public input to ensure the recommendations are clinically sound, practical and implementable.

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Acknowledgement

- CAP Quality Center and member of the Expert Panel and Advisory Panel of the Quantitative Image Analysis Guideline
- Digital Pathology Association
- Moffitt Cancer Center Analytic Microscopy Core (Joseph Johnson, slides sharing and Jonathan Nguyen, file transferring)
- Dr. Liron Pantanowitz, slides sharing
- Ms. Nicole Thomas, slides preparation

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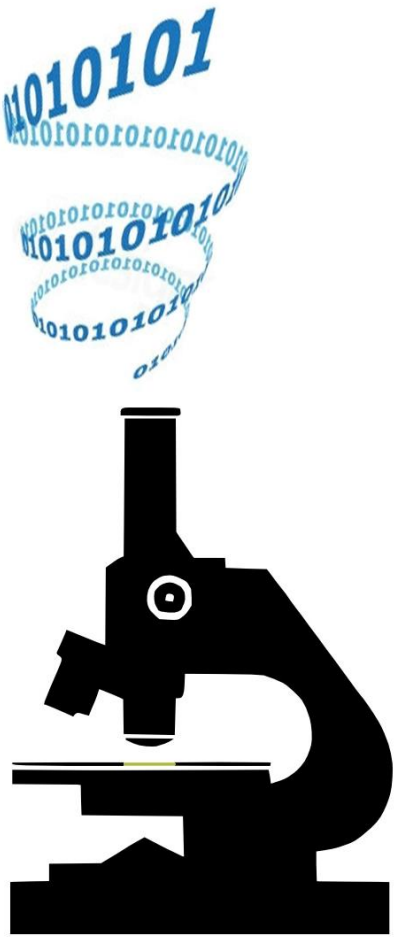
Questions?



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