Quality Assurance of Breast Biomarkers Assays in Immunohistochemistry: Adapting Standards for Bangladesh laboratories

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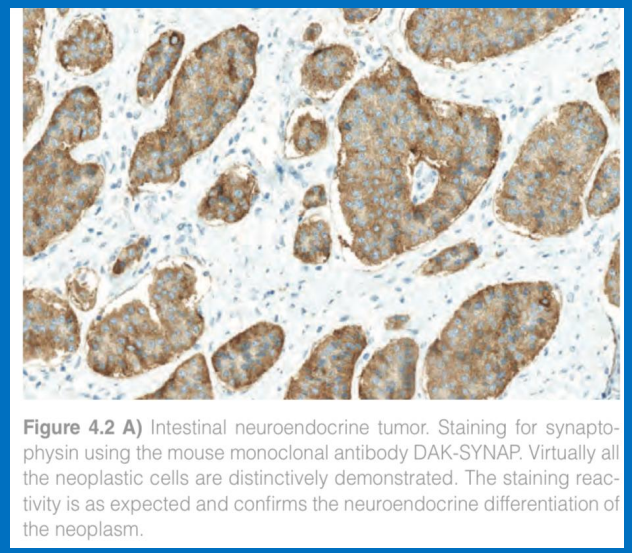
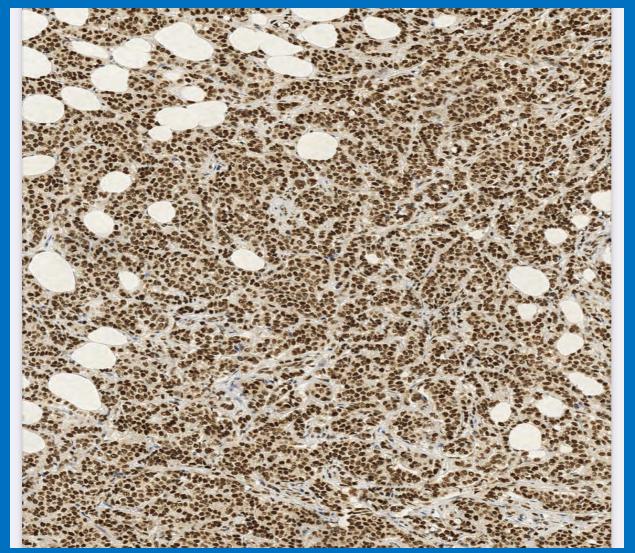


Figure 4.2 A) Intestinal neuroendocrine tumor. Staining for synaptophysin using the mouse monoclonal antibody DAK-SYNAP. Virtually all the neoplastic cells are distinctively demonstrated. The staining reactivity is as expected and confirms the neuroendocrine differentiation of the neoplasm.



Biomarker testing is at the crux of the successful management of patients with breast cancer.

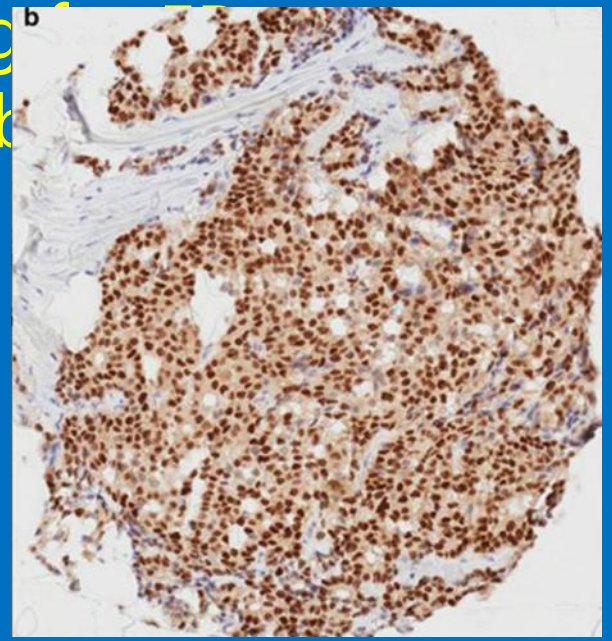
An estimation of approximately 20% of results for estrogen receptor (ER) and progesterone receptor (PR) and a third of human epidermal growth factor 2 (HER2) results may not be accurate (false negative or false positive).

Variation in testing biomarkers in breast carcinoma is a universal issue and is not restricted to low-resources countries only.

ERs are expressed in about 70% of invasive breast carcinoma cases.

-Strongly predictive factor of hormone therapy response as well as a favorable prognostic factor
(ASCO-CAP): the cut-off that indicates patients who will benefit from endocrine therapy remains at 1% of cancer nuclei stained for ER.

-2–3% of breast carcinomas will have 1–10% cell staining f
low reproducibility of the results between laboratories, b
benefit of antiestrogenic therapy for these patients

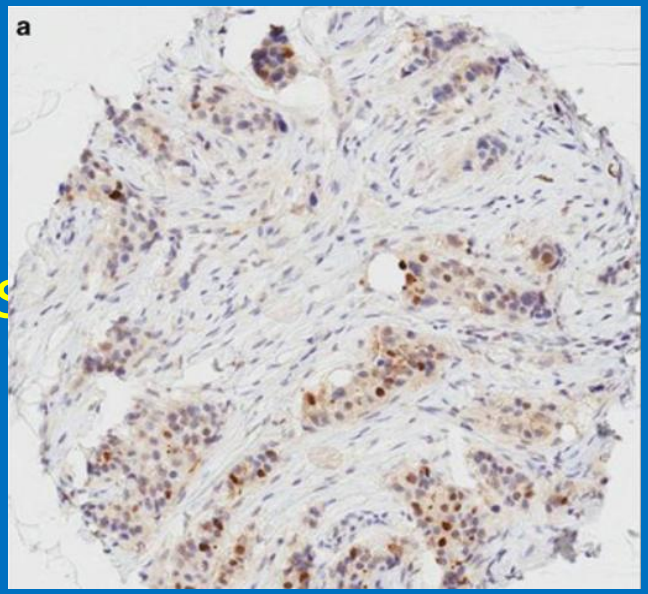


PR is expressed in about 60% of cases of IDC

10% of cases may prove to be ER-positive and PR-negative. These patients have a higher risk of recurrence than ER-positive, PR-positive cases.

<5% of patients PR-positive, ER-negative.

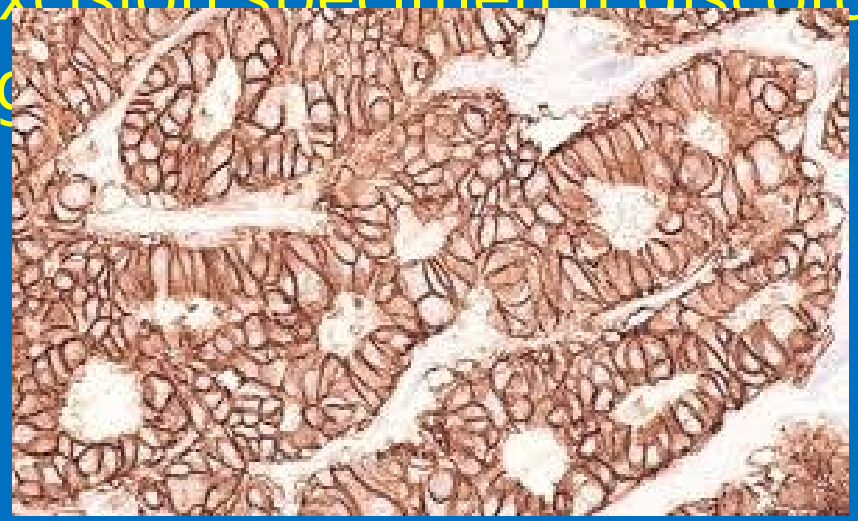
Recent studies PR expression <20% might have advers prognostic implications.



HER2 status is routinely assessed using a combination of IHC protein expression levels and in situ hybridization (ISH) to assess *HER2* gene status.

High concordance (98–99%) between HER2 results in core biopsies and surgical specimen score; biopsy sample is material of choice

HER2 test be repeated on the excision specimen if discordance between histopathologic findin



Tumors until now reported as HER2-negative might be re-defined as HER2-low, with HER2 negativity being limited to IHC-0

Based on this definition, up to 55% of breast cancers are HER2-low, comprising a majority of hormone receptor-positive (HR-positive) tumors (65–83%) with different intrinsic subtypes

HER2-low tumors do not benefit from adjuvant trastuzumab but T-DXd are effective in HER2-positive disease and also in HER2-low tumors with no amplification

2023 ESMO expert consensus statements of HER2-low recommend classifying all levels of HER2 expression [23]. However, the score 0 versus 1+ must be reported in all HER2-negative cases

All breast cancer patients diagnosed between 2013 and 2018 in **Sweden** were identified in the National Quality Register for Breast Cancer (29 labs)

Cases with data on ER, PR, HER2, Ki67, grade, and treatment were selected (43,261 cases).

The ER positivity rates (84.2% to 97.6%) with 6/29 labs

PR rates 64.8% to 86.6% with 7/29 labs

HER2 positivity rates ranged from 9.4% to 16.3%,

Median Ki67 (15% and 30%) showed significant intra-laboratory

Original Article | Published: 19 November 2004

Immunohistochemical detection of Ki67 in breast cancer correlates with transcriptional regulation of genes related to apoptosis and cell death

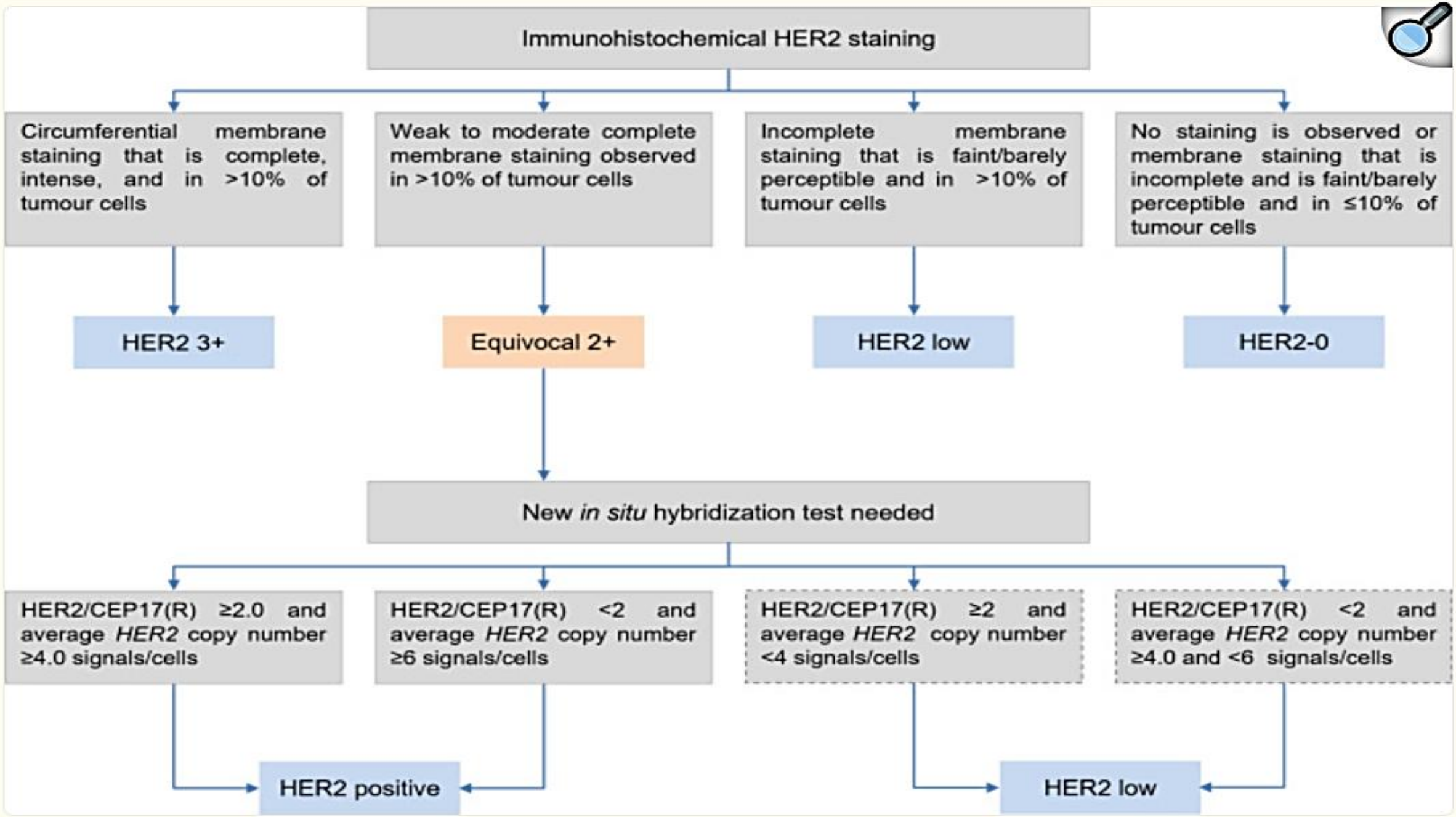
Puay-Hoon Tan, Boon-Huat Bay, ... Kuo-Bin Li + Show authors

Modern Pathology **18**, 374–381 (2005) | Cite this article

In conclusion, our study has shown that immunohistochemical protein detection of Ki67 in invasive breast cancer correlates with *Ki67* gene expression profiles only when immunohistochemical reactivity is >10% of tumor cell nuclei stained, validating at the molecular level, the usage of this cutoff in immunohistochemical scoring systems for Ki67. However, the association

Ki67 IHC Clinical Utility

Although other indications (eg, predicting benefit from radiation therapy) are the subject of active investigation, at present there are fundamentally 3 intended uses for Ki67 IHC: 1) to estimate prognosis in early-stage disease regarding whether further adjuvant chemotherapy is warranted, 2) to predict whether chemotherapy may or may not be active, and 3) to monitor patients during or after neoadjuvant endocrine or chemotherapy to determine if the regimen chosen is working or an alternative should be considered.


Immunohistochemical HER2 staining
Circumferential membrane staining that is complete, intense, and in >10% of tumour cells
Weak to moderate complete membrane staining observed in >10% of tumour cells
Incomplete membrane staining that is faint/barely perceptible and in >10% of tumour cells
No staining is observed or membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumour cells
HER2 3+
Equivocal 2+
HER2 low
HER2-0
New *in situ* hybridization test needed
HER2/CEP17(R) ≥2.0 and average *HER2* copy number ≥4.0 signals/cells
HER2/CEP17(R) <2 and average *HER2* copy number ≥6 signals/cells
HER2/CEP17(R) ≥2 and average *HER2* copy number <4 signals/cells
HER2/CEP17(R) <2 and average *HER2* copy number ≥4.0 and <6 signals/cells
HER2 positive
HER2 low

Pre-analytic phase

Pre-fixation
Fixation
Post-Fixation/Decalcification
Processing
Dehydration & clearing
Paraffin embedding
Sectioning
Drying/Storage

Analytic phase

Platform (manual/ Automated)
Epitope retrieval
Blocking
Primary Antibody
Detection system
Chromogen
Counterstain
Mounting

Post-analytic phase

Design of controls
Critical stain indicators
Internal/External control
Interpretation
Positive/Negative
Localization
Quantification
Cutt-of levels
Reporting

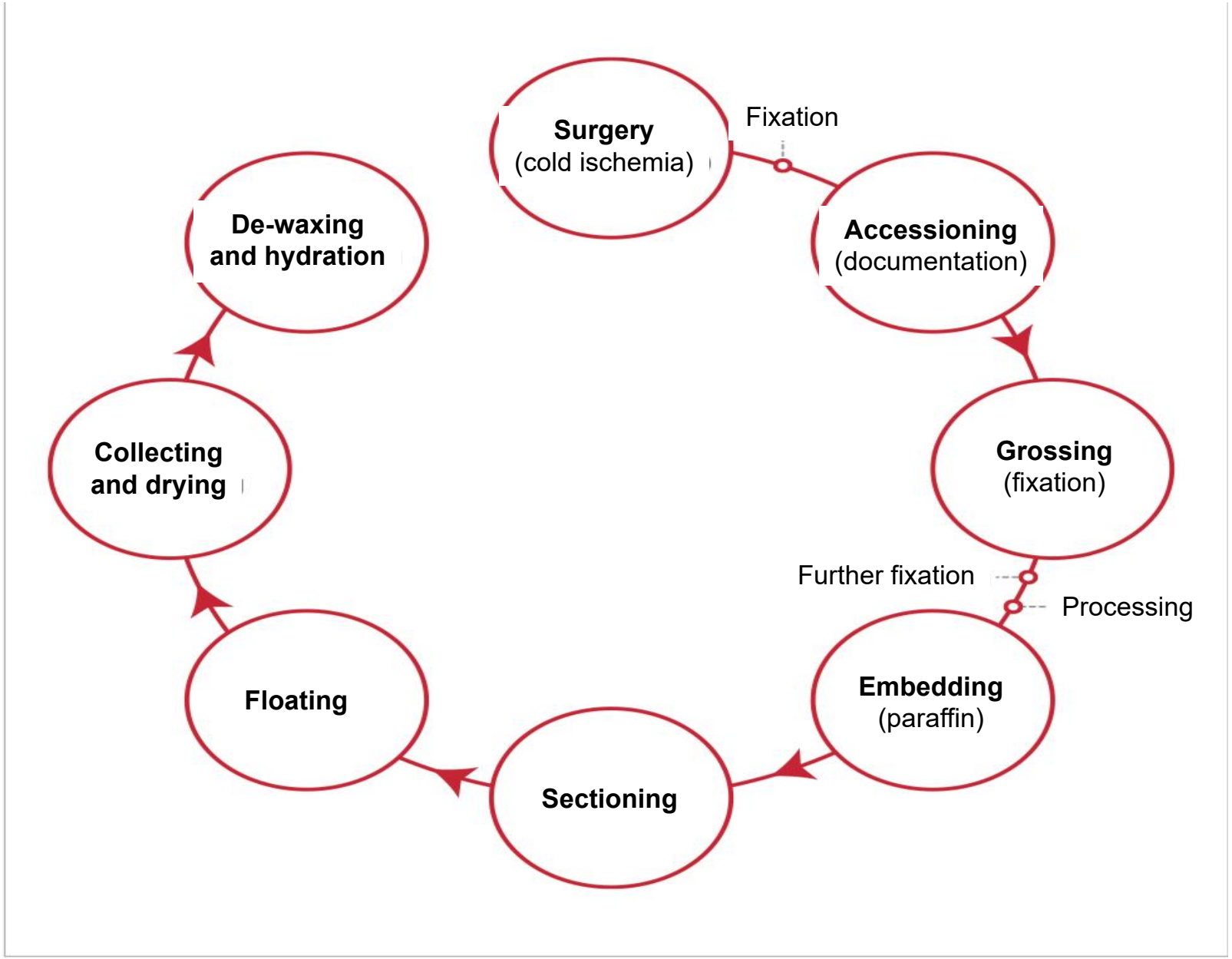


Figure 2.1 An overview of the processing steps included in the pre-analytical phase.

- Pre-analytical variables are considered the ‘weakest link’ of IHC

- Poorly fixed tissue compromises the accuracy of IHC results. Reproducibility of staining between laboratories also becomes difficult.

Causes of deficient IHC

- Weak Staining: Causes and Solutions
- Non-Specific Staining
- High Background: How to Minimize?
- **Tissue thickness can cause artefact**
- Fixation time should be <72 h

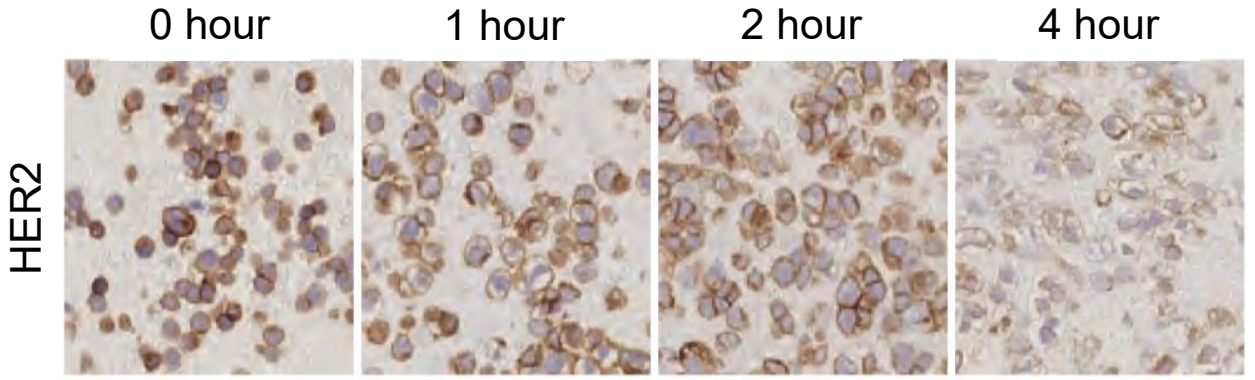
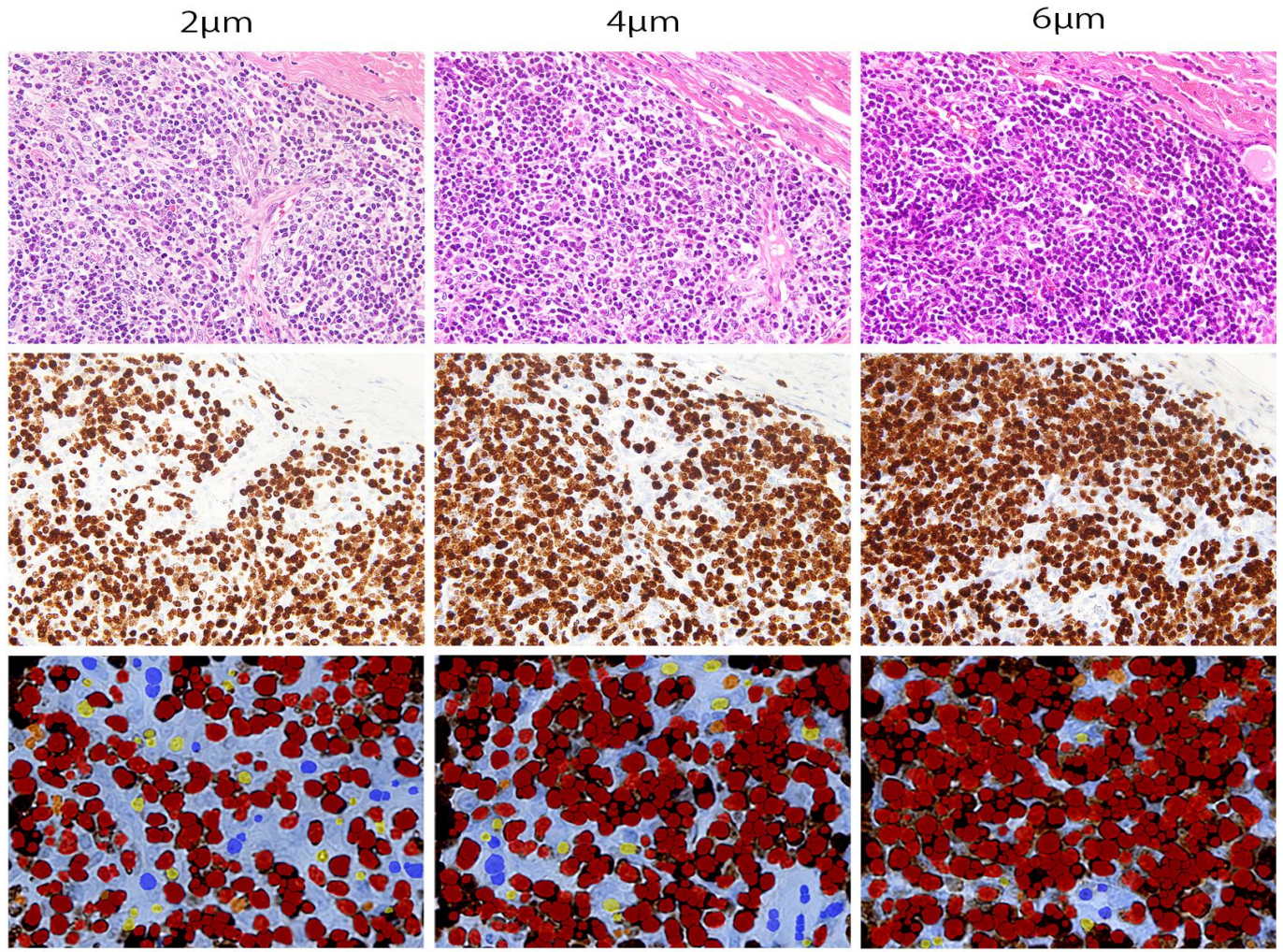


Figure 2.2 Cold ischemia alters the staining intensity of HER2 in MDA-MB-453 cells (2+ cell line). Weak to moderate membrane immunoreactivity on approximately half of the cells is observed in a cell pellet fixed immediately in 10% NBF (0 hour). With as little as one hour cold ischemia (the cell pellet was kept moist under saline-damped gauze), the morphology is already deteriorating and there appears to be increased number of cells with membrane staining.



Proportion of Ki 67 positive cells

Negative	17 (5.8%)	12 (3.3%)	3 (0.7%)
Weak	20 (6.8%)	13 (3.6%)	5 (1.1%)
Moderate	8 (2.7%)	6 (1.7%)	4 (0.9%)
Strong	248 (84.6%)	328 (91.4%)	434 (97.3%)

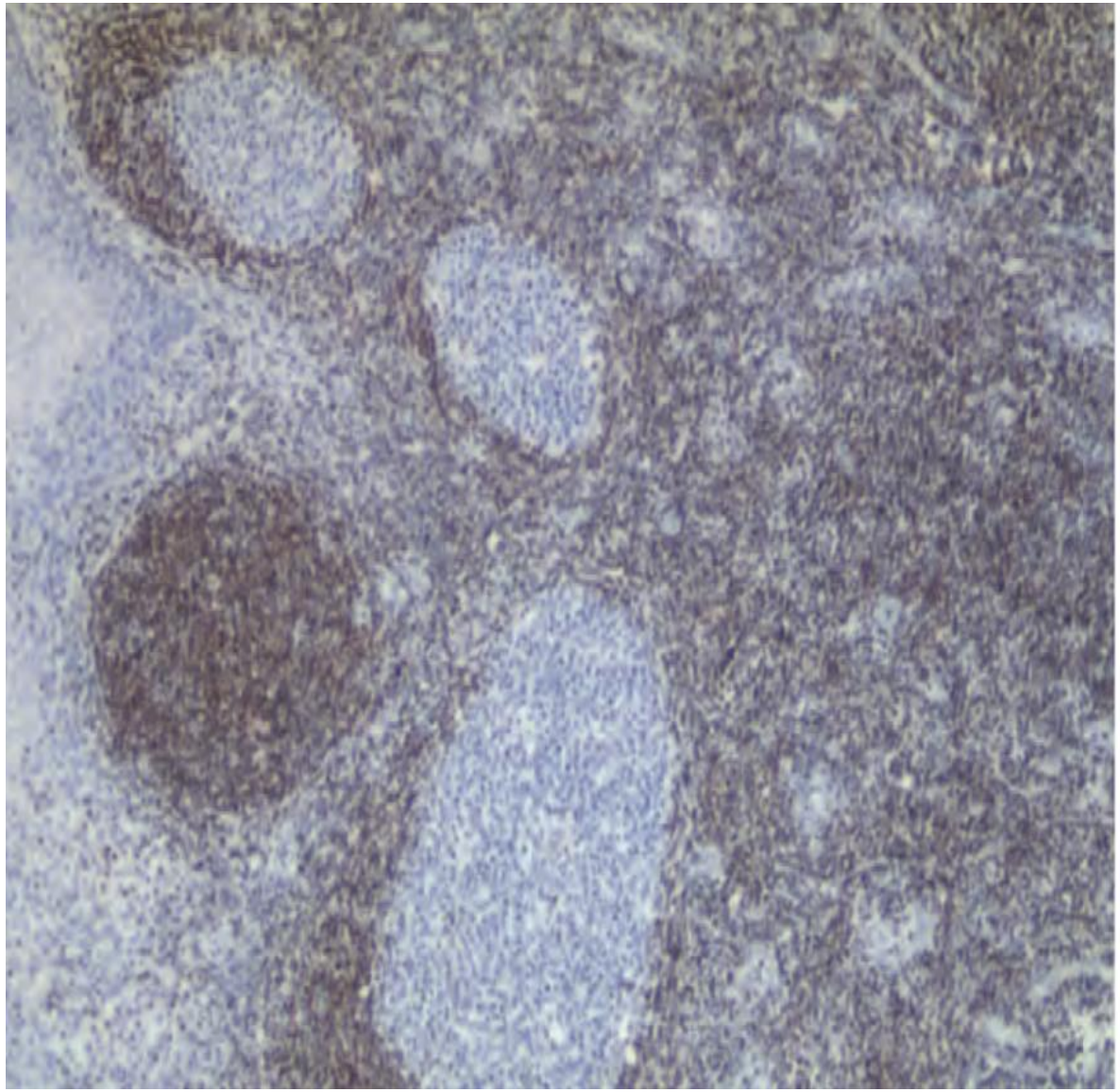
* distribution of positive cells are significantly different depending on section thickness.
chi-square test ($p < 0.00001$)

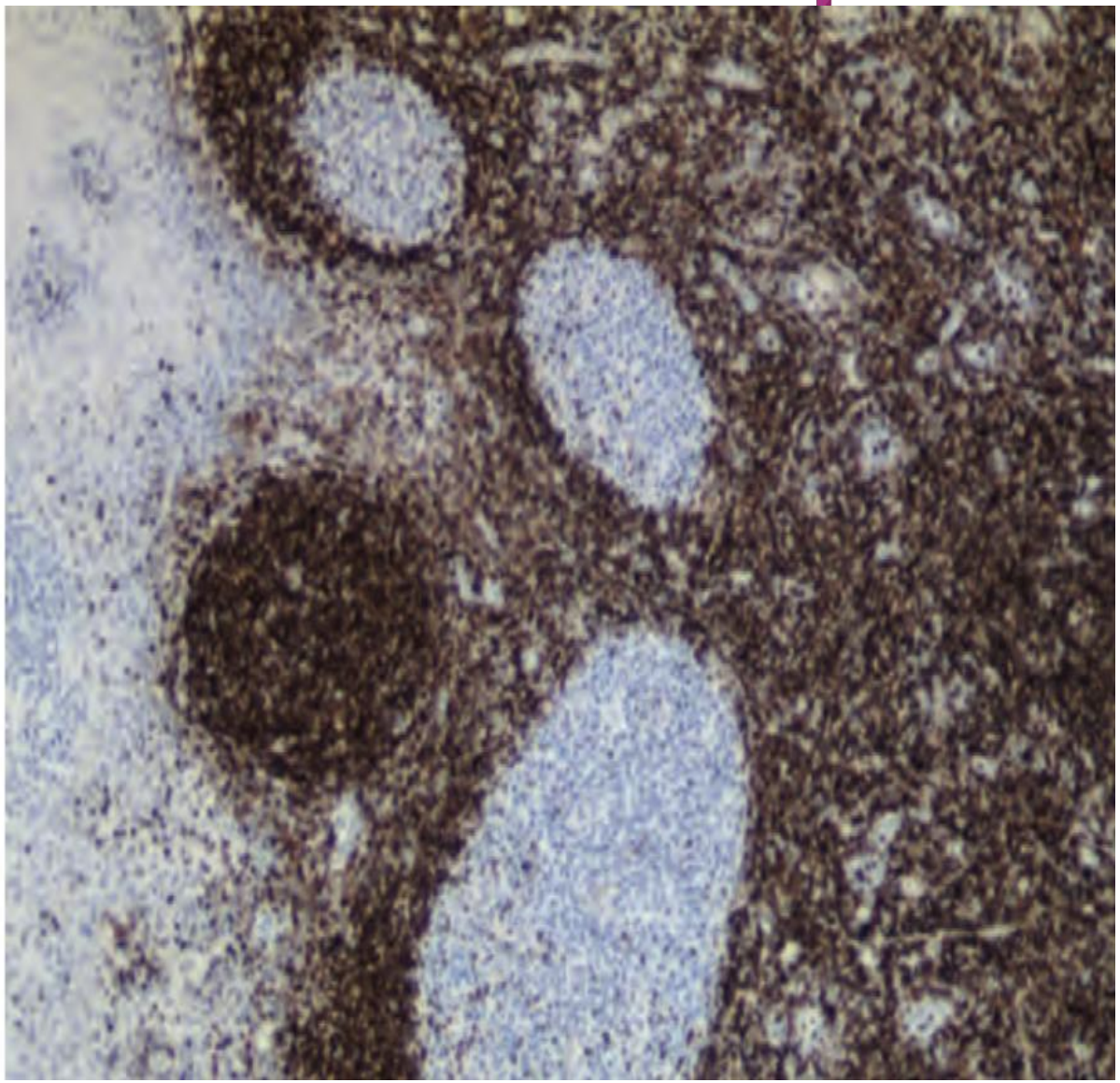
Primary antibodies and procedures

Various factors in the IHC process may affect the results:

Condition of fixation, type of primary antibody, condition of antigen retrieval, and type of detection method.

For example, the commercial primary ER antibodies against ER, are mouse monoclonal antibodies (clones 6F11 and 1D5) and rabbit monoclonal antibodies from clones SP1 and EP1; the sensitivity and specificity of these antibodies are not the same





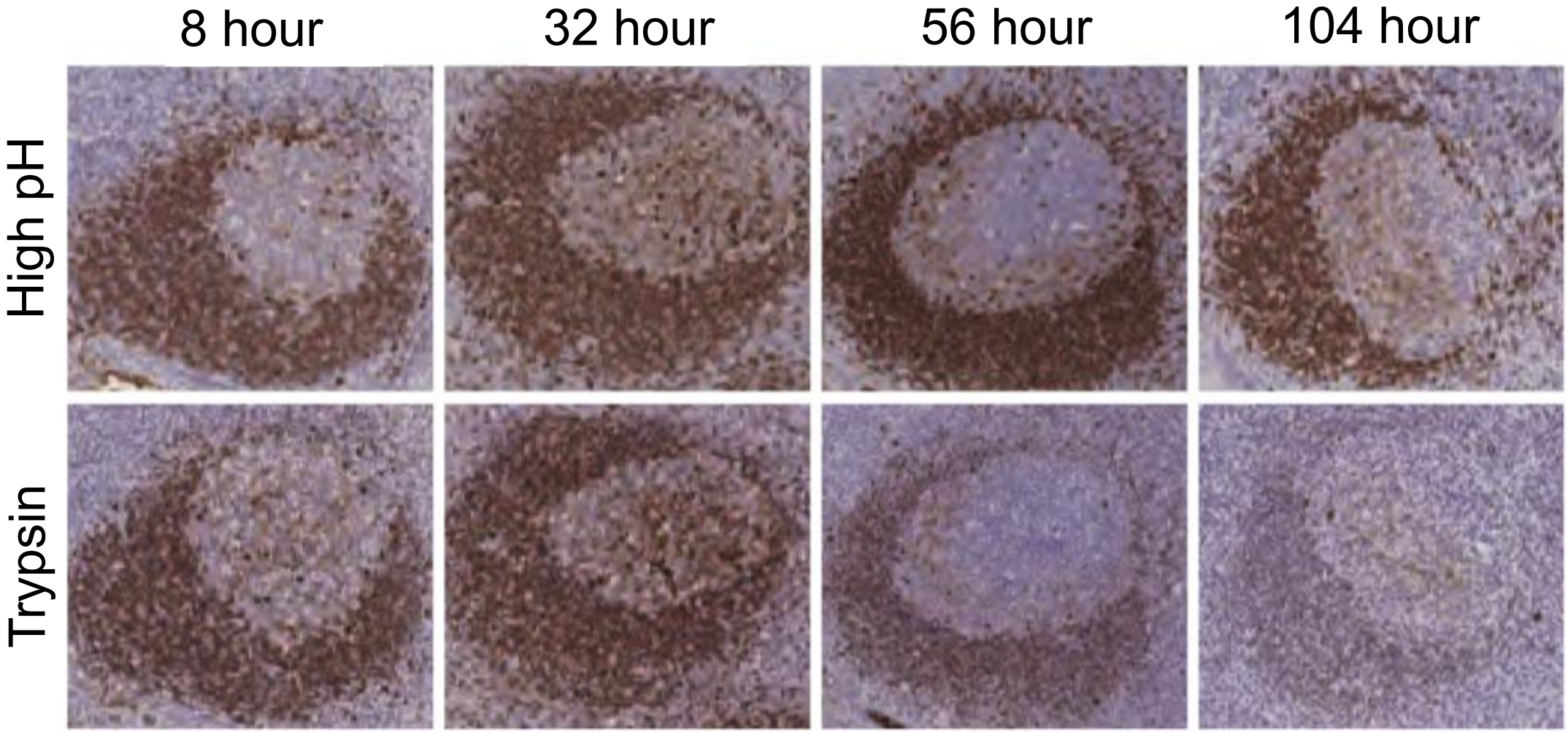


Figure 2.5 Length of fixation affects immunoreactivity for IgM in tonsil mantle zone B cells. This figure illustrates reduced immunoreactivity for

Antibody optimization for IHC

- Adjusting the antibody concentration, diluent, incubation time and temperature is crucial for successful IHC staining

Most optimal dilution →

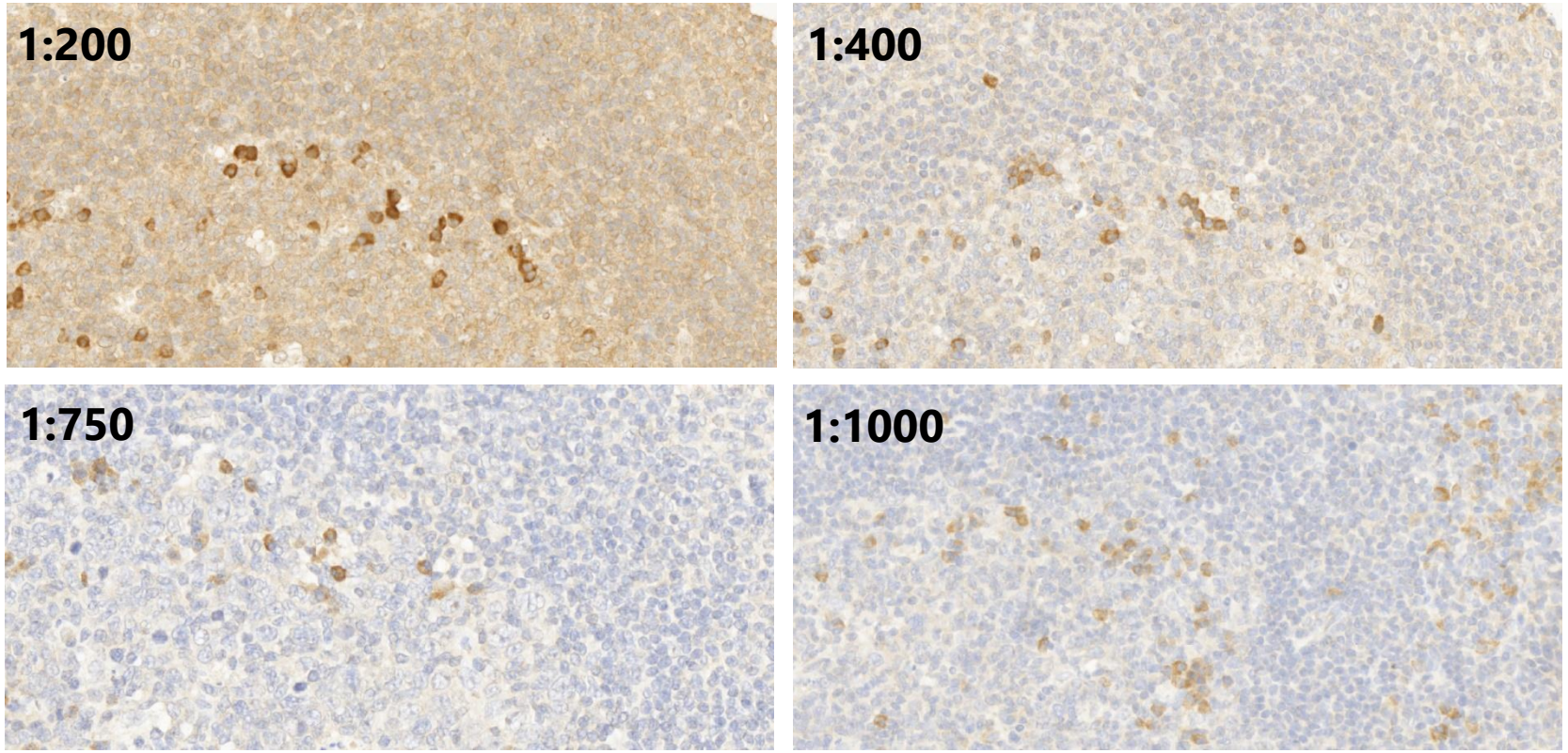


Figure (?) Various Embigin antibody dilutions affect the staining quality on Tonsil FFPE

The Fundamental Challenge in the Pathological Landscape of HER2-Low Breast Cancer: Accurate Definition

IHC Testing is the Primary Method for Identifying HER2-Low Breast Cancer

T-Dxd

- T-Dxd is the 2nd U.S. FDA-approved HER2-targeted ADC in metastatic HER2-positive BC and the first HER2-targeted agent in metastatic or inoperable HER2-low BC.
- Composed of an anti-HER2 immunoglobulin G1 antibody, a tetrapeptide-based cleavable linker and a membrane permeable topoisomerase I inhibitor with a drug-to-antibody ratio of 8:1.
- A randomized phrase III, DB-04 trial evaluated T-Dxd in 557 patients (494 HR positive and 63 TNBCs) with HER2-low unresectable or metastatic BC previously treated with one or two lines chemotherapy

- Laboratories should revalidate their HER2 IHC tests for HER2-low assessment before using.
- Non-specific background staining can make it more difficult to detect the faint membrane staining.
- Important to use adequate controls for consistent and reproducible results.
- In predictive IHC testing, there is a need for reliable controls at relevant clinical decision cut-off values.

External Quality Assurance (EQA) Helps Improve Infrastructure for Testing Breast Biomarkers Across a Lower- and Middle-Income Country

ASCO-CAP recommends mandatory participation in an EQA program ≥ 2 testing events per year.

EQA programs essentially evaluate analytic validity (rather than preanalytic) processes, like cold ischemia time, but ensure that good pathology practices are followed in a continued manner.

"Our experience demonstrates that laboratory performance improves with participation in an EQA system even in less perfect settings, and this drives the placement of more proficient practices across the country"

Arch Pathol Lab Med (2024) 148 (9): 1028–1034.

What proficiency testing (PT) or alternative assessment is required for predictive immunohistochemistry markers?

The updated IHC predictive marker PT requirements are **for breast ER, HER2; gastric HER2; and high sensitivity ALK**

- Laboratories performing **both** IHC staining/interpretation must enroll in PT.
- Laboratories **only** performing predictive marker IHC staining must perform alternative performance assessment.
- Slides are stained at a different laboratory must perform alternative performance assessment

Part 2: Adapting Immunostains for Bangladesh

"As is your pathology, so is your medicine."

-

Sir William Osler

Am J Clin Pathol January 2017;147:15-32

- Pathology is vital to national policy planning through, for instance, surveillance programs (eg, Ebola, Zika).

- Pathology is an integral part of any clinical care system, and without it, the system is greatly undermined

- Pathology is also vital for research across communicable to noncommunicable diseases.

- Pathologists play a key role in linking the clinical services with the laboratory, providing **leadership** and capitalizing on the opportunities arising from the **rapidly emerging, new technologies**.

Challenges in Bangladesh: Infrastructure and Resources

Power outages, temperature control issues, water quality, Equipment maintenance challenges.

Impact of these challenges on reagent stability, equipment function, and assay performance.

Shortage of trained pathologists and technicians.

- **Need for specialized training in IHC techniques, interpretation, and QA.**
- **challenges in procuring reagents and supplies:**
- **logistical difficulties, unreliable supply chains, cost considerations.**

These challenges impact on assay availability and consistency.

Manual staining techniques as a viable option in resource-limited settings.

Strategies for improving temperature control and water quality in laboratories.

Training and quality control considerations for manual techniques.

Training and Capacity Building in Bangladesh

- Plan for developing and implementing comprehensive training programs for pathologists and technicians.
- Collaboration with international organizations and institutions for training and mentorship.
- Use of telepathology for remote consultation and training.

Adapting IHC: Quality Assurance and Standardization in Bangladesh

- Implementation of standardized IHC protocols and guidelines for all laboratories.
- Development of affordable/sustainable QA programs tailored to the local context.
- Participation in external quality assessment schemes where feasible

Collaboration and Sustainability: The Path Forward: building a Sustainable Future for IHC in Bangladesh

Strengthen Infrastructure and Resources:

- Targeted Investment: Advocate for increased government funding specifically allocated for IHC infrastructure
- Regional Hubs: Establish regional or zonal IHC centers equipped to serve multiple smaller clinics.
- Equipment Upgrades: Call for investment in robust, low-maintenance equipment suitable for the local environment, including backup power systems (eg solar)

Supply Chain Improvements:

Enhancing Technical Expertise and Capacity Building:

- National Training Program: Training program for pathologists and laboratory technicians. This program could include:

-Hands-on Workshops:

- Mentorship Program: link experienced pathologists/technicians with those in training or working in resource-limited settings.

-Certification/Accreditation: possibility of developing a certification Telepathology and Remote Consultation

The basal fundament for a technical optimal IHC performance:

- Appropriate tissue fixation and processing
- Appropriate and efficient epitope retrieval
- Appropriate choice & titre of antibody/clone
- Robust, specific & sensitive detection system
- Appropriate choice of control material

Preanalytical	Analytical	Postanalytical
Validated fixation protocol <72hr formalin 1:10 tissue ratio	Automated platform reduces variability CV 5% vs 15%	Digital scoring algorithms K=0.89 vs manual

1. Minimize prefixation delays
2. Sections <5mm thickness
3. Fix in neutral buffered formalin
4. Fix 6-72 hrs

... The biomarker protocol trap – Caution: not for faint-hearted lab personel !!!!!

Fixation
Time, Type, Volume

ication
Preparation

Pre-analytic

Pre-treatment

Primary antibody
Clone, Dilution
Buffer, Time, Temp

Tissue
Type, Dimension,
Laser resection,
De-differentiation

Section
Thickness
Storage
Drying

Manual
Stainer

Analytic

Visualization
Sensitivity, Specificity

Development
Sensitivity,
Localization

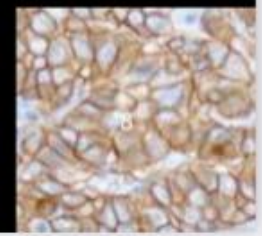
With 3 choices for 5 variables in each phase = > 4 million protocols….

Controlment

Post-analytic

Quantification
Reporting

Interpretation
Localization
Positive/Negative - cut-off level



Call to action: believe in quality

"Governments don't often appreciate the importance of diagnostics, and it is taken for granted, because the private providers have filled this vacuum

- Multicenter validation of protocols
- IHC vs FISH concordance
- Long term QA monitoring