



AALBORG
UNIVERSITY HOSPITAL

IHC post-analytical phase with focus on controls

Søren Nielsen, Consultant at NordiQC

Kristi Bøgh Anderson MD, Scientific Associate, NordiQC

kristi.anderson@rn.dk



How to measure accuracy, precision, quality...?



Time

Hour/min./sec.

Unit standard



Weight

Kilo/gram/..

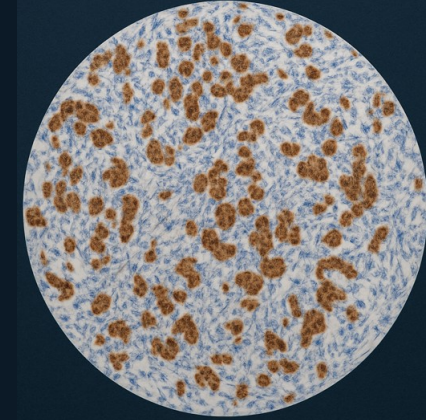
Unit standard



Length

Meter/centi./mili.

Unit standard



"IHC"

"pos/neg"

"No standard"

Access to calibrators / standard institutes

*Test Performance
Characteristics in
Tissue controls**

* Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine
Part 2: Immunohistochemistry Test Performance Characteristics. Torlakovic et al AIMM 2017 Feb;25(2):79-85

*Standardization of positive controls in diagnostic immunohistochemistry:
recommendations from the International Ad Hoc Expert Committee. Torlakovic et al AIMM 2015 Jan;23(1):1-18.

Inspiration by Steve Bogen
Boston Cell Standards

POSITIVE CONTROLS

Confirm the assay can detect the antigen when present

External **positive** tissue controls

High expressor → confirms detection of abundant antigen

Low expressor → confirms analytical sensitivity, avoids false negatives

Internal **positive** tissue controls

Endogenous elements expected to stain.
Confirm staining worked under the same **pre-analytical and analytical** conditions as the tumor.

NEGATIVE CONTROLS

Identify non-specific staining and prevent false positives

External **negative** tissue controls

Internal **negative** tissue controls

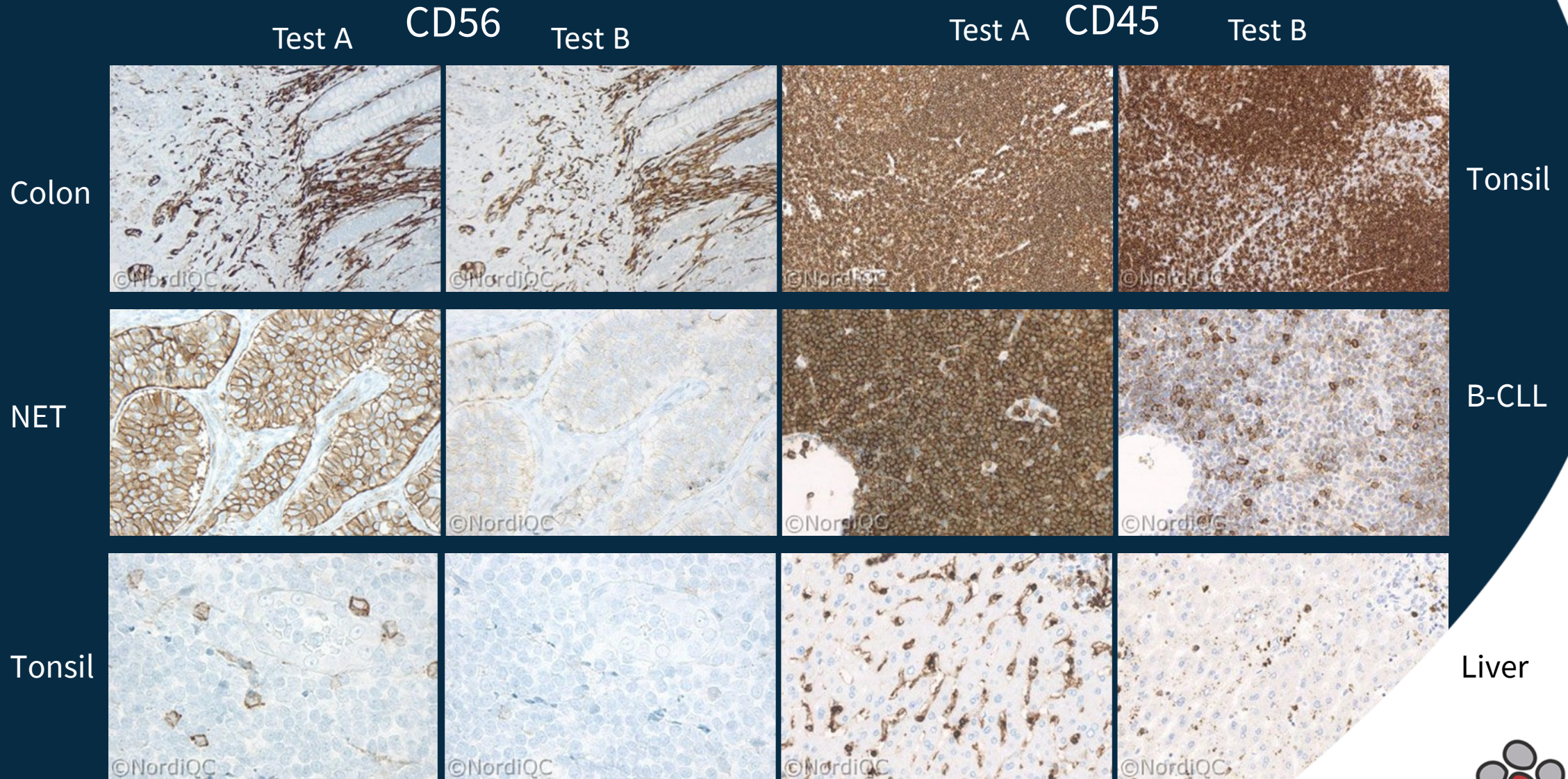
Negative reagent control

Primary antibody omitted

Antibody diluent **or serum** used instead

Detects background from: secondary antibody, detection system, endogenous tissue components, pigment in tissue

The central role of tissue controls for IHC



iCAPCs concept

Test Performance Characteristics

REVIEW ARTICLE

Standardization of Positive Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Committee

Emina E. Torlakovic, MD, PhD,† Søren Nielsen, HT, CT,‡§ Glenn Francis, MBBS, FRCPA, MBA, FFSc (RCPA),¶# John Garratt, RT,†** Blake Gilks, MD, FRCPC,††† Jeffrey D. Goldsmith, MD,‡‡ Jason L. Hornick, MD, PhD,*§§ Elizabeth Hyjek, MD, PhD,* Merdol Ibrahim, PhD,|| Keith Miller, FIBMS,|| Eugen Petcu, MD, PhD,|| Paul E. Swanson, MD,¶## Xiaoge Zhou, MD,***††† Clive R. Taylor, MD, PhD,‡‡‡ and Mogens Vyberg, MD‡§*

Appl Immunohistochem Mol Morphol • Volume 23, Number 1, January 2015

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

Kimberly H. Allison, MD¹; M. Elizabeth H. Hammond, MD²; Mitchell Dowsett, PhD³; Shannon E. McKernin⁴; Lisa A. Carey, MD⁵; Patrick L. Fitzgibbons, MD⁶; Daniel F. Hayes, MD⁷; Sunil R. Lakhani, MD^{8,9}; Mariana Chavez-MacGregor, MSc¹⁰; Jane Perlmutter, PhD¹¹; Charles M. Perou, PhD⁵; Meredith M. Regan, ScD¹²; David L. Rimm, MD, PhD¹³; W. Fraser Symmans, MD¹⁰; Emina E. Torlakovic, MD, PhD^{14,15}; Leticia Varela, MD¹⁶; Giuseppe Viale, MD^{17,18}; Tracey F. Weisberg, MD¹⁹; Lisa M. McShane, PhD²⁰; and Antonio C. Wolff, MD²¹

J Clin Oncol 38:1346-1366. © 2020 by American Society of Clinical Oncology

IHC Critical Assay Performance Controls (iCAPCs)

Which tissues are recommended ?

Which tissues / cells are critical ?

Right antibody

What is the expected staining pattern ?

Appropriate level of sensitivity

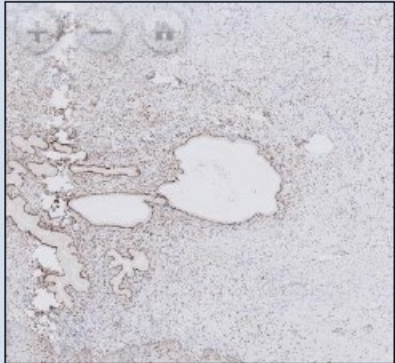
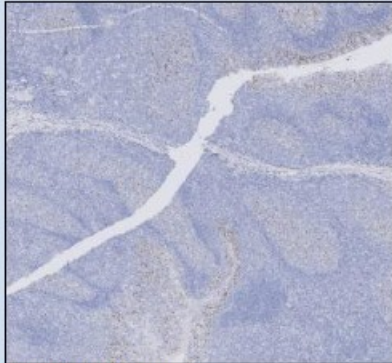
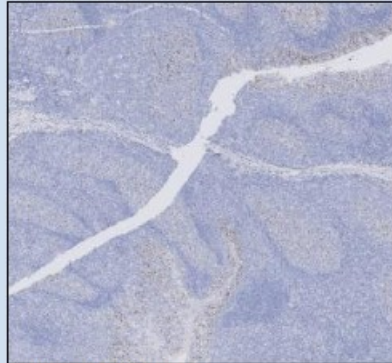
Guidance level of specificity

External positive tissue controls



Info ▾ Modules Assessments Protocols **Controls** Events ▾ [KBA](#)

ER - Estrogen Receptor

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Cervix	Tonsil	Tonsil
Description	Virtually all squamous epithelial cells, columnar epithelial cells and stromal cells (except lymphocytes and endothelial cells) must show a moderate to strong nuclear staining reaction.	The vast majority of squamous epithelial cells and dispersed follicular dendritic cells / T-cells within the germinal centers must show an at least weak, distinct nuclear staining reaction.	No staining reaction in mantle zone and germinal center B-cells should be seen.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

IHC controls

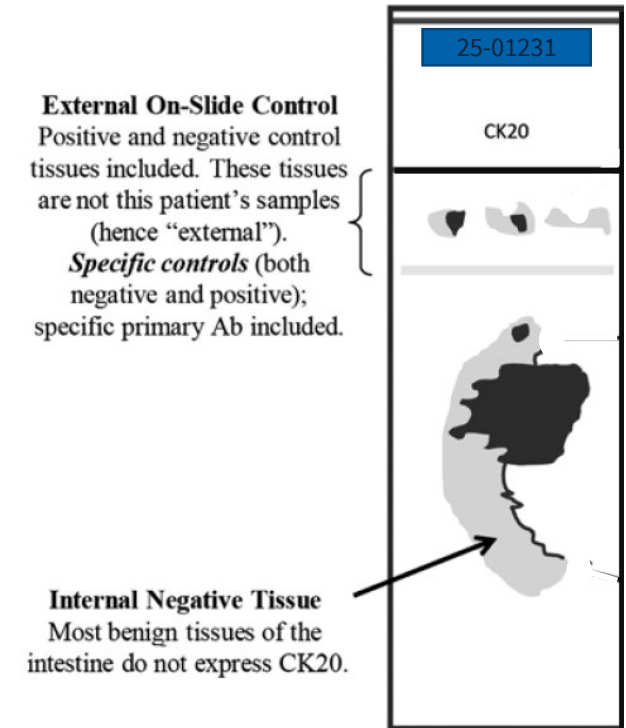
Tissue controls are the most valuable tools
to monitor the
analytical specificity and sensitivity for IHC

Internal positive and negative tissue control

Cells/structures within the patient material

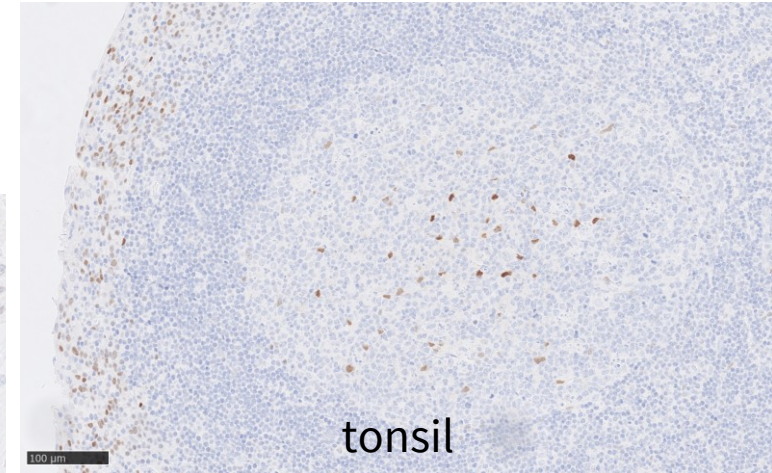
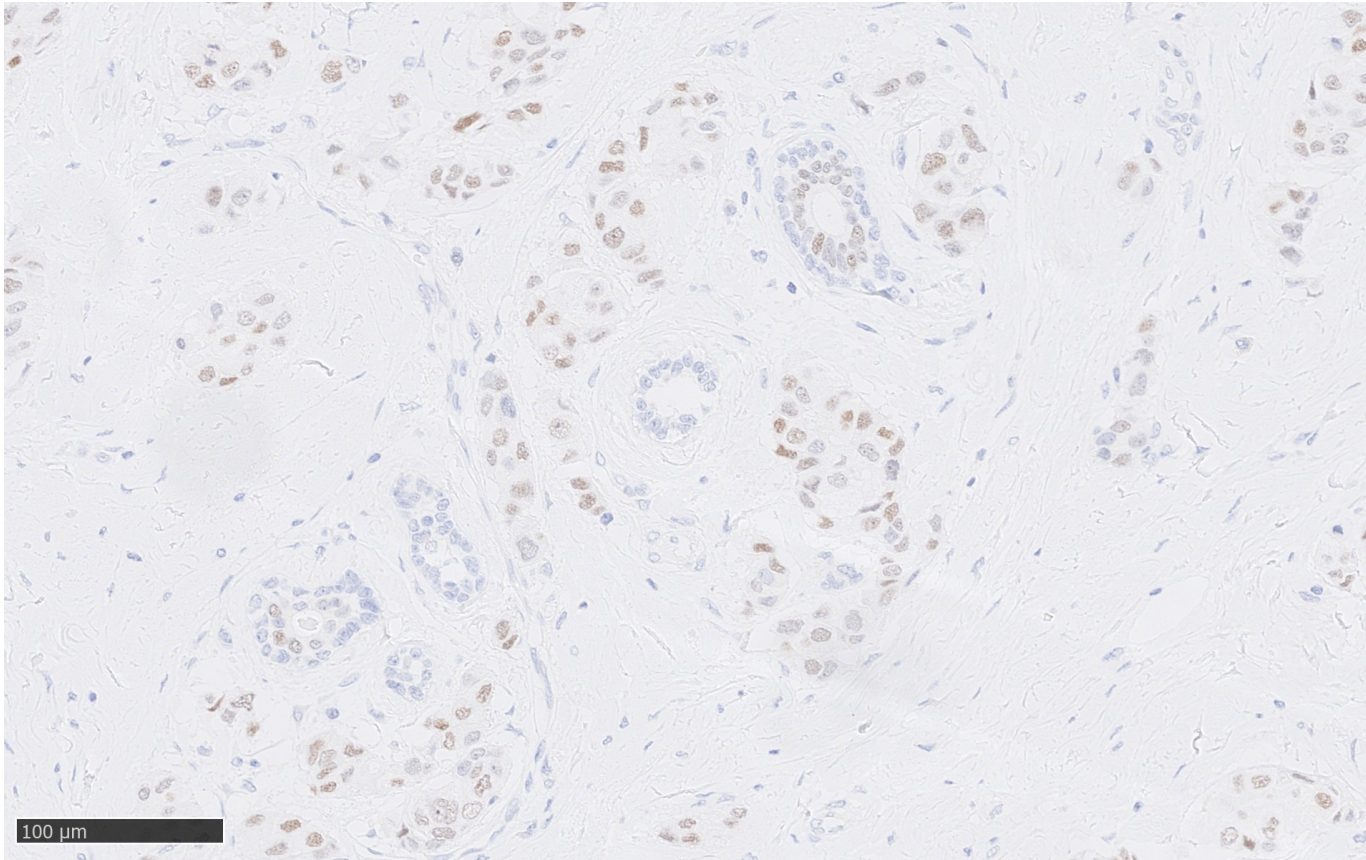
External positive and negative tissue control

Slide next to patient material

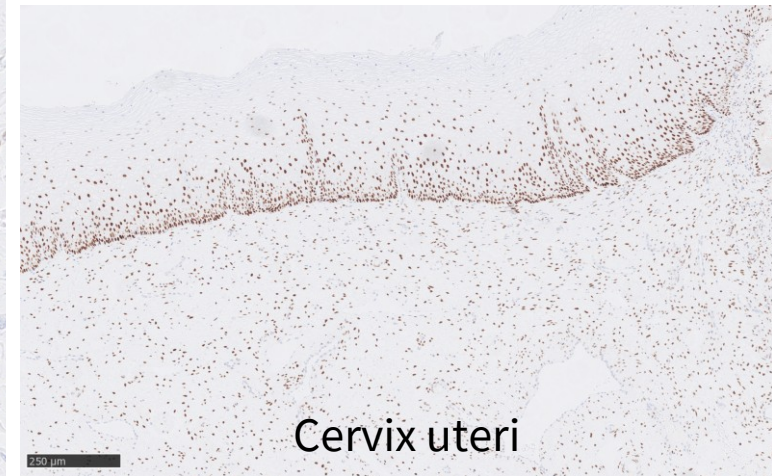


ER

Internal tissue controls



External tissue controls



Estrogen and Progesterone Receptor Testing in Breast Cancer Guideline Update

Background

An expert panel was convened to evaluate evidence in an update and reaffirmation of the 2010 “ASCO/CAP Guideline Recommendations for Immunohistochemical (IHC) Testing of Estrogen and Progesterone Receptors (ER/PgR) in Breast Cancer.”

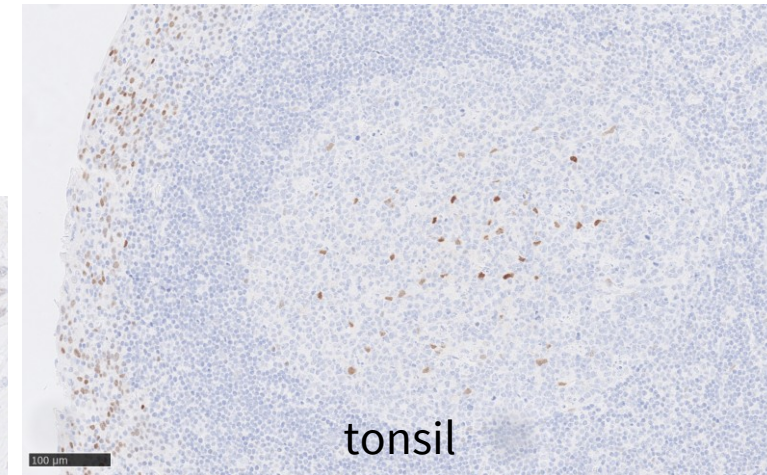
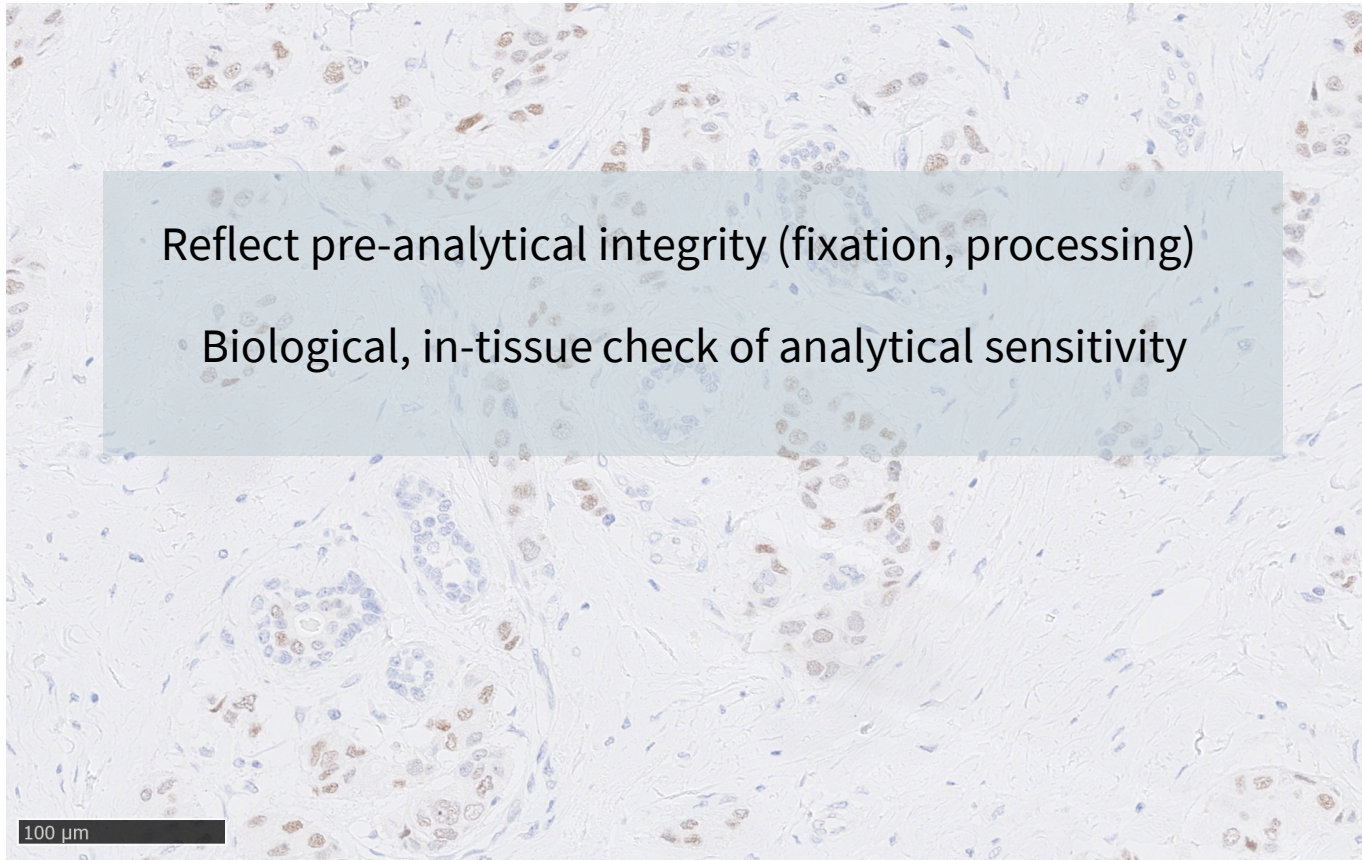
Notably the **“Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology / College of American Pathologists Clinical Practice Guideline Update”** differences impact pathologists and patients, including:

1. A new recommendation for laboratories to establish a specific standard operating procedure to ensure the validity of low positive (1-10%) or negative (0 or < 1%) interpretations and results. Correlation of ER staining with the histologic features (as well as attention to other standard quality control measures) is also recommended and unusual/discordant results worked up.
2. New reporting recommendations are made for cases with 1-10% ER expression to acknowledge the more limited data on endocrine responsiveness in this group and overlapping features with ER negative cancers. The status of internal controls should also be reported for cases with 0-10% staining (with a special comment for those lacking internal controls).
3. 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.
4. The update recommends ER testing for patients diagnosed with ductal carcinoma in situ without invasion; PgR testing is optional.

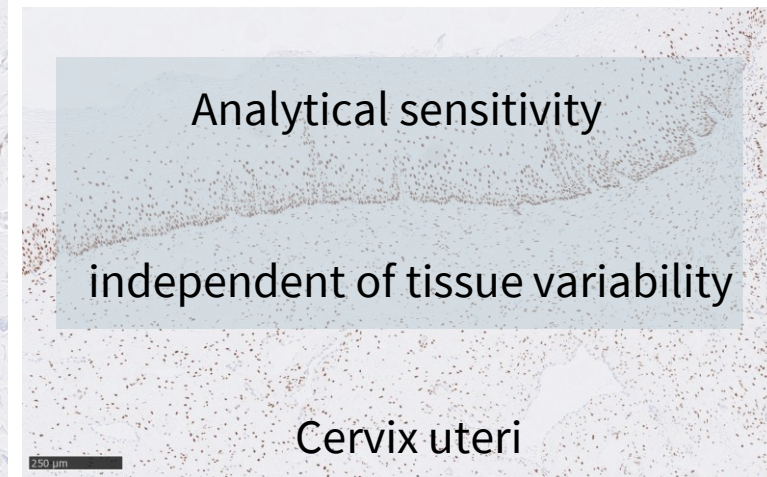
- **Guideline status:** Active
- **Originally published:** June 2010
- **Update published online ahead of print:** January 13, 2020
- **Update published in print:** May 2020
- **Joint Partner:** American Society of Clinical Oncology

ER

Internal tissue controls



External tissue controls



Patient samples that test negative or weak for ER in normal glands may be reported as insufficient for IHC evaluation of ER

Research Article

The Value of Internal Positive Controls and Synthetic Calibrators for Performance Assessment of Breast Carcinoma Estrogen Receptor Immunohistochemistry Assay

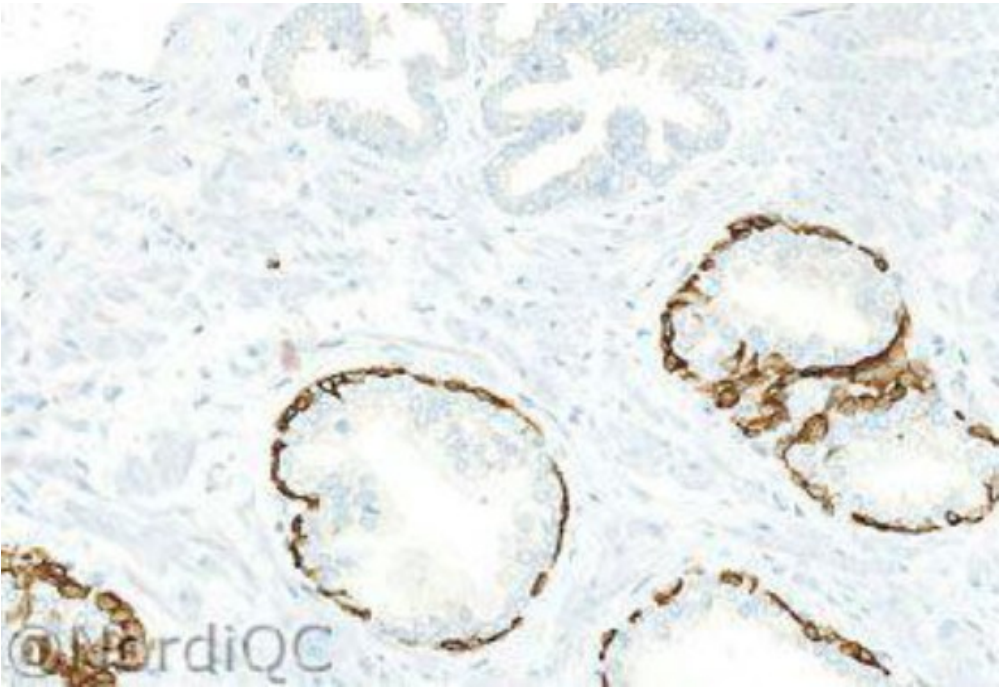
Emina E. Torlakovic^{1 2}  , Seshi R. Sompuram³ , Søren Nielsen⁴ , Steven Bogen³ 

How to use internal positive tissue controls

Target analyte	Application	Internal control to confirm "true" loss
BAP1, MTAP	Mesothelioma	Stromal cells
p53	Gynecological carc.	Stromal cells
PTEN	Lung and gynecological carc.	Stromal and benign cells
MMR (MLH1, MSH2, MSH6,PMS2)	Lynch syndrome, IO treatment	Stromal cells / lymphocytes
SMAD4	Pancreas and GI carc.	Stromal and benign cells
SMARCB1/INI1	Sarcoma, PNST, carcinoma..	Stromal and benign cells

TABLE 2. Examples of IHC Assays Where Preferential Use of Internal Positive Controls Recommended

IHC Assay	Use	Comments
Cytokeratin 5	Demonstration of basal cells in glandular structures of prostate to differentiate between benign (positive) and malignant (negative) glands	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control Tested sample may be completely negative if no normal tissue is present
Mismatch repair proteins (MLH1, MSH2, PMS2, MSH6)	Absence of expression in the cells of colon or endometrial adenocarcinoma is abnormal; patients referred for molecular testing to rule out Lynch Syndrome	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control



Limitations of internal positive tissue controls

In general **not applicable as positive controls** as levels of expression may not be relevant for level and range required.

CD5

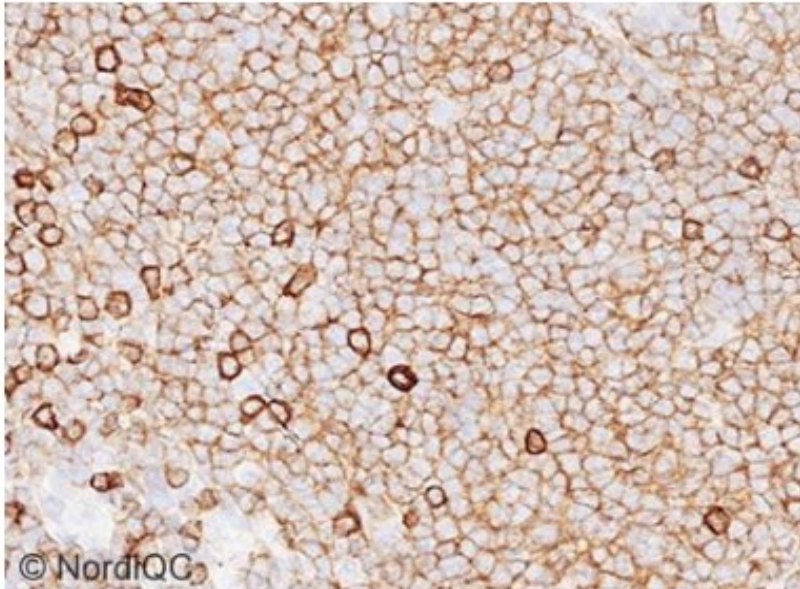


Fig. 2a
Optimal staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

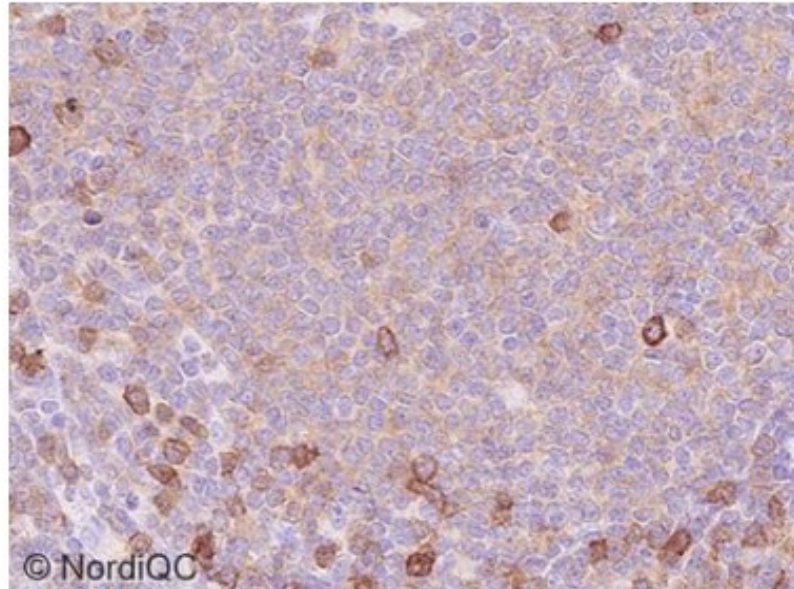


Fig. 2b
Insufficient staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1b. The intensity of the neoplastic cells is significantly reduced and displays an indistinct staining reaction pattern - compare with Fig. 2a (same field). T-cells intermingling with the neoplastic cells retain a strong membranous staining reaction.

Limitations of internal positive tissue controls

In general **not applicable as positive controls** as levels of expression may not be relevant for level and range required.

CD5

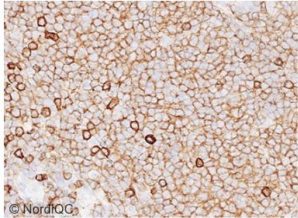


Fig. 2a
Optimal staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

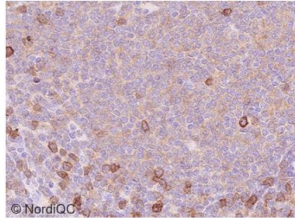
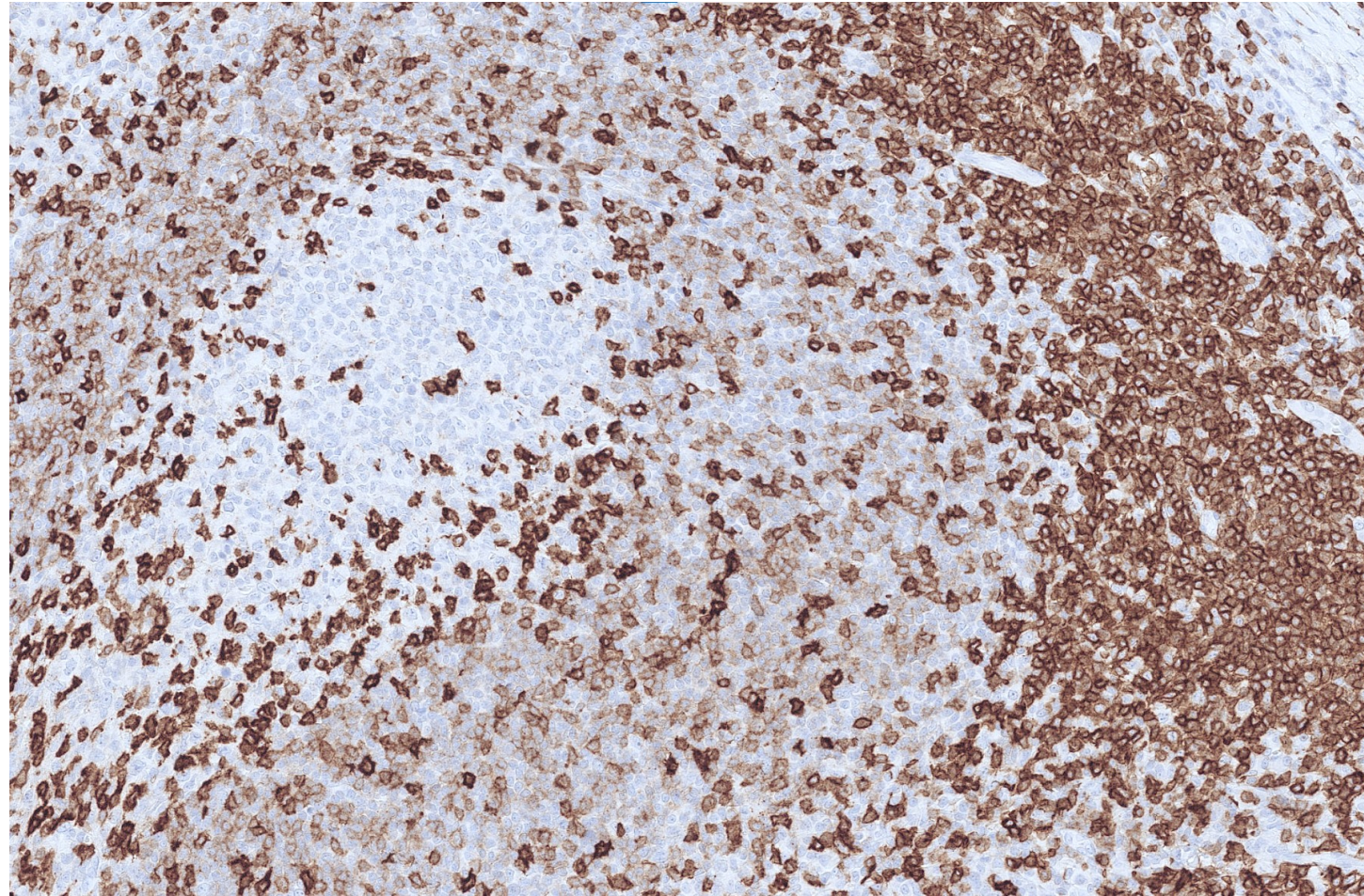


Fig. 2b
Insufficient staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1b. The intensity of the neoplastic cells is significantly reduced and displays an indistinct staining reaction pattern - compare with Fig. 2a (same field). T-cells intermingling with the neoplastic cells retain a strong membranous staining reaction.



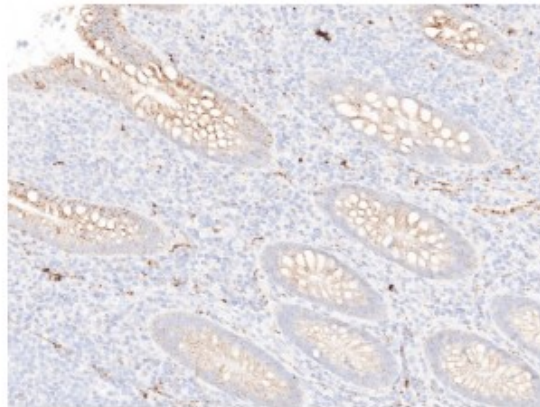
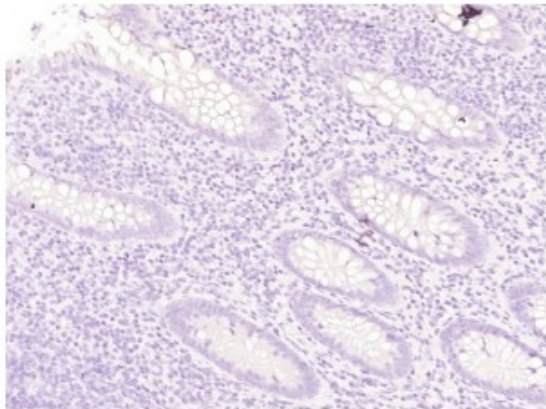
Tonsil, mantle zone B-cells are the critical control

Internal & external tissue controls

Internal/external negative tissue controls are a basic test for IHC assay and system specificity, but with limited potential as demonstration of any aberrant false positive reaction will depend on presence of the target analyte in the selected tissues

ALK (Lu)

Colon/Appendix	
Pos. tissue control	Neg. tissue control
Nerves	Epithelial and muscle

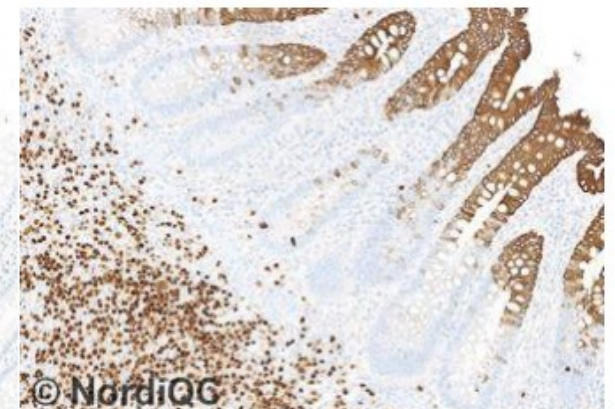
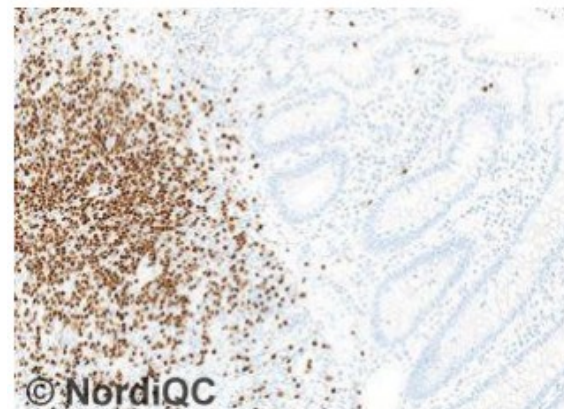


ALK (Lu) run 73.

Detection system (OptiView) being contaminated with secondary Ab cross-reacting with mucin in colon

PAX5

Colon/Appendix	
Pos. tissue control	Neg. tissue control
B-cells	Epithelial and muscle



PAX5 run 41.

rmAb clone SP34 for PAX5 being contaminated with a CK20 Ab reacting with the epithelial cells in colon

IHC controls to guide reliability of data

PAX8

An important marker when working up carcinoma of unknown primary.

Commonly points toward tumors of:

Ovary

Uterus

Kidney

IHC controls to guide reliability of data

But can **breast carcinoma** also show PAX8 expression?

But can breast carcinoma show PAX8 expression?

Comparison of PAX8 Expression in Breast Carcinoma Using MRQ50 and BC12 Monoclonal Antibodies

*Kamaljeet Singh, MD, Linda C. Hanley, MD, C. James Sung, MD,
and M. Ruhul Quddus, MD, MPhil (Path)*

41% MRQ-50
0% BC12

Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

James R. Conner, MD, PhD and Jason L. Hornick, MD, PhD

< 5%

Unexpected PAX8 Immunoreactivity in Metastatic High-grade Breast Cancer

*Mark R. Kilgore, MD, Dustin E. Bosch, MD, PhD, Kathi H. Adamson, MD, Paul E. Swanson, MD,
Suzanne M. Dintzis, MD, PhD, and Mara H. Rendi, MD PhD*

31% MRQ-50
11% pAb CM

Immunohistochemistry in the Differential Diagnosis of Triple Negative Breast Carcinoma and High-grade Serous Carcinoma: Old and New Markers

Pragya Virendrakumar Jain, MD,* Mariel Molina, MD,* Michelle Moh, MD, MS,†
Erin Bishop, MD,‡ Janet S. Rader, MD,‡ and Julie M. Jorns, MD*

(Appl Immunohistochem Mol Morphol 2024;32:456–461)

TRPS1

PAX8 (MRQ-50)

TNBC

HGSC

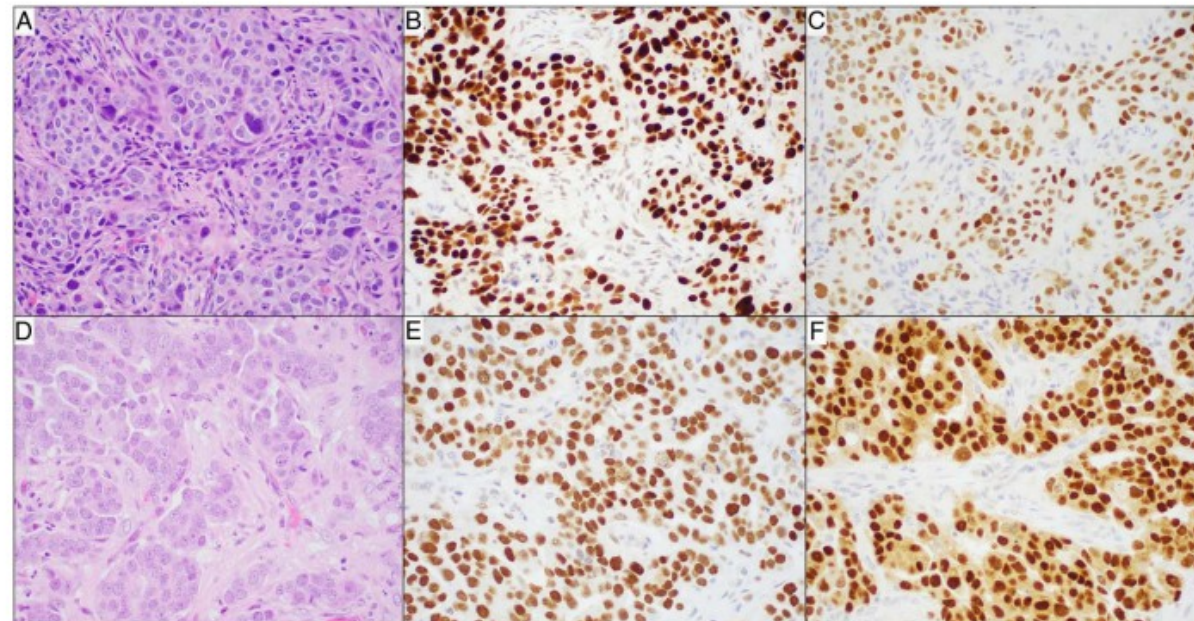


FIGURE 1. A–C, An example case of a triple-negative invasive ductal carcinoma (A) with diffuse, strong nuclear TRPS1 expression, with background breast stromal cells showing weak, nuclear TRPS1 staining (B); breast carcinoma cells also show diffuse, moderate to strong nuclear staining with PAX8 via the MRQ50 clone (C). D–F, An example case of high-grade serous carcinoma (D) with diffuse, moderate to strong nuclear TRPS1 (E), and PAX8 (MRQ50 clone) expression (F).

TABLE 4. Immunohistochemical (IHC) Profiles of Triple-negative Breast Cancers (TNBC) (N = 151) and High-grade Serous Carcinomas (HGSC) of Müllerian Origin (N = 185)

IHC marker	TNBC positive, N (%)	HGSC positive, N (%)
Keratin 7	147 (97.4)*	184 (99.5)†
Focal (1%-10%)	4 (2.7)	1 (0.5)
> 10%	143 (94.7)	183 (98.9)
Keratin 20	2 (1.3)‡	2 (1.1)§
Focal (1%-10%)	2 (1.3)	2 (1.1)
> 10%	0 (0)	0 (0)
GATA3	102 (67.5)	2 (1.1)
Focal (1%-10%)	16 (10.6)	2 (1.1)
> 10%	86 (56.9)	0 (0)
GCDFP15	29 (19.2)	0 (0)
Focal (1%-10%)	2 (1.3)	0 (0)
> 10%	27 (17.9)	0 (0)
Mammaglobin	27 (17.9)	5 (2.7)
Focal (1%-10%)	0 (0)	5 (2.7)
> 10%	27 (17.9)	0 (0)
SOX10	110 (72.8)	0 (0)
Focal (1%-10%)	2 (1.3)	0 (0)
> 10%	108 (71.5)	0 (0)
TRPS1	149 (98.7)¶	75 (40.5)
Focal (1%-10%)	4 (2.7)	12 (6.5)
> 10%	145 (96)	63 (34)
WT1	7 (4.6)	171 (92.4)
Focal (1%-10%)	4 (2.7)	1 (0.5)
> 10%	3 (1.9)	170 (89.9)
PAX8 (BC12 clone)	0 (0)	164 (88.6)
Focal (1%-10%)	0 (0)	0 (0)
> 10%	0 (0)	164 (88.6)
PAX8 (MRQ50 clone)	61 (40.4)	179 (96.8)
Focal (1%-10%)	4 (2.7)	0 (0)
> 10%	57 (37.7)	179 (96.8)
p53 (aberrant mutant, null or cytoplasmic pattern)	98 (64.9)	174 (94.1)

*The 4 keratin 7 negative TNBC cases had 1 breast marker positive each, including SOX10 (2), GATA3 (1), and GCDFP15 (1).

†The single keratin 7 negative HGSC was also keratin 20 negative.

‡Both keratin 20 (focal) positive TNBCs were also keratin 7 (diffuse) positive.

§The 2 keratin 20 (focal) positive HGSC were also keratin 7 (diffuse) positive.

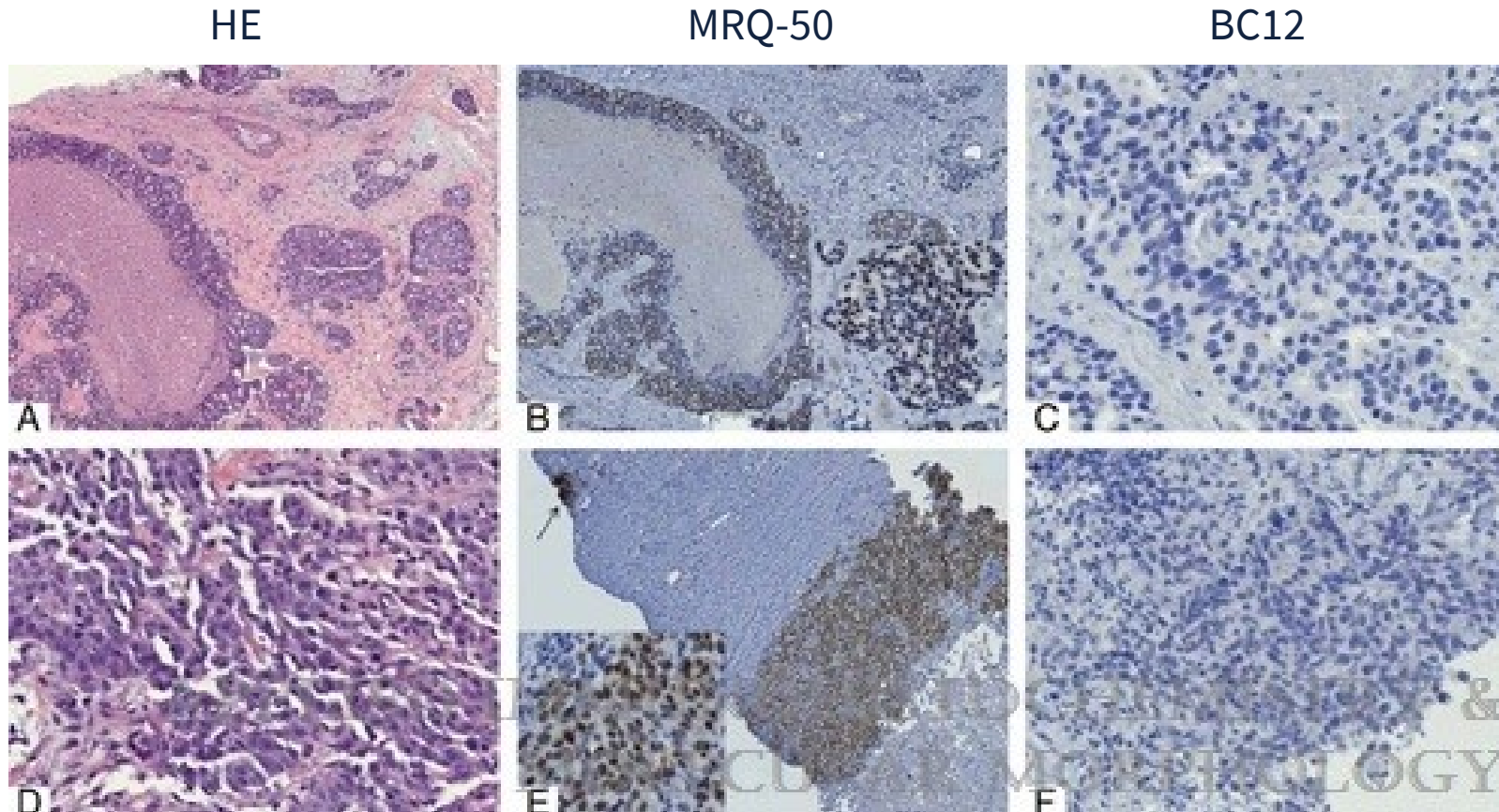
¶The 2 TRPS1 negative TNBC cases showed positivity for breast-specific markers GATA3, SOX10, and GCDFP15, respectively.

Aberrant Immunostaining of Breast Carcinoma by MRQ-50 PAX8 Antibody

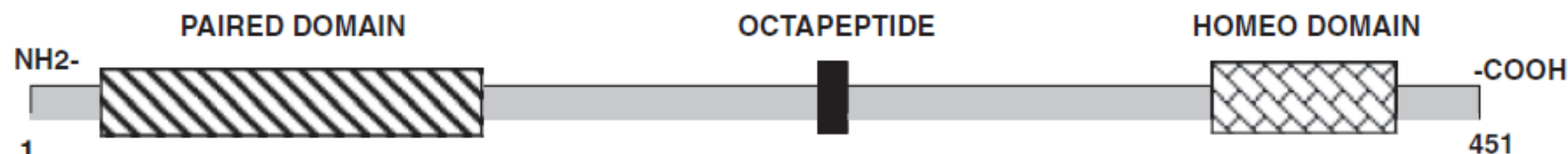
Singh, Kamaljeet MD; Hansen, Katrine MD; Quddus, M. Ruhul MD MPhil (Path)

Author Information

Applied Immunohistochemistry & Molecular Morphology 28(4):p e37-e38, April 2020. | DOI: 10.1097/PAI.0000000000000682



Photomicrographs from 2 breast carcinomas with aberrant PAX8 expression by MRQ-50 clone. On staining with hematoxylin and eosin (A, D) both tumors were high grade with necrosis. Immunohistochemistry for PAX8 with **MRQ-50 antibody (B, E) showed nuclear positivity in tumor cells and lymphocytes** (arrow). PAX8 IHC with **BC12 clone (C, F) did not stain tumor or lymphocytes.**



N – Terminal Region

PAX5	MDLEK NY PTPRTS RT GHGGV NQLGGV FVNGRPL PDVVRQ RIV ELAHQ GVRPCD ISRQLRV	61
PAX8	--MPH N -----S IR SGHG LNQLGGA FVNGRPL PEVVRQ RIV DLAHQ GVRPCD ISRQLRV	54
PAX5	SHGCVSKILGRYYETG SIK PGVIGGSKPKVATPKVVEK IAEYKRQ NPTMFAWEIRD LLA	121
PAX8	SHGCVSKILGRYYETG SIR PGVIGGSKPKVATPKVVEK IGDYKRQ NPTMFAWEIRD LLA	114
PAX5	ERVCDNDTVPSVSSINRIIRTKV QQP PN Q PV-----ASS HS IVSTGS VTQ VSS VST	173
PAX8	EGVCDNDTVPSVSSINRIIRTKV QQP FN L PMDS CVATKSLSPGH TLIPSSAV TPPES PQS	174
PAX5	DSAGSS YSISG ILGITS PSAD TNKRKRDEGI QES	233
PAX8	DSL GSTYS INGLLGIA Q PGSD--KRKMDDSD QDS	232

Immunogen:
Residues 1-212

70% Homology

C – Terminal Region

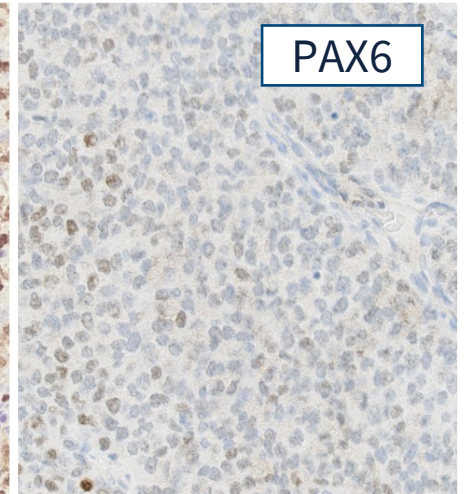
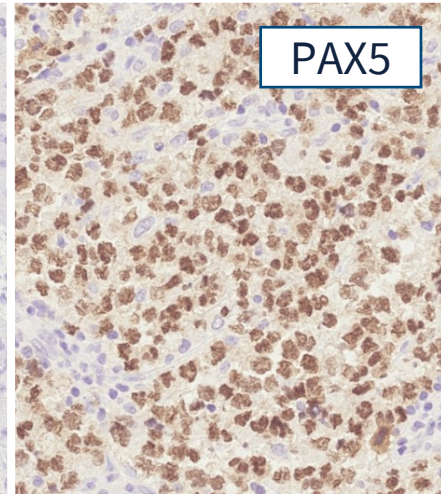
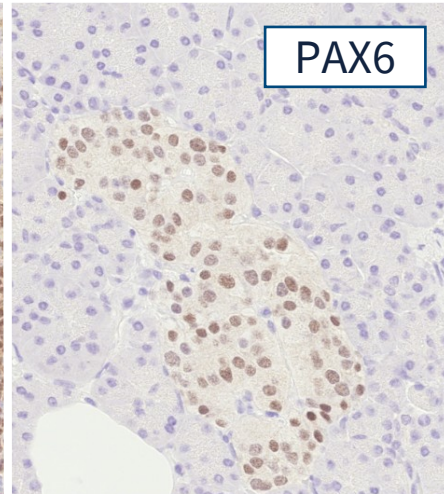
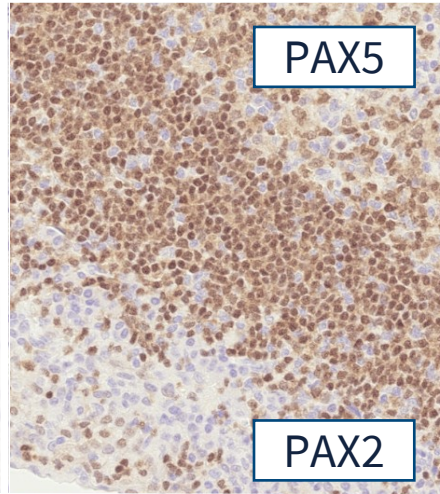
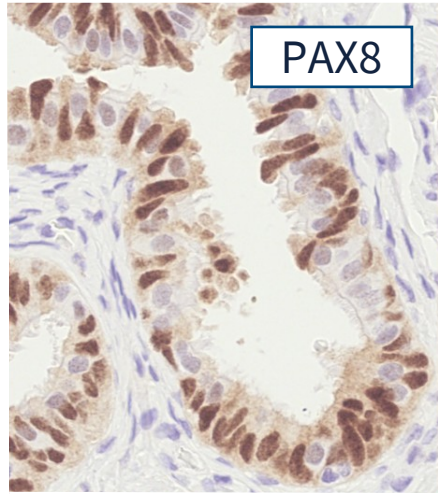
PAX5	QLEVLDRVFERQHYS DIFTT TEPIKPE Q -----TTEYSAMASLAGGLDDMKANLAS	284
PAX8	HLEPLECPFERQHYPEAYASPSHTKGE QGLYPLPL NSTLDDGKATLTPSNTPLGRNLST	292
PAX5	-----PTPAD----- I GS S VPGPQSY P -----	301
PAX8	HQTYPVVADPHSPFAIKQETPEVSSSS STPSSLSSSA FLDLQ QVGS GVPFNAF PHAASV	352
PAX5	-----IVTGRDLAST TLPGYPPH VP PAGQGSY SAPT LTGMV PGSEFSGSPY SH PQYS	353
PAX8	YGQFTGQALLS GRE MVGP TLPGYPPH I PTS G QGSY ASSA IAGM VAGSEYSGNAY GHT TPYS	412
PAX5	SYNDS WRFPN PGL L GSPYYYSAAARGAAPPAAATAYDRH	392
PAX8	SYSE AWRFPN SS LL SSPYYYSSTRPSAPPTTATAFDHL	451

Immunogen:
Residues 318-426

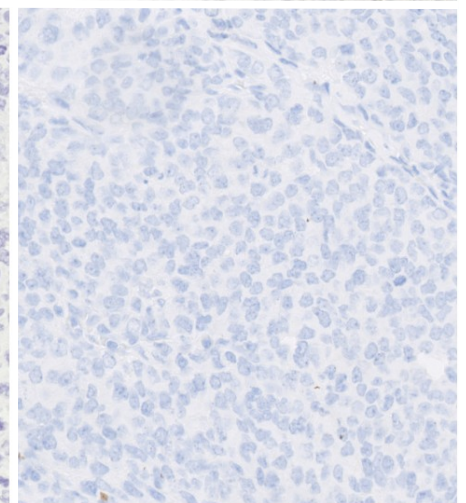
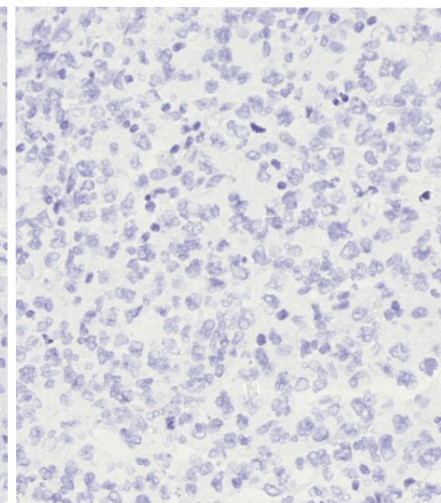
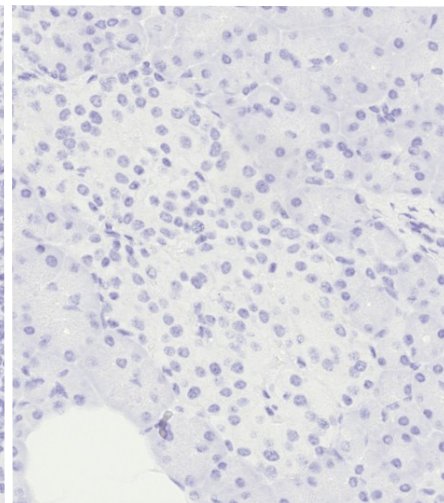
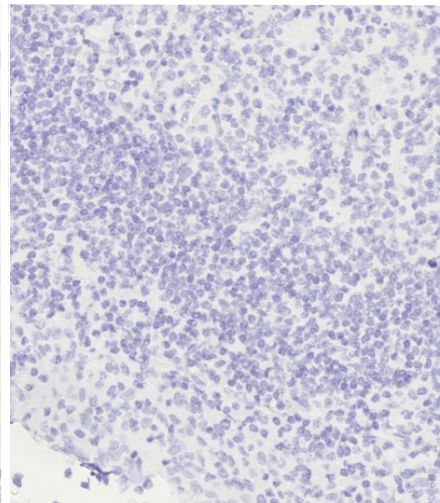
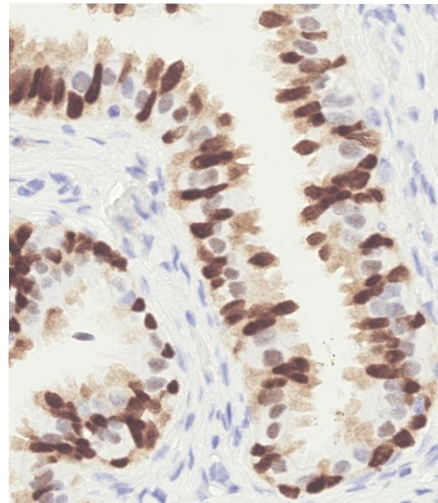
39% Homology

IHC controls to guide reliability of data

MRQ-50
(& pAb)



SP348
/BC12



Fal. Tube

Tonsil

Pancreas

DLBCL

Breast carc.

NordiQC Assessments of PAX8 Immunoassays

Rasmus Røge, MD,† Ole Nielsen, HT,‡ Michael Bzorek, HT,§ Søren Nielsen, HT,*
and Mogens Vyberg, MD*†*

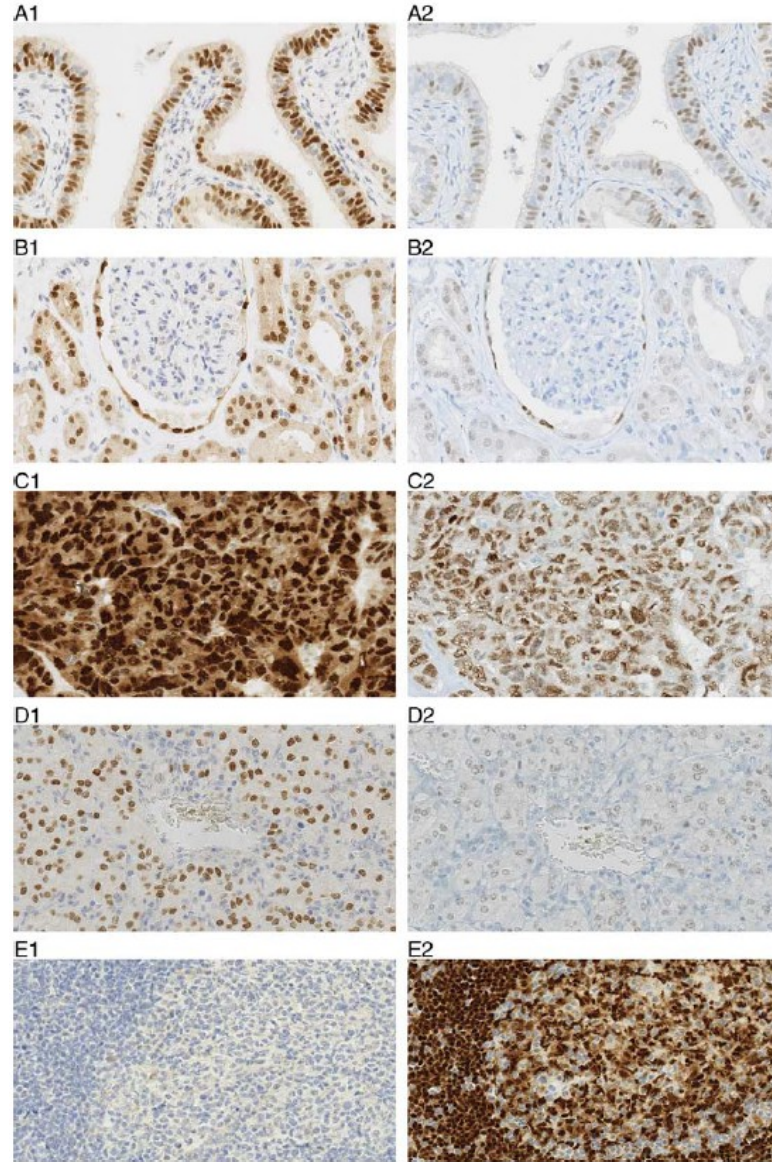
Positive tissue control 1
Fallopian tuba

Positive tissue control 2
Kidney

Tumour type 1
Ovarian carc.

Tumour type 2
Renal cell carc.

Negative tissue control 1
Tonsil



SP348/ BC12

MRQ-50 / pAb

Level of analytical
sensitivity

Level of analytical
specificity

But can breast carcinoma show PAX8 expression?

Comparison of PAX8 Expression in Breast Carcinoma Using
MRQ50 and BC12 Monoclonal Antibodies

Kamaliyet Singh, MD, Linda C. Hanley, MD, C. James Sung, MD.

No data about controls

Une

High-grade Breast Cancer

*Mark R. Kilgore, MD, Dustin E. Bosch, MD, PhD, Kathi H. Adamson, MD, Paul E. Swanson, MD,
Suzanne M. Dintzis, MD, PhD, and Mara H. Rendi, MD PhD*

31% MRQ-50
11% pAb CM

mary:
chemistry

hD

The importance of IHC controls has been neglected

Diagnostic Cytopathology, Vol 39, No 4 2011

Documentation of Immunocytochemistry Controls in the Cytopathologic Literature: A Meta-Analysis of 100 Journal Articles

Carol Colasacco, M.L.I.S., S.C.T.(A.S.C.P.), C.T.(I.A.C.),^{1*} Sharon Mount, M.D.,^{1,2}
and Gladwyn Leiman, M.B.B.C.H., F.I.A.C., F.R.C.Path.^{1,2}

Absent: Controls were not mentioned.

Vague: Statement such as “appropriate positive and negative controls were included.”

Identical: Controls identical to study samples were described.

Other: Controls were dissimilar or partially similar (i.e., tissue control with smears or tissue control with cell block and ThinPrep samples run), or samples were too scant to include controls.

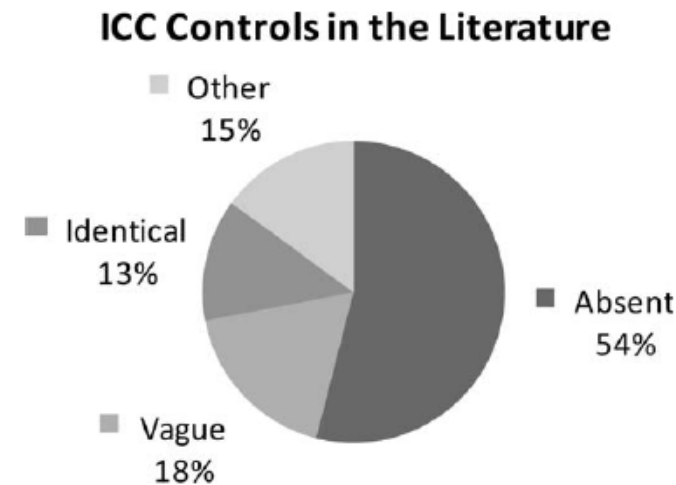


Fig. 1. Description of immunocytochemistry controls in articles reviewed.

> 70% of publications based on IHC do not describe controls used to verify data and conclusions

Canadian Association of Pathologists-Association canadienne des pathologistes National Standards Committee/Immunohistochemistry

Best Practice Recommendations for Standardization of Immunohistochemistry Tests*

Emina Emilia Torlakovic, MD, PhD,¹ Robert Riddell, MD, FRCPath, FRCPC,²
Diponkar Banerjee, MBChB, FRCPC, PhD,³ Hala El-Zimaity, MD, MS, FRCPC,⁴
Dragana Pilavdzic, MD, FRCPC,⁵ Peter Dawe, MS,⁶ Anthony Magliocco, MD, FRCPC,⁷
Penny Barnes, MD, FRCPC,⁸ Richard Berendt, MD, FRCPC,⁹ Donald Cook, MD, FRCPC,¹⁰
Blake Gilks, MD, FRCPC,¹¹ Gaynor Williams, MD, PhD,¹² Bayardo Perez-Ordóñez, MD, FRCPC,¹³
Bret Wehrli, MD, FRCPC,¹⁴ Paul E. Swanson, MD,¹⁵ Christopher N. Otis, MD,¹⁶
Søren Nielsen, HT, CT,¹⁷ Mogens Vyberg, MD,¹⁷ and Jagdish Butany, MBBS, MS, FRCPC¹³

CME/SAM

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 1: Fit-for-Purpose Approach to Classification of Clinical Immunohistochemistry Biomarkers

Carol C. Cheung, MD, PhD, JD,*† Corrado D'Arrigo, MB, ChB, PhD, FRCPath,‡§||
Manfred Dietel, MD, PhD,¶|| Glenn D. Francis, MBBS, FRCPA, MBA, FFS (RCPA),#***††
C. Blake Gilks, MD,‡‡ Jacqueline A. Hall, PhD,§§|| Jason L. Hornick, MD, PhD,¶¶||
Merdol Ibrahim, PhD,## Antonio Marchetti, MD, PhD,*** Keith Miller, FIBMS,##
J. Han van Krieken, MD, PhD,††† Søren Nielsen, BMS,‡‡‡§§§ Paul E. Swanson, MD,|| || ||
Clive R. Taylor, MD,¶¶¶ Mogens Vyberg, MD,‡‡‡§§§ Xiaoge Zhou, MD,###****
and Emina E. Torlakovic, MD, PhD,*††††††††

From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
and International Quality Network for Pathology (IQN Path)

Abstract: Technical progress in immunohistochemistry (IHC) as well as the increased utility of IHC for biomarker testing in precision medicine avails us of the opportunity to reassess clinical IHC as a laboratory test and its proper characterization as a special type of immunoassay. IHC, as used in current clinical applications, is a descriptive, qualitative, cell-based, usually nonlinear, in situ protein immunoassay, for which the readout of the results is principally performed by pathologists rather than by the instruments on which the immunoassay is performed. This modus operandi is in contrast to other assays

original purpose for which an IHC test is developed and its subsequent clinical uses, as well as the role of pathologists in the analytical and postanalytical phases of IHC testing. This paper is the first of a 4-part series, under the general title of "Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine."

Key Words: biomarkers, quality assurance, quality control, validation, immunohistochemistry

(Appl Immunohistochem Mol Morphol 2017;25:4-11)

Two main IHC categories

Type I / Class I IHC tests:

Interpreted in the context of histo- or cytomorphologic and clinical data. Results interpreted and used by pathologists. E.g. TTF1, SOX10, CDX2, PAX8, TRPS1, p40, CGA etc

Type II / Class III IHC tests:

Stand-alone tests being interpreted (largely) to provide predictive and prognostic information. Results interpreted by pathologists and used by clinicians to give tailored treatment. E.g. ER, HER2, ALK, PD-L1, CLDN18.2 etc.

Am J Clin Pathol 2010;133:354-365

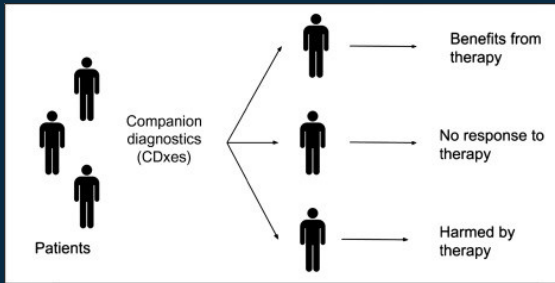
Two main IHC categories

In practice more and more IHC tests become both Type I and Type II tests:

IHC	Area	Type I	Type II	Drug
ALK	Lymphoma	ALCL	NSCLC	Crizotinib
CD30	Lymphoma	HL, ALCL	HL, ALCL	Brentuximab
CD56	Carcinoma	Neuroendo.	SCLC	Lorvotuzumab
MMR	CRC	Lynch	Solid carc.	Pembrolizumab

Indirectly indicated typically due to personalized treatment e.g.

IHC	Area	Type I	Type II	Comment
p40 - lung	Carcinoma	Squamous		
TTF1- lung	Carcinoma	Adeno	Crizotinib,....	ALK, EGFR, ROS1...



Two main IHC categories

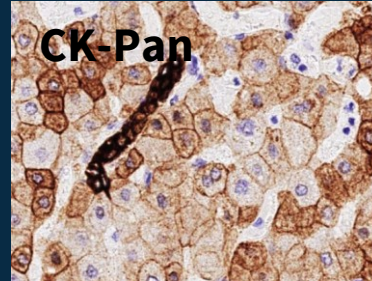
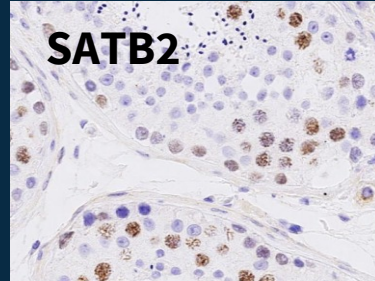
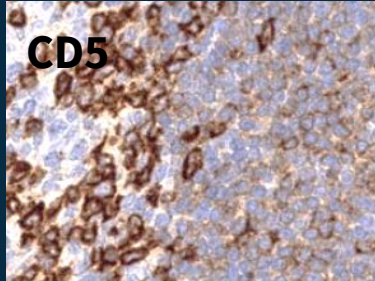
Type II / Class III, IHC companion diagnostics (CDx):

IHC-target	Area	Demonstration	Drug	
ER	Breast	Estrogen receptor	Tamoxifen, ...	1998
HER2	Breast and gastric	HER2 protein overexpression	Herceptin, Enhertu...	
CD117	GIST	Protein second to gene mut.	Glivec,...	
ALK, ROS1	NSCLC	Fusion protein from gene mut.	Crizotinib,...	2015
PD-L1	NSCLC, TNBC	PD-1 receptor	Pembrolizumab,..	
MMR	Solid carcinoma	PD-L1 receptor	Pembrolizumab	
Claudin 18.2	Gastric cancer	Claudin 18.2 protein	Zolbetuximab	
FOLR1....s	Ovarian cancer	Folate receptor alpha	Mirvetuximab	2025

Positive and negative tissue controls – Potentials and Limitations

External tissue controls have **highly recommended for most IHC assays type I**

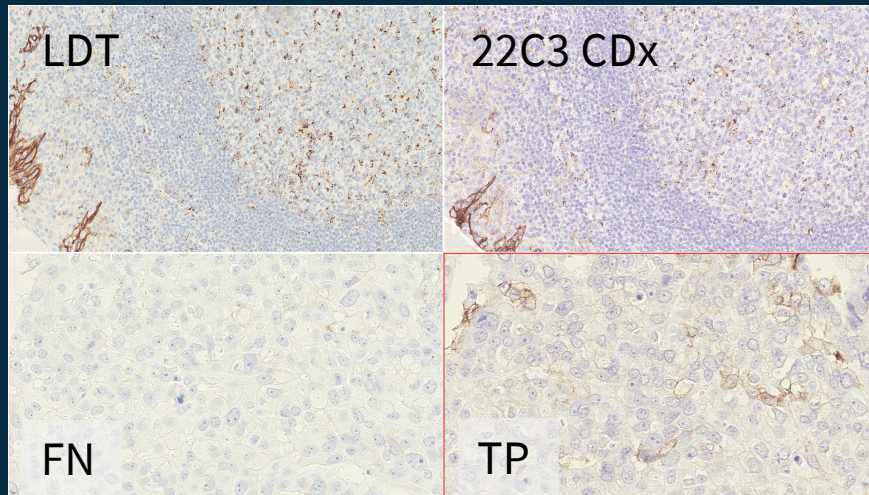
CD markers, transcription factors, tumour lineage markers...



Not found fully reliable in most type II predictive and semi-quantative IHC assays

HER2, PD-L1, Claudin 18.2...

Control ok



Tumour
PD-L1 neg
CPS <10

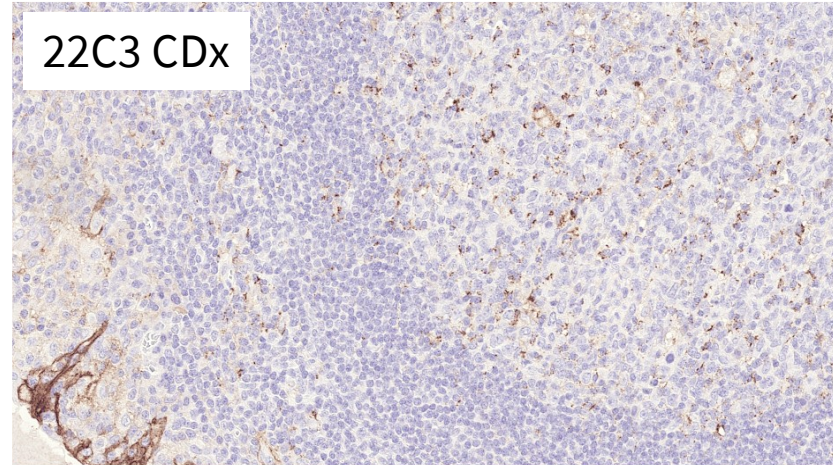
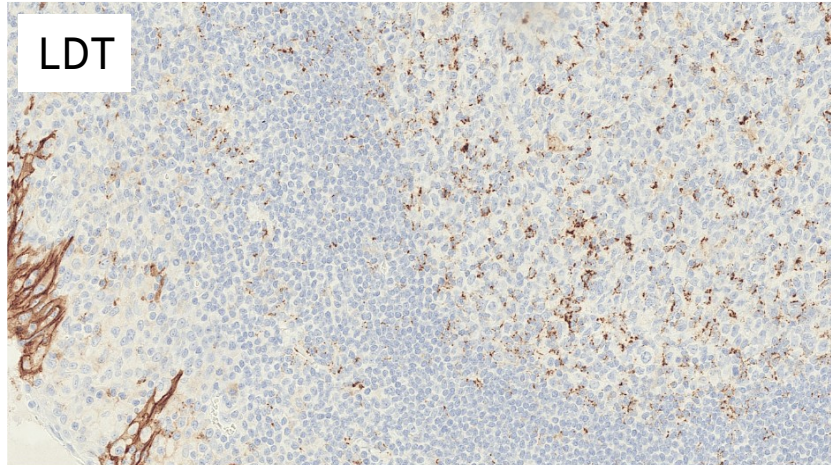
Control ok

Tumour
PD-L1 pos
CPS ≥10

Positive and negative tissue controls

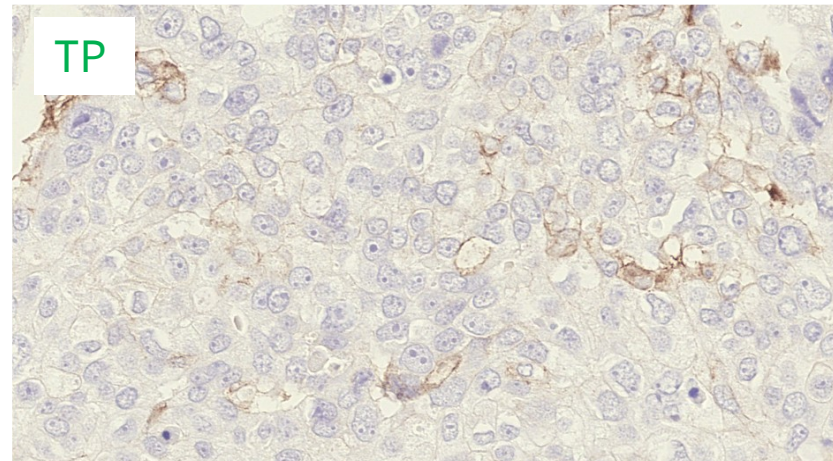
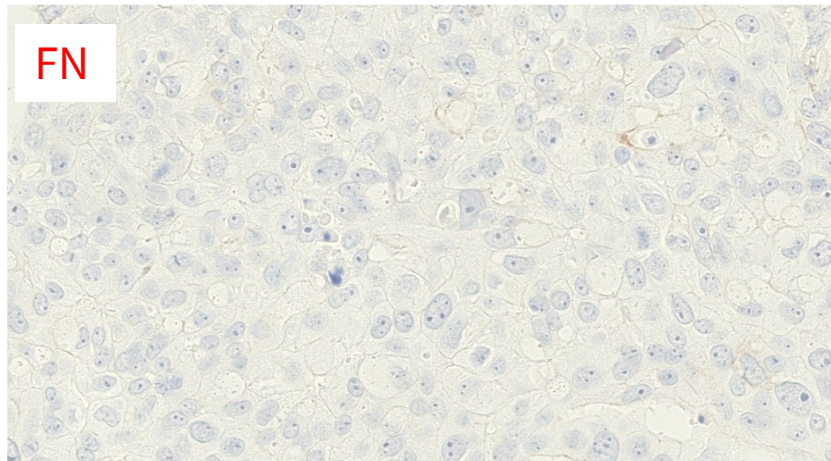
Not found fully reliable in most type II predictive and semi-quantitative IHC assays

Control ok



Control ok

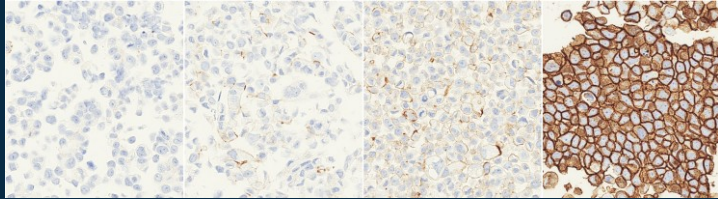
Tumour
PD-L1 neg
CPS <10



Tumour
PD-L1 pos
CPS ≥10

Controls for precision testing

Cell lines



www.histocyte.com

ALK and ROS1 being +/-
HER2, ER, PR, PD-L1 with dynamic
range

www.arrayscience.com

MMR

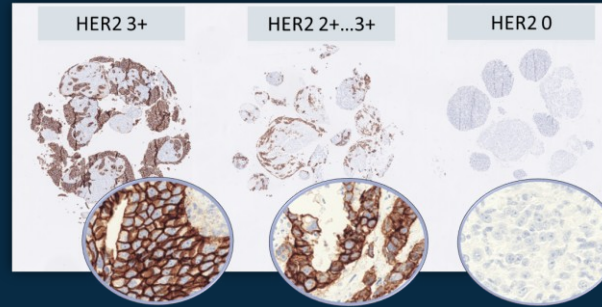
Melanoma (PRIME, MelanA/MART-1,
HMB-45, BRAF V600E, SOX10,
P53 (null mutant, missense mutant,
WT)

HER2, ER, PR, PD-L1, Ki67 with dynamic
range

www.acepixbio.com

HER2, PDL1...

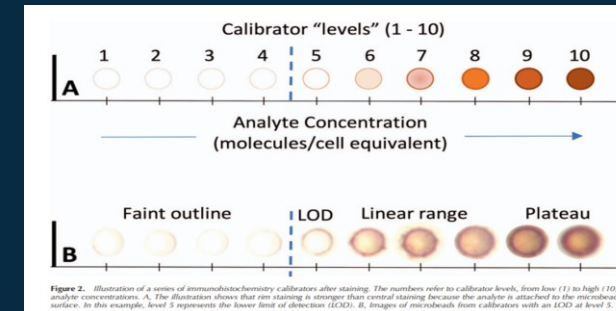
Histoids/ Faux tissue



www.statlab.com

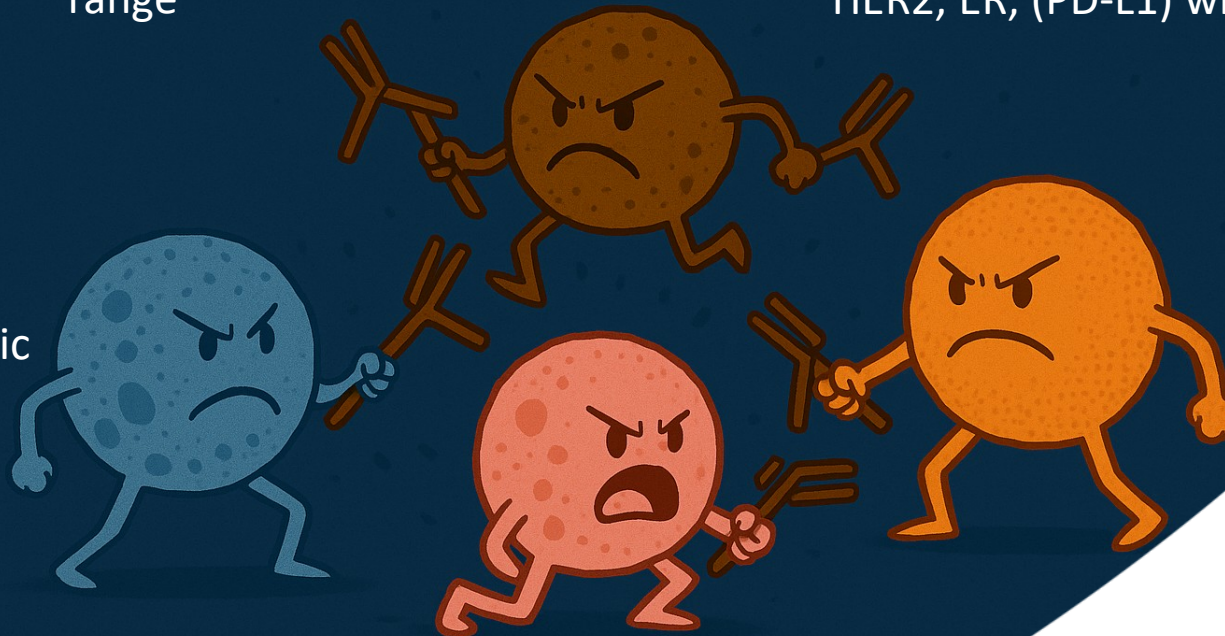
ALK +/-
HER2, PD-L1 with dynamic
range

Microbeads



www.bostoncellstandards.com

ALK, p53, PD-L1 +/-
HER2, ER, (PD-L1) with dynamic range



Role of microbeads in IHC

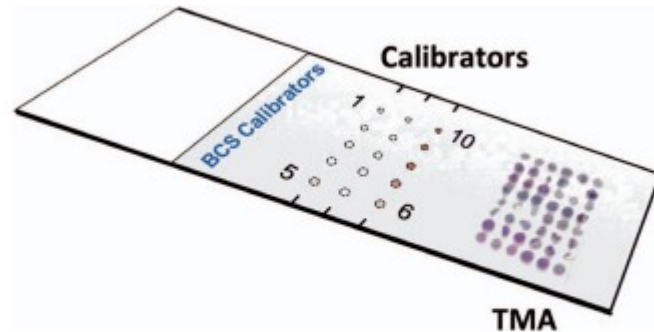


Figure 5. Illustration of the survey tool for correlating clinical accuracy (from the tissue microarray data) with analytic sensitivity (from the calibrator data). The calibrators are at up to 10 different concentrations, for example levels 1–10. The middle row depicts negative controls. Abbreviations: BCS, Boston Cell Standards; TMA, tissue microarray.

A Consortium for Analytic Standardization in Immunohistochemistry

Steven A. Bogen, MD, PhD; David J. Dabbs, MD; Keith D. Miller, FIBMS; Søren Nielsen, BLS; Suzanne C. Parry, BSc(Hons), MSc, FIBMS; Matthias J. Szabolcs, MD, PhD; Nils t'Hart, MD, PhD; Clive R. Taylor, MD, PhD; Emina E. Torlakovic, MD, PhD

(Arch Pathol Lab Med. doi: 10.5858/arpa.2022-0031-RA)

* LOD; Limit of detection

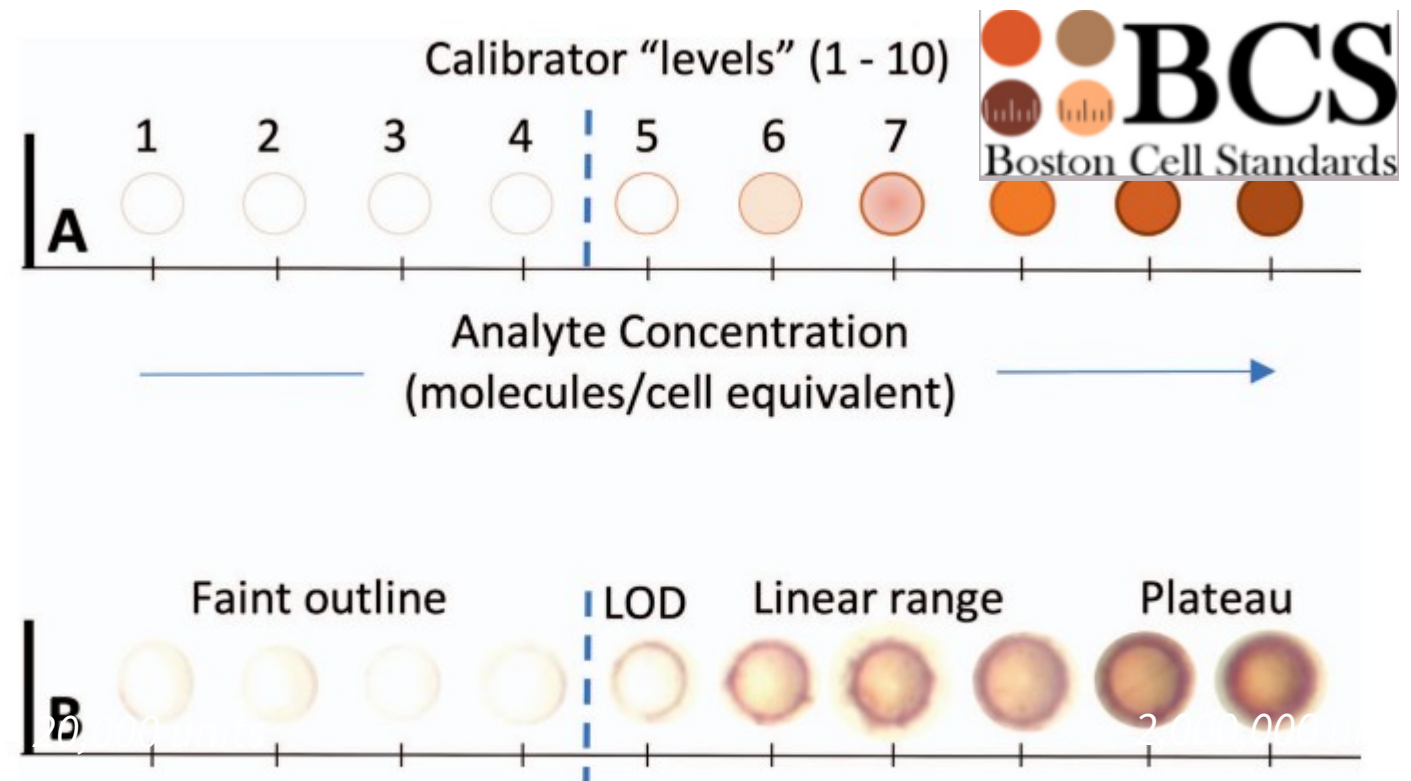


Figure 2. Illustration of a series of immunohistochemistry calibrators after staining. The numbers refer to calibrator levels, from low (1) to high (10) analyte concentrations. A, The illustration shows that rim staining is stronger than central staining because the analyte is attached to the microbead surface. In this example, level 5 represents the lower limit of detection (LOD). B, Images of microbeads from calibrators with an LOD at level 5.

Developmental and validation phase to correlate LOD*/analytical sensitivity in microbeads versus diagnostic accuracy and sensitivity for;

ER, HER2, PD-L1 and p53

Reference standard materials for IHC; Calibrators – LOD* - PD-L1



CERTIFICATE OF ANALYSIS

DESCRIPTION

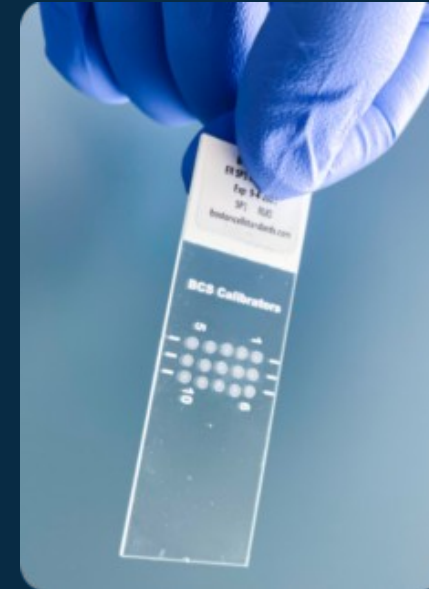
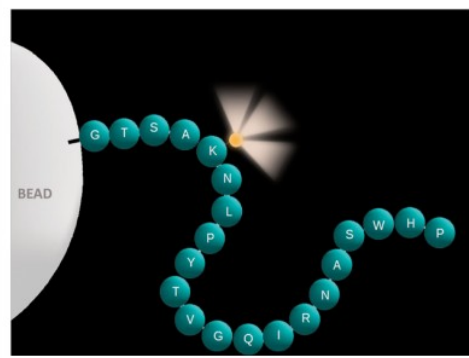
Product Description:	IHCalibrators (10 levels)	Catalog Number: PDEXR
Mean Diameter:	7-8 micron	Lot Number: 001
Target:	PD-L1 (extracellular Domain)	Antibody: 22C3 and 28-8 clones

CONCENTRATION

Average PD-L1 Molecules per Microbead

Level 10	603,077
Level 9 -	598,591
Level 8 -	479,714
Level 7 -	356,351
Level 6 -	228,502
Level 5 -	116,354
Level 4 -	53,551
Level 3 -	22,149
Level 2 -	9,550*
Level 1 -	2,197*

*The values of levels 1 and 2 are estimates based on the amount of PD-L1 analyte applied during coupling, extrapolating from higher levels. The concentrations were too low for direct measurement by fluorescence quantification.



- Bogen, SA. 2019. A root cause analysis into the high error rate in clinical immunohistochemistry. *Appl. Immunohistochem. Mol. Morphol.* 27(5) 329-338.
- Sompuram, SR, K Vani, AK Schaedle, A Balasubramanian, & SA Bogen. 2019. Selecting an optimal positive IHC control for verifying retrieval. *J. Histochem. Cytochem.* 67(4):273-283.
- Sompuram, SR, K Vani, AK Schaedle, A Balasubramanian, & SA Bogen. 2018. Quantitative assessment of immunohistochemistry laboratory performance by measuring analytic response curves and limits of detection. *Arch Pathol Lab Med.* 142 (7):851-862.

Reference standard materials for IHC: Calibrators – LOD – PD-L1 22C3 11

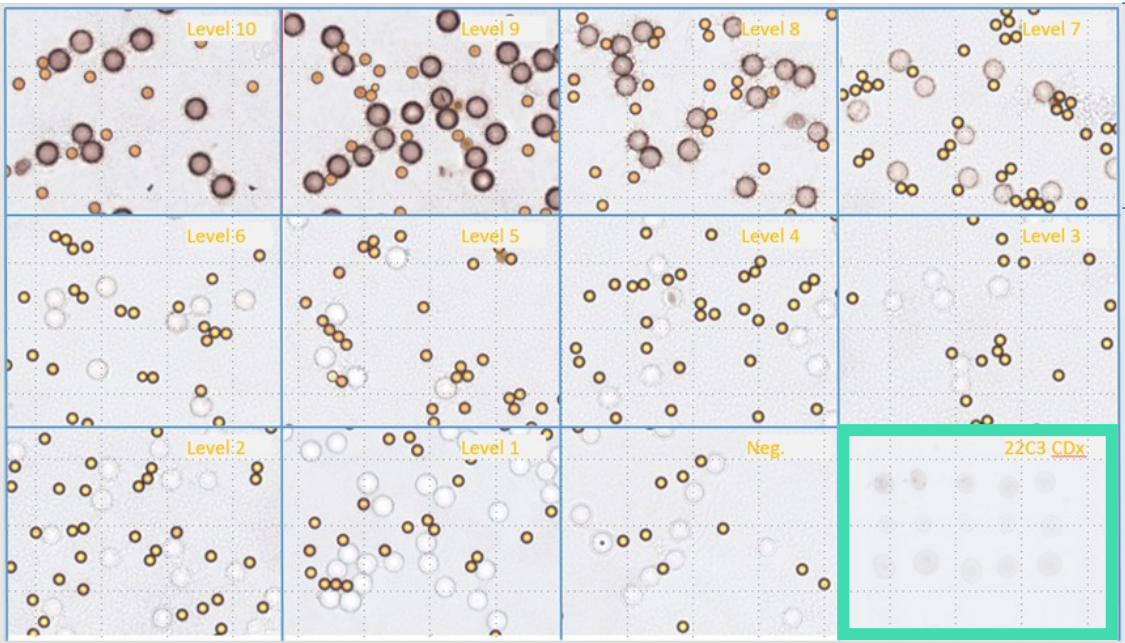
ARTICLE OPEN

Check for updates

Quantitative comparison of PD-L1 IHC assays against NIST standard reference material 1934

Seshi R. Sompuram¹, Emina E. Torlakovic^{2,3}, Nils A. 't Hart⁴, Kodela Vani¹ and Steven A. Bogen¹✉

© The Author(s), under exclusive licence to United States & Canadian Academy of Pathology 2021, corrected publication 2021



22C3 LOD
356.351 mol.
pr microbead

Average PD-L1 Molecules per Microbead

Level 10	603,077
Level 9	598,591
Level 8	479,714
Level 7	356,351
Level 6	228,502
Level 5	116,354
Level 4	53,551
Level 3	22,149
Level 2	9,550*
Level 1	2,197*

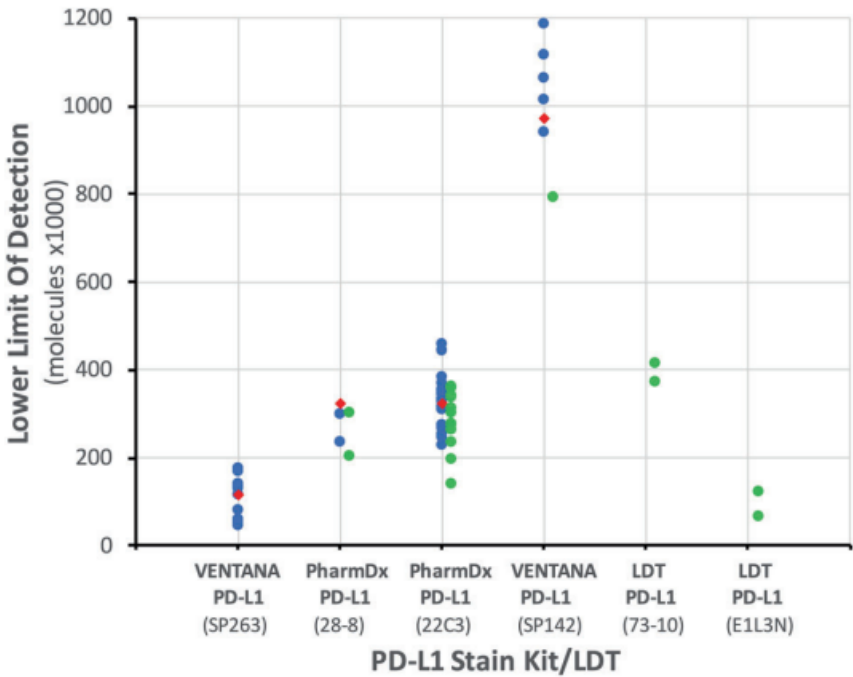


Fig. 2 Lower limit of detection (LOD) of various PD-L1 assays (x axis). Lower numbers (on the y axis) equate to greater sensitivity. Each dot represents a separate IHC laboratory test. Blue dots depict FDA-cleared assays in clinical laboratories, green dots for laboratory-developed tests (LDTs), and red diamonds for FDA-cleared assays as performed by a reference laboratory. Tissue staining in Fig. 2 was performed by these reference labs. For enhanced clarity, the LDT data are positioned slightly to the right of the vertical lines.

Correlation of IHC for HER2 – Microbeads – Accuracy/Precision



Breast carcinomas

N=15 (NordiQC runs B31, B32, B33)

HER2 classical	HER2 Low		PATHWAY Standard LOD 1,981,264 HER2 mol.	PATHWAY – red. HIER & Ab LOD 2,669,835 HER2 mol.	PATHWAY + OptiView LOD 1,274,947 HER2 mol.
		0	2	5	0
		1+	3	3	3
		2+ Unamplified	1	2	3
		2+ Amplified	3	1	3
		3+ Amplified	6	4	6

Reduced analytical sensitivity (LOD) provided a less accurate HER2 result for both classical overexpression and HER2 low

Increased analytical sensitivity (LOD) provided a less accurate HER2 result for HER2 low

Correlation of IHC for HER2 Microbeads – Accuracy/Precision

Breast
carcinomas

Unamplified

1+

Amplified

2+

Amplified

3+

PATHWAY Standard
LOD 1,981,264 HER2 mol.

PATHWAY – red. HIER & Ab
LOD 2,669,835 HER2 mol.

PATHWAY + OptiView
LOD 1,274,947 HER2 mol.

1+	0	2+
2+	1+	2+
3+	2+	3+

Correlation of IHC for HER2 – Microbeads – Accuracy/Precision

HER2 molecules

pr microbead

8. 2,669,835

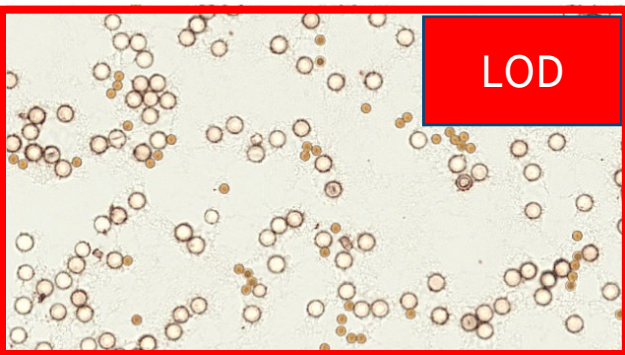
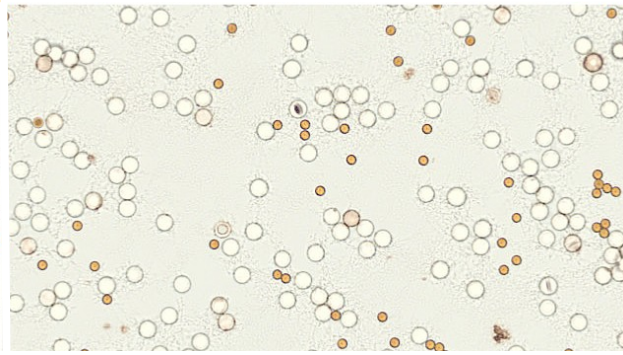
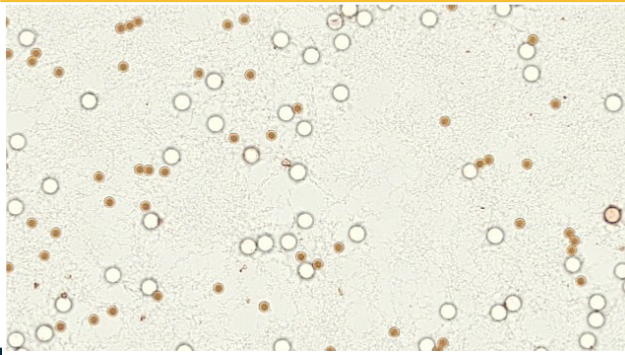
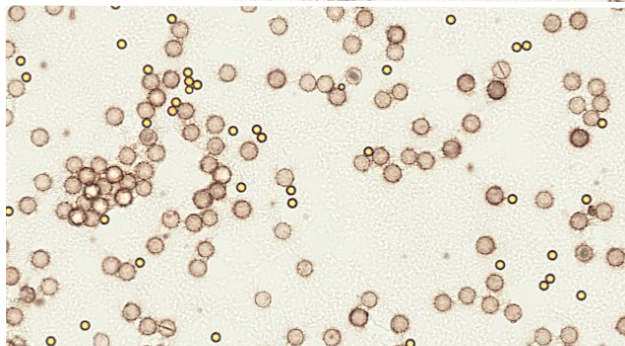
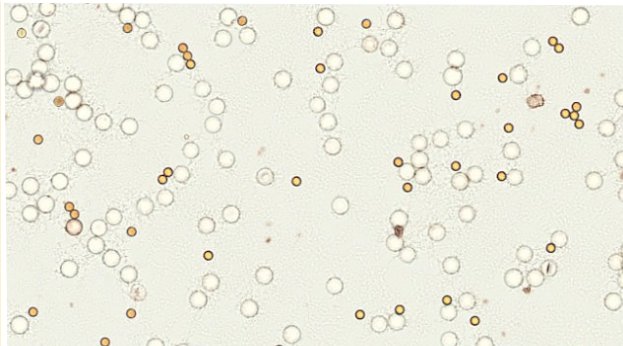
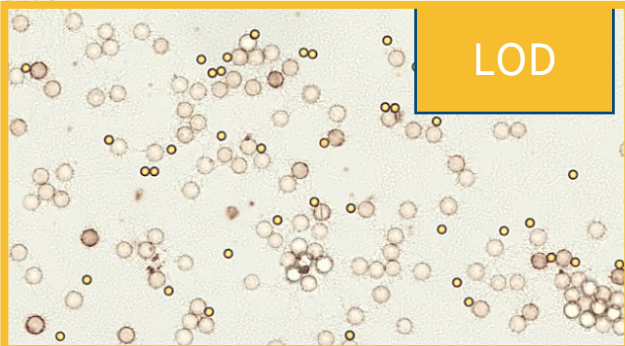
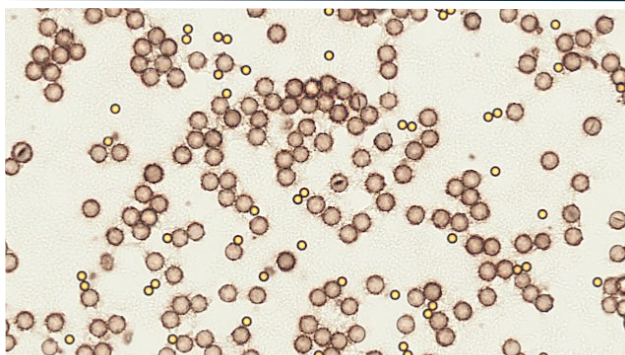
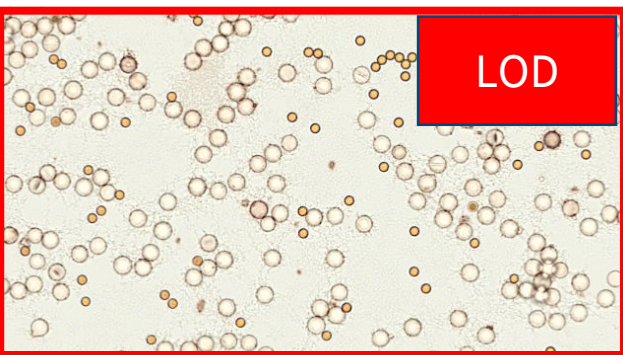
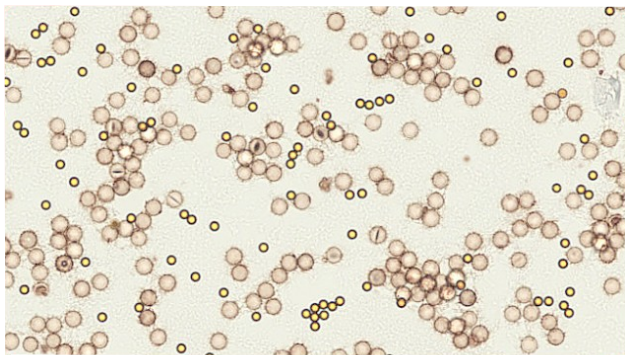
7. 1,981,264

6. 1,274,947

PATHWAY Standard

PATHWAY – red. HIER & Ab

PATHWAY + OptiView



510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY ONLY

I Background Information:

A 510(k) Number

K220163

B Applicant

Boston Cell Standards, Inc.

C Proprietary and Established Names

HER2/ER/PR IHCControls® - Level H
HER2/ER/PR IHCControls® - Level M
HER2/ER/PR IHCControls® - Level L

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NJW	Class II	21 CFR 864.1860 - Immunohistochemistry Reagents and Kits	PA - Pathology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Positive controls to monitor performance of the immunohistochemical (IHC) staining process for HER2, ER and PR IHC assays.

C Type of Test:

Peptide based on-slide controls to monitor the performance of the analytic components (antigen retrieval and immunostaining) of certain human epidermal growth factor receptor, type II (HER2), estrogen receptor (ER) and progesterone receptor (PR) IHC stains.

HER2/ER/PR IHCControls® - Level H, HER2/ER/PR IHCControls® - Level M and HER2/ER/PR IHCControls® - Level L (HER2/ER/PR IHCControls®) are unassayed positive controls for HER2, ER, and PR IHC assays which are provided in vials as a liquid suspension. The suspension contains microscopic glass microbeads that are approximately the same size as cells and coated with purified analyte(s). HER2/ER/PR IHCControls® are not biological cells or tissues. The microbead surface bears the molecule(s) being measured. Peptides representing the epitopes of HER2, ER, and PR antibodies used by clinical IHC laboratories are incorporated into the products. The same immunohistochemical reaction that occurs on a tissue or cellular sample also occurs on the HER2/ER/PR IHCControls®. As a result of immunostaining, the HER2/ER/PR IHCControls® bearing the analyte turn the same color as cells expressing the analyte. The HER2/ER/PR panel includes controls at 3 different analyte concentrations, which allows matching the analyte concentration to the dynamic range (range of staining intensity) of the particular HER2, ER, or PR IHC stain with which it is intended to be used.

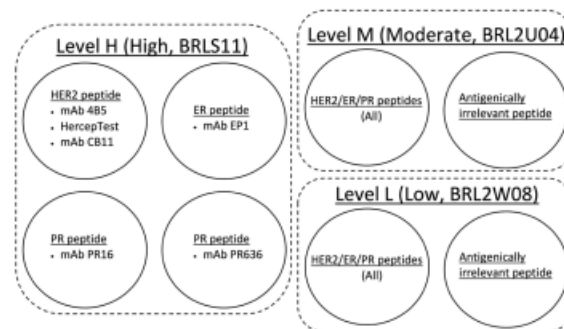


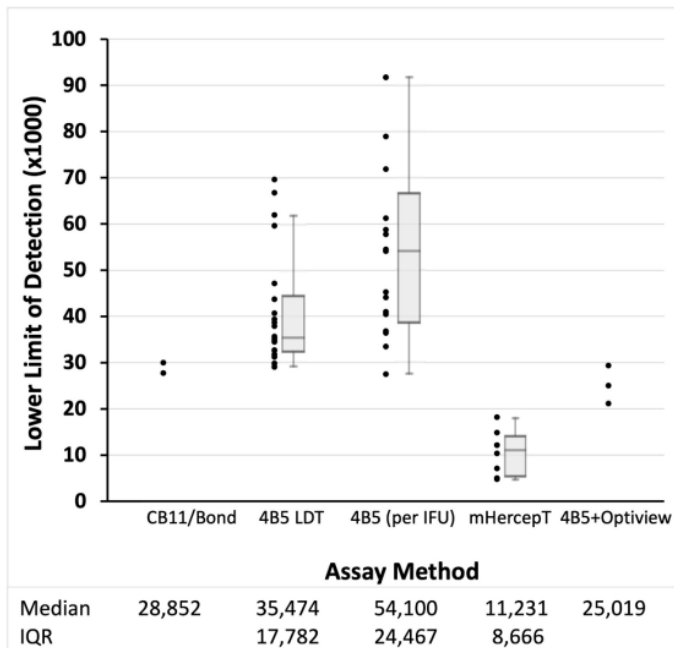
Figure 1. The 3 levels of HER2/ER/PR IHCControls® and their microbeads are schematically illustrated. Each circle represents a test microbead, coated with one or more peptide analytes. The primary antibodies immunoreactive with the peptide coatings are listed within the circles. Level H has 4 different test microbeads, each coated with a single peptide. Levels M and L have only two different test microbeads, coated with a cocktail of HER2/ER/PR peptides, although the concentration is lower in Level L.

1' large feasibility study published on calibrators as IHC QC tool

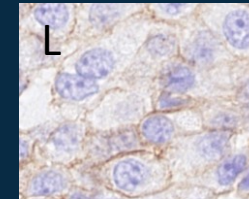
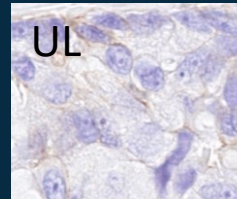
www.thelancet.com Vol 120 October, 2025

New standards in HER2-low testing: the CASI-01 comparative methods study

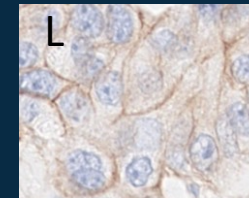
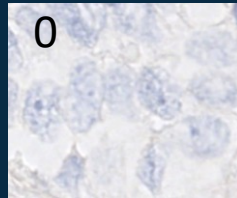
David J. Dabbs,^{a,b} Emina Torlakovic,^{a,c} Søren Nielsen,^{a,d} Suzanne C. Parry,^{a,e} Jing Yu,^b Catherine Stoos,^f Beth Clark,^b Henrik Høeg,^g Jeppe Thagaard,^g Seshi R. Sompuram,^h Stephen P. Naber,ⁱ Yukako Yagi,^j James Sayre,^k Kodela Vani,^h Mélissande Cossutta,^l Françoise Soussaline,^l Alexandre Papine,^l Nils A. t'Hart,^{a,m} Matthias J. Szabolcs,^{a,n} Bharat Jasani,^{a,o} Mary Kinloch,^{a,c} Luis Chiriboga,^{a,p} Keith Miller,^{a,q} and Steve Bogen^{a,h,i,*}



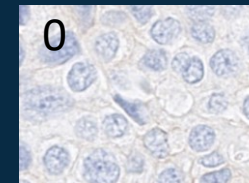
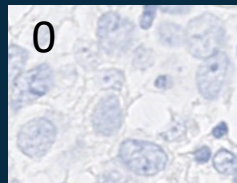
LOD
11.000



LOD
54.000



LOD
90.000



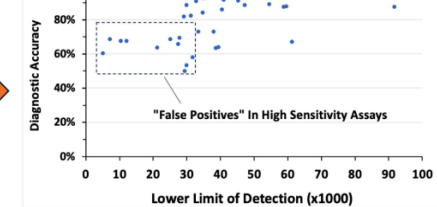
BC 1

BC 2

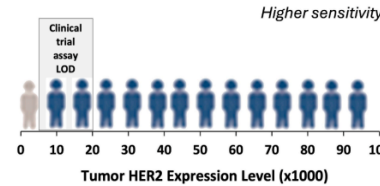
A Clinical Trial Assay Sensitivity



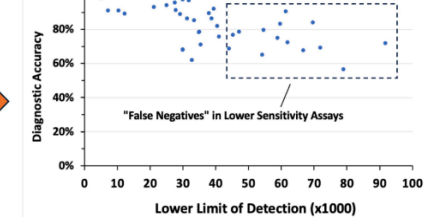
B Commercial Lab Assay Sensitivity



C Clinical Trial Assay Sensitivity



D Commercial Lab Assay Sensitivity



TMA slides with 80 Breast carcinomas and calibrators with 10 different levels of HER2 molecules send to 54 laboratories (mainly from NordiQC and UK NEQAS)

HER2-Low/Ultralow and "HER2 classical" IHC testing for; ENHERTU/TDX (Trastuzumab deruxtecan) and Herceptin



* LOD; Limit of detection

You cannot manage what you do not measure

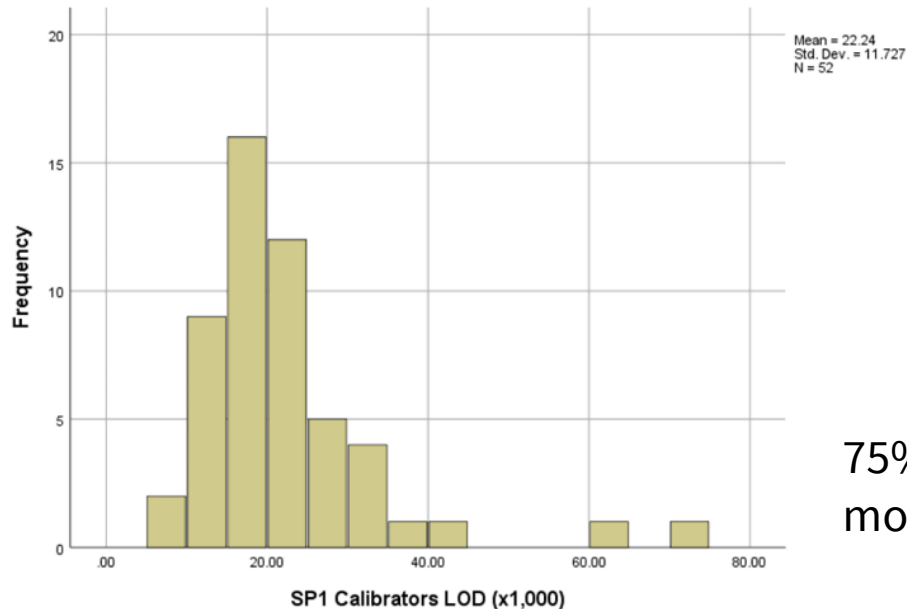
Calibrators make sensitivity measurable!

Research Article

The Value of Internal Positive Controls and Synthetic Calibrators for Performance Assessment of Breast Carcinoma Estrogen Receptor Immunohistochemistry Assay

Emina E. Torlakovic^{1,2}, Seshi R. Sompuram³, Søren Nielsen⁴, Steven Bogen³

Labs showed up to 10-fold differences in LOD:
LODs ranging from 7,310 to 74,790 molecules!



75% of labs had IHC protocols with LOD $\leq 25,000$, indicating a more sensitive assay

TMA design:

80 cores of breast carcinomas

28 ER negative

20 ER low positive

32 ER high positive

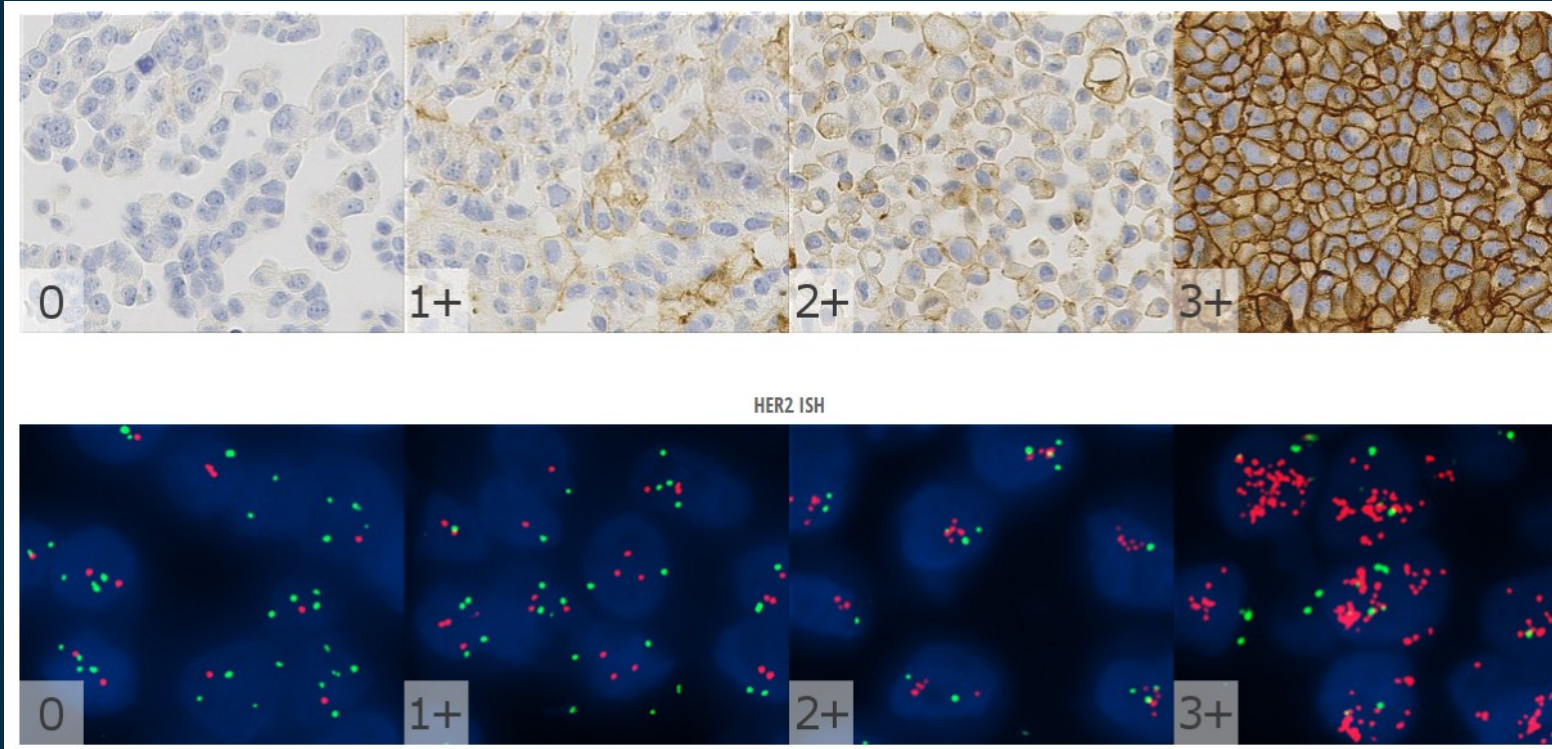
5 cores of benign breast epithelium

2 cores of benign ER negative tissue
liver
kidney cortex

+ ER calibrators

52 different laboratories using SP1 based protocols

Role of cell lines for IHC



**In NordiQC run B39
15% of the
participants used
cell lines as onslide
control**

Still need evidence/proof (VALIDATION) how to correlate any change in staining pattern in cell lines for accuracy in tissues of breast carcinoma. Tissue and cell line expression robustness (too fragile or too stabile)?

What expression levels characterizes a successful vs unsuccessful test?

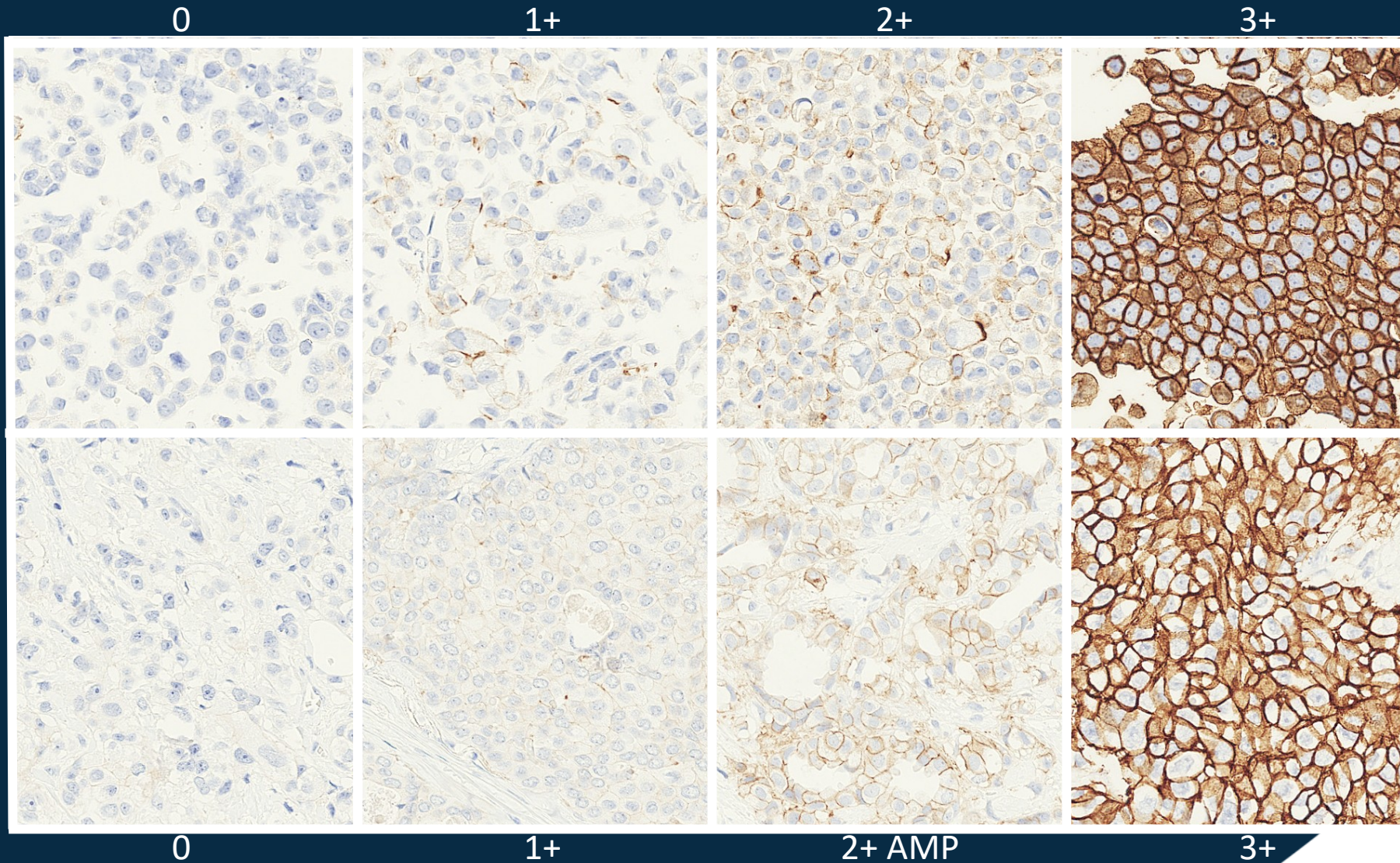
Impact on section thickness?

Pattern on different assays?

Correlation of IHC for HER2 PATHWAY – cell lines and tissues

Cell lines
(HCL028
Histocyte)

Breast
carcinomas
NordiQC
run B34



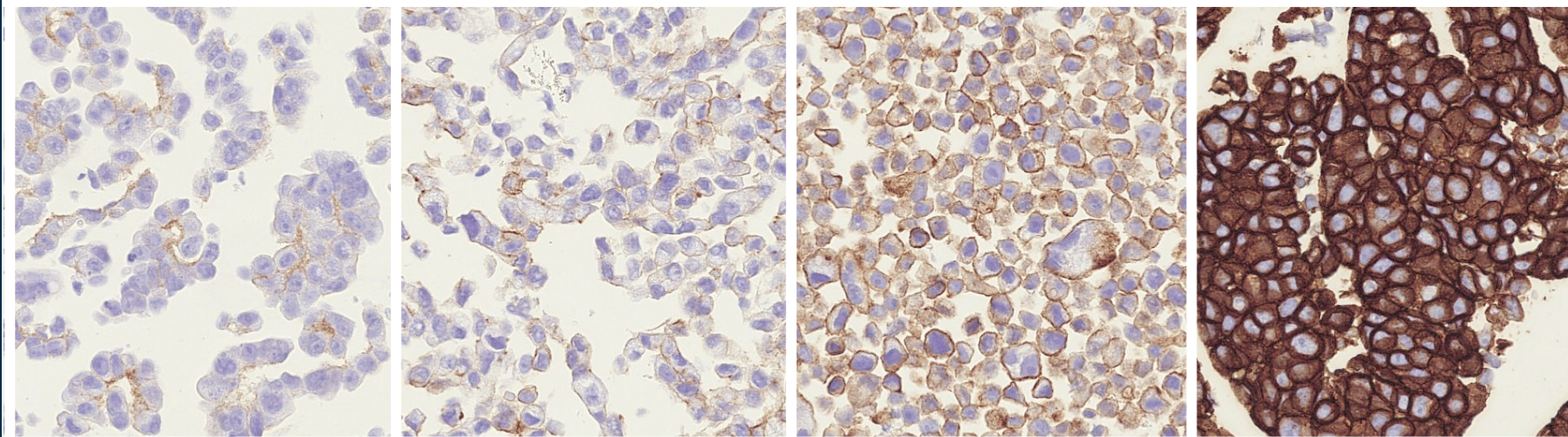
Correlation of IHC for HER2 HercepTest 2' Gen – cell lines and tissues

1+ (0)

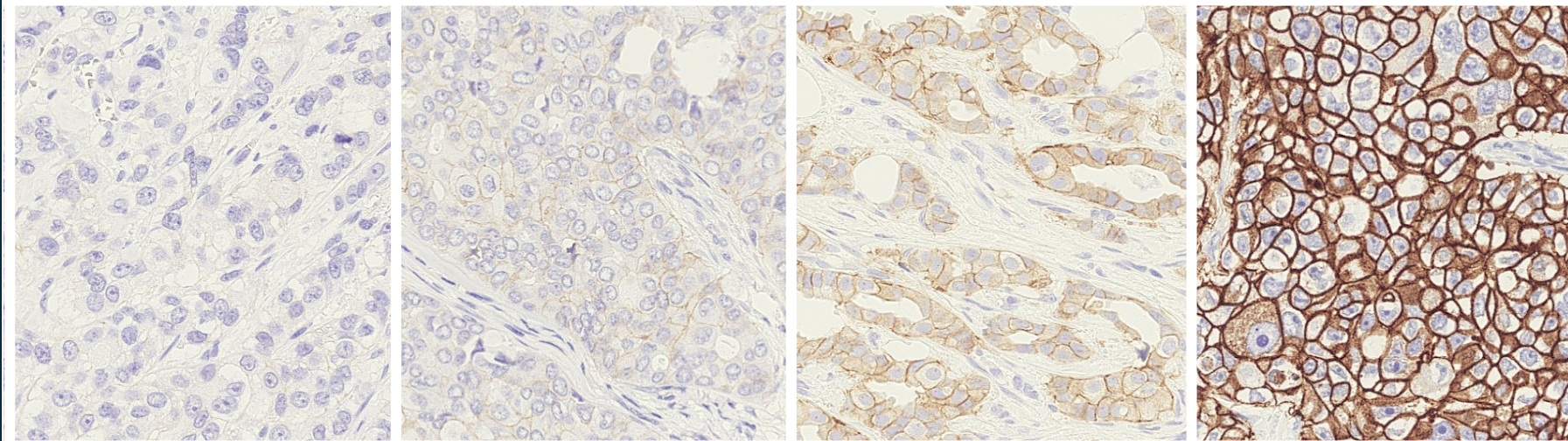
1+

2+

3+



Cell lines
(HCL028
Histocyte)



Breast
carcinomas
NordiQC
run B34

0

1+

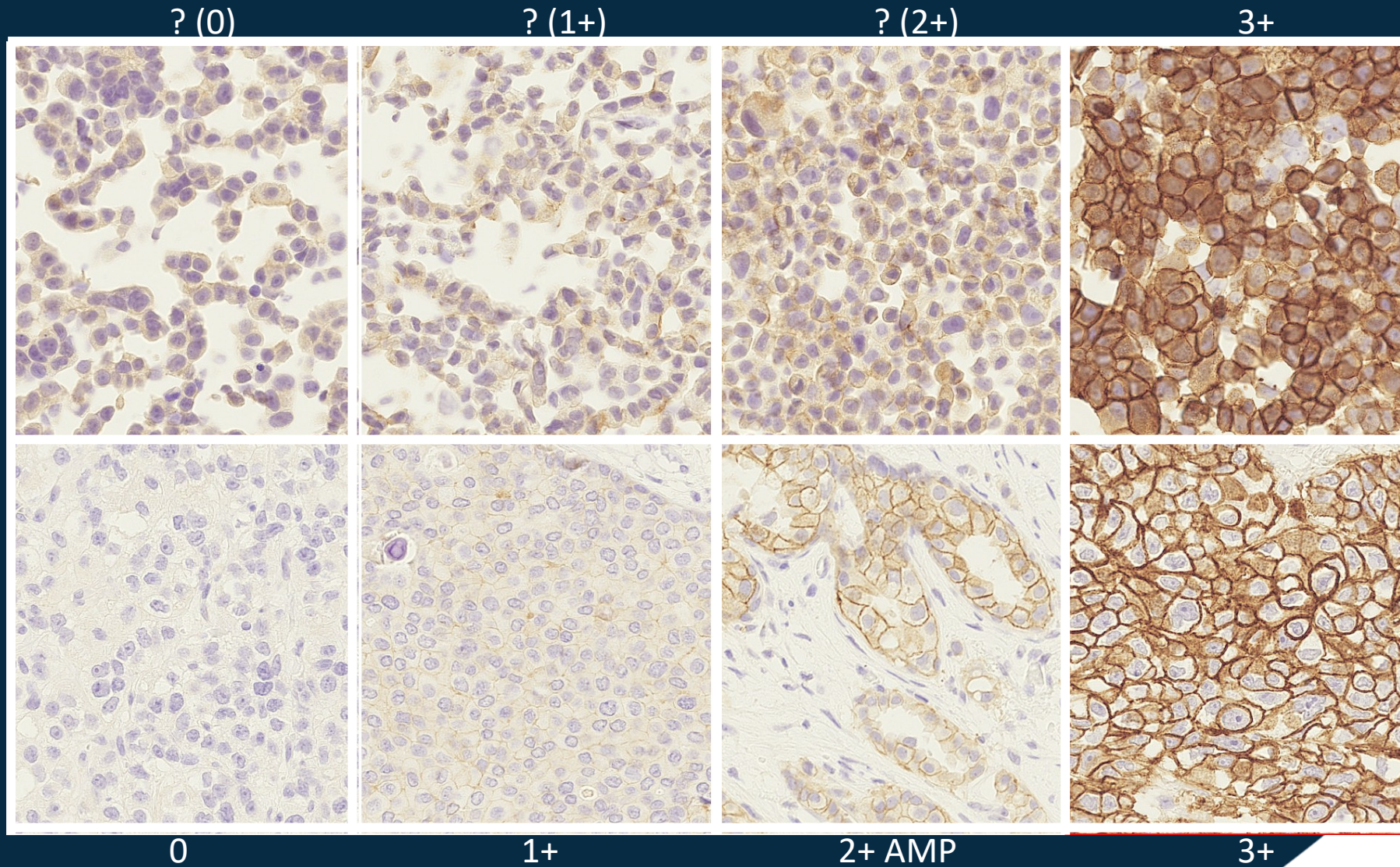
2+ AMP

3+

Correlation of IHC for HER2 SP3 – cell lines and tissues

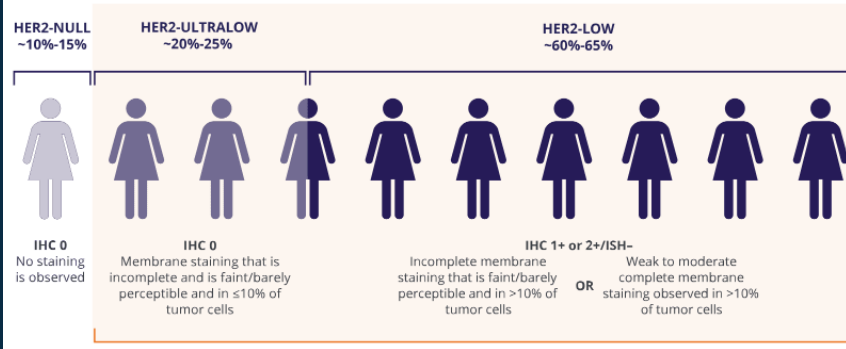
Cell lines
(HCL028
Histocyte)

Breast
carcinomas
NordiQC
run B34



ENHERTU expands the opportunity for HER2-directed therapy for eligible patients with mBC

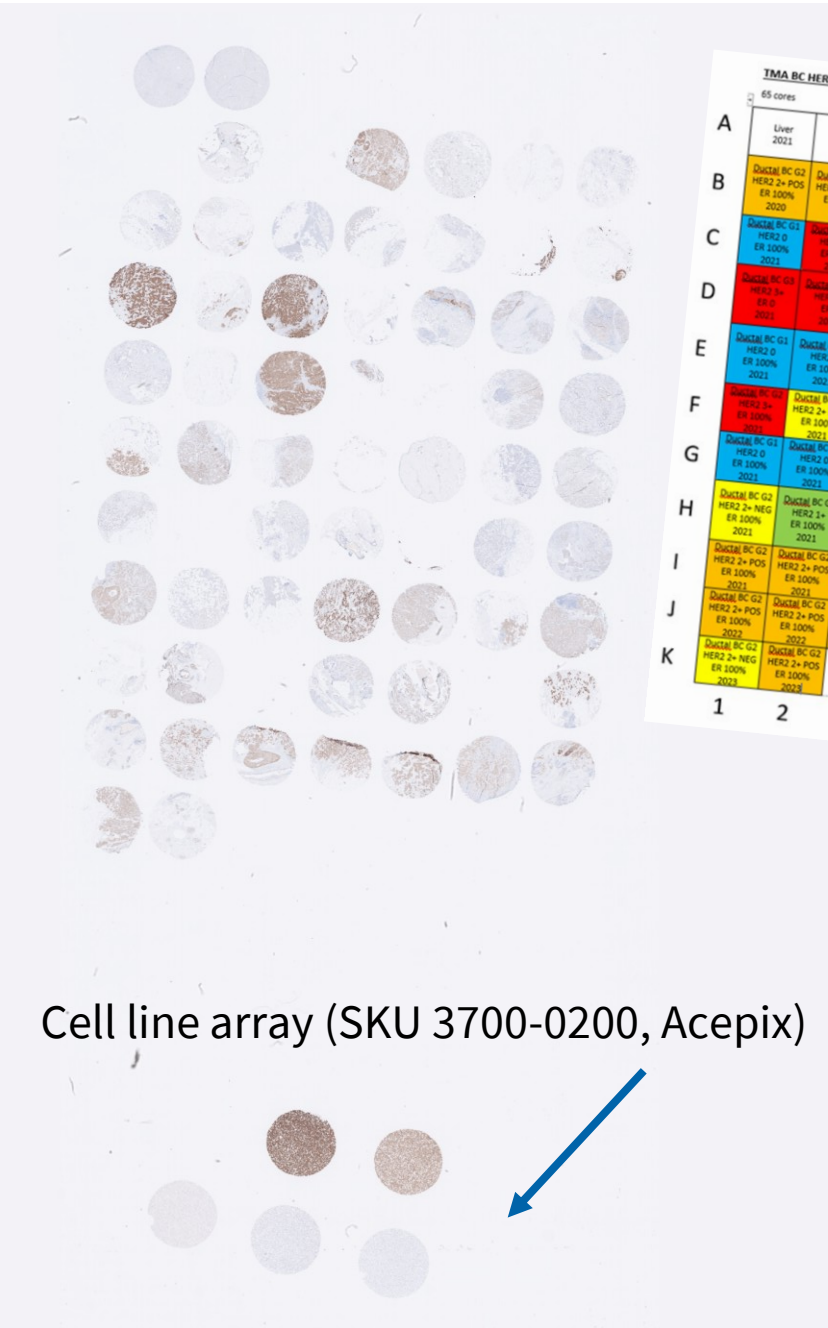
HR+/HER2-negative mBC^{2,a}



Acepix HER2 Array

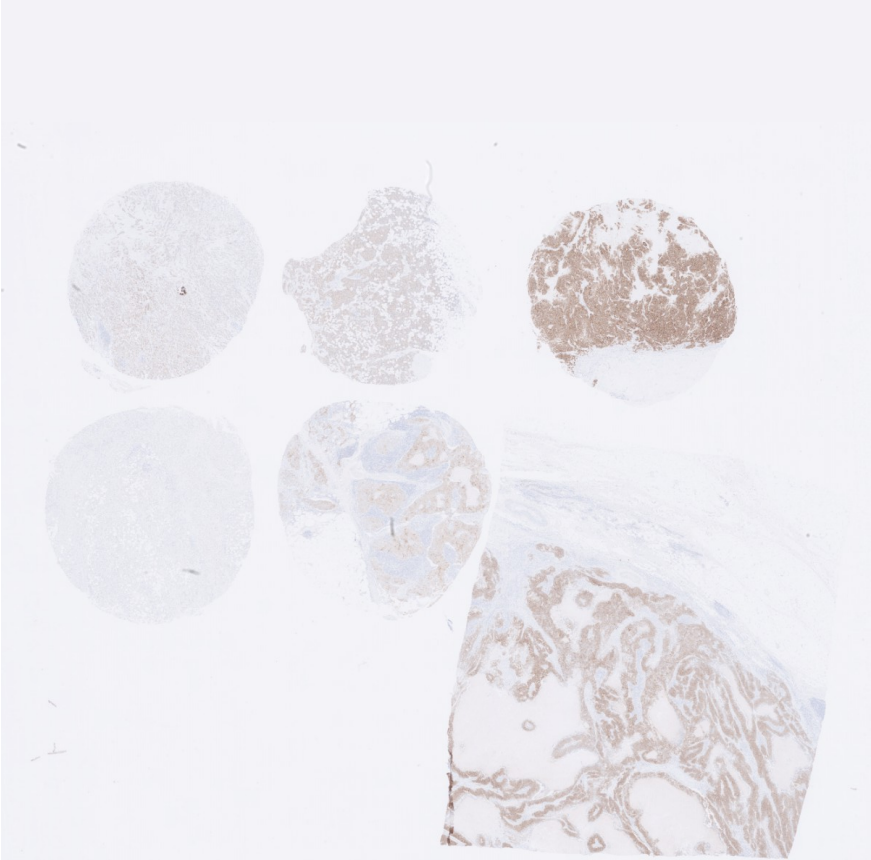
HER2 Ultra low, Low, Classical Pos

Score 0	Score 1+	Score 2+	Score 3+	
No staining	Incomplete/faint staining in ≤10% tumor cells	Incomplete/faint staining in >10% tumor cells	Weak/moderate complete in >10% tumor cells OR Intense membrane staining ≤10% tumor cells	Complete intense staining in >10% tumor cells
Zero	Ultra low	Low	Equivocal	Positive
			ISH	



TMA BC HER2 0-3+ ductal carcinoma (non-TNBC)
65 cores

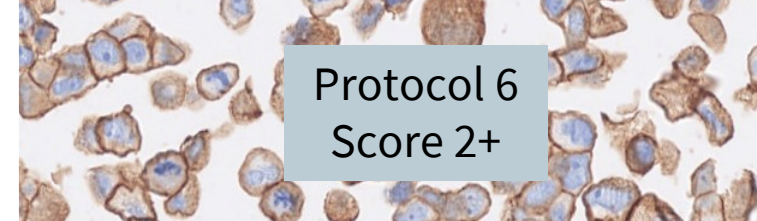
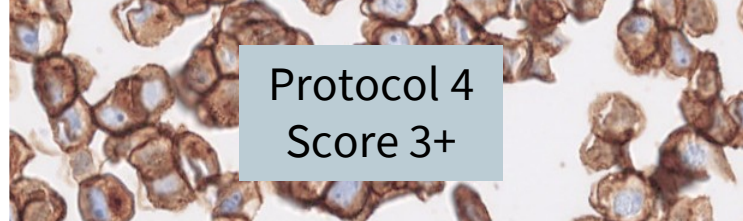
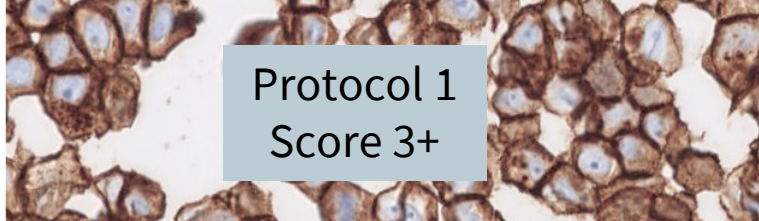
A	Liver 2021	Liver 2021					
B	Ductal BC G2 HER2 2+ POS ER 100% 2020	Ductal BC G2 HER2 2+ POS ER 100% 2020	Ductal BC G3 HER2 2+ POS ER <1% 2020	Ductal BC G3 HER2 2+ POS ER 100% 2020	Ductal BC G1 Luminal A HER2 1+ ER 100% 2021	Ductal BC G2 Luminal B HER2 0 ER 100% 2021	Ductal BC G2 HER2 0 ER 100% 2021
C	Ductal BC G1 HER2 0 ER 100% 2021	Ductal BC G1 HER2 3+ ER 15% 2021	Ductal BC G3 HER2 0 ER 100% 2021	Ductal BC G3 HER2 0 ER 100% 2021	Ductal BC G3 HER2 0 ER 100% 2021	Ductal BC G3 HER2 3+ ER 40% 2021	Ductal BC G3 HER2 3+ ER 100% 2021
D	Ductal BC G3 HER2 3+ ER 0 2021	Ductal BC G2 HER2 3+ ER 0 2021	Ductal BC G3 HER2 3+ ER 100% 2021	Ductal BC G1 HER2 3+ ER 100% 2021	Ductal BC G3 HER2 3+ ER 1% 2021	Ductal BC G3 Luminal B HER2 1+ ER 100% 2021	Ductal BC G3 HER2 1+ ER 100% 2021
E	Ductal BC G1 HER2 0 ER 100% 2021	Ductal BC G1 HER2 0 ER 100% 2021	Ductal BC G3 HER2 3+ ER 100% 2021	Ductal BC G3 Luminal B HER2 2+ NEG ER 100% 2021	Ductal BC G1 HER2 1+ ER 100% 2021	Ductal BC G2 Luminal B HER2 1+ ER 100% 2021	Ductal BC G2 HER2 1+ ER 100% 2021
F	Ductal BC G3 HER2 3+ ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G3 HER2 0 ER 100% 2021	Ductal BC G2 HER2 1+ ER 100% 2021	Ductal BC G3 HER2 0 ER 100% 2021	Ductal BC G3 HER2 1+ ER 100% 2021	Ductal BC G2 HER2 1+ ER 100% 2021
G	Ductal BC G1 HER2 0 ER 100% 2021	Ductal BC G1 HER2 0 ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 90-100% 2021	Ductal BC G3 HER2 2+ NEG ER 100% 2021	Ductal BC G3 HER2 3+ ER 100% 2021	Ductal BC G2 HER2 1+ ER 100% 2021	Ductal BC G3 HER2 2+ ER 100% 2021
H	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 1+ ER 100% 2021	Ductal BC G3 Luminal B HER2 1+ ER 75% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021
I	Ductal BC G2 HER2 2+ POS ER 100% 2021	Ductal BC G2 HER2 2+ POS ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G1 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021	Ductal BC G2 HER2 2+ NEG ER 100% 2021
J	Ductal BC G2 HER2 2+ POS ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 95% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022
K	Ductal BC G2 HER2 2+ NEG ER 100% 2022	Ductal BC G2 HER2 2+ POS ER 100% 2022					
	1	2	3	4	5	6	7



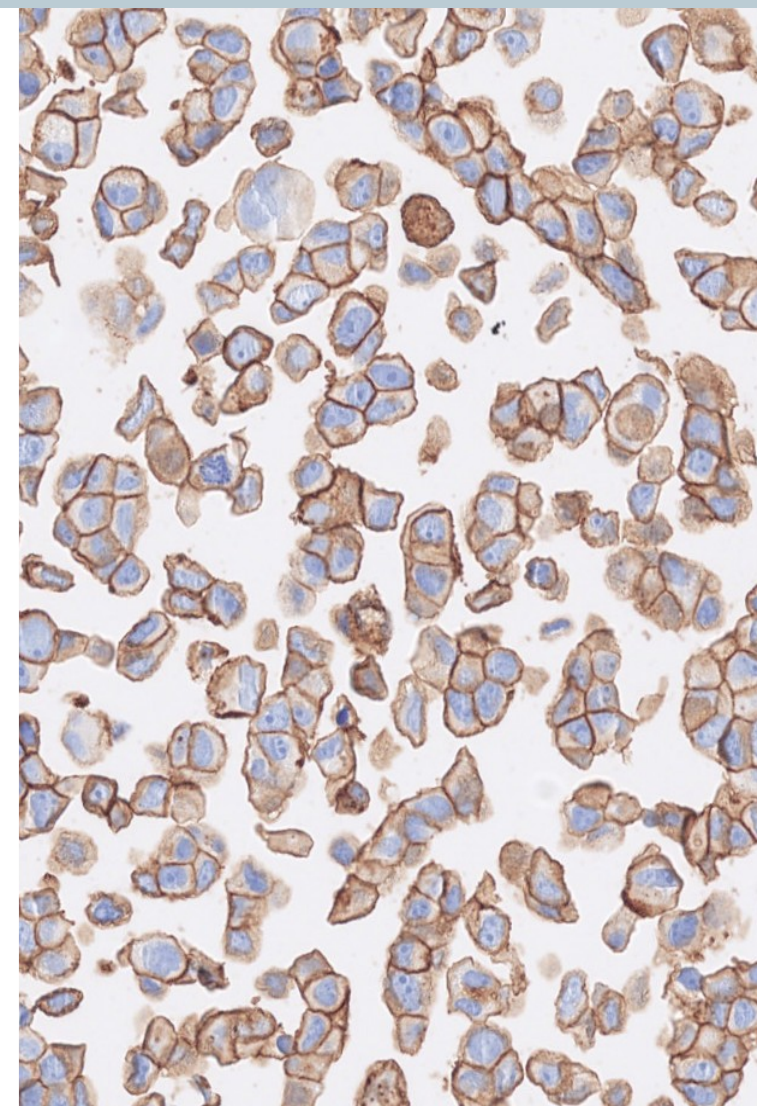
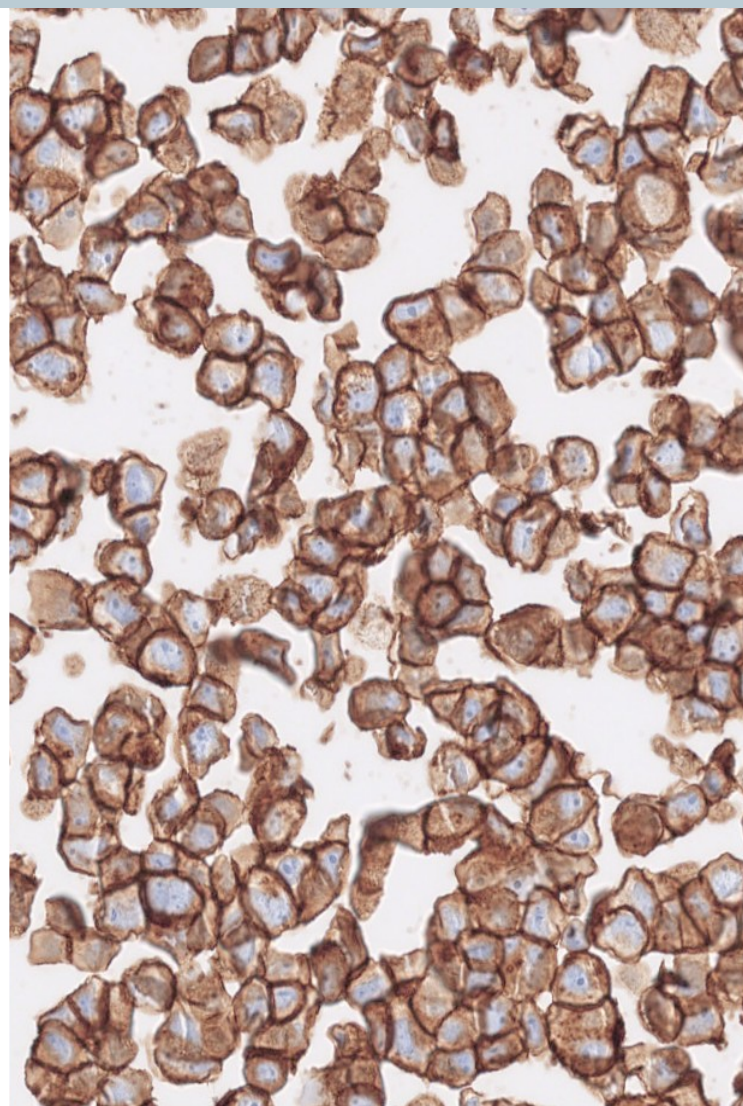
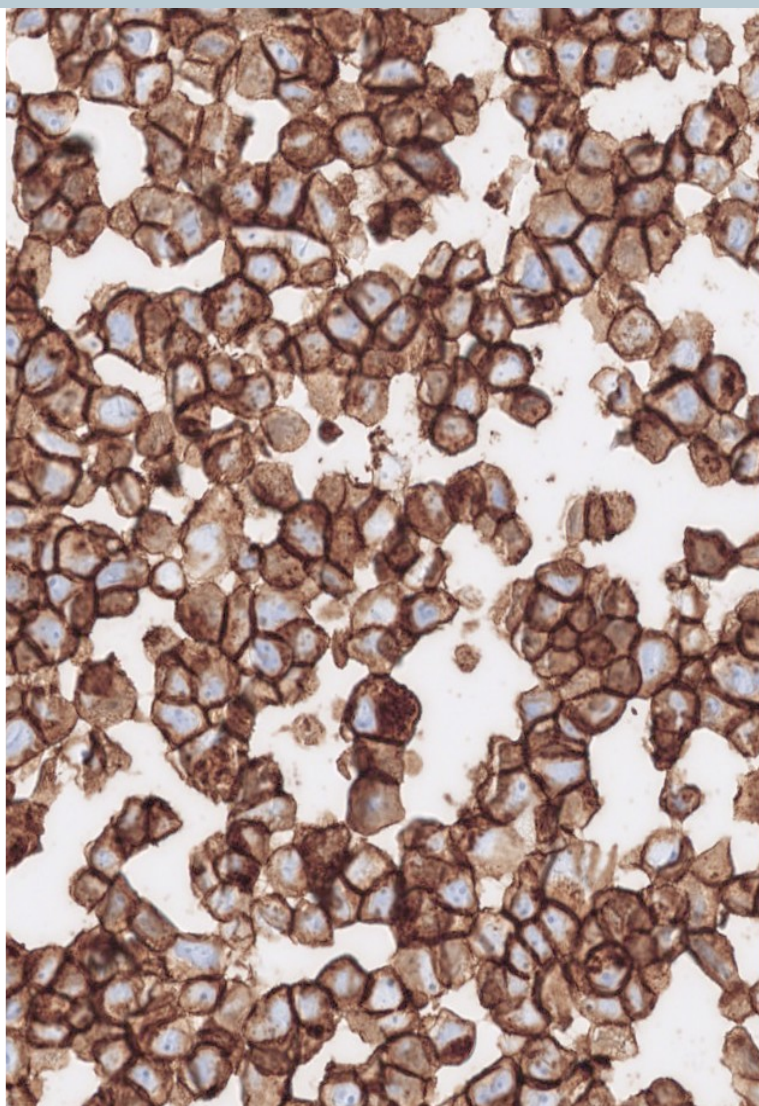
Cell line array (SKU 3700-0200, Acepixon)

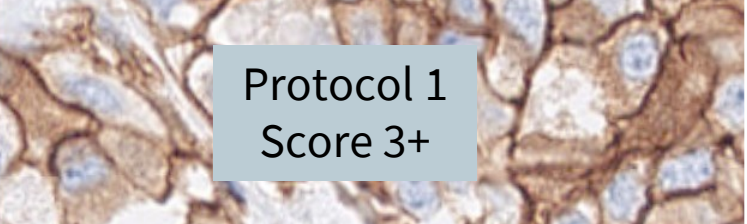


	IHC: HER2 Score* (0, 1+, 2+, 3+)
Breast carc. no. 1	1-2+
Breast carc. no. 2	1-2+
Breast carc. no. 3	3+
Breast carc. no. 4	0
Breast carc. no. 5	1-2+
Breast carc. no. 6	2-3+

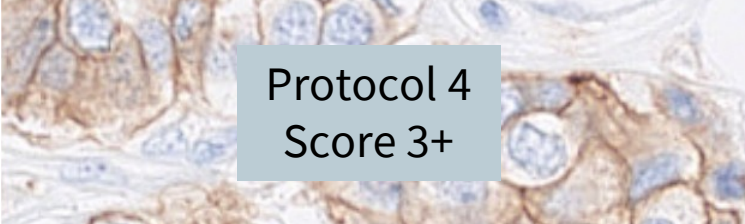


Cell lines **Core 3+**

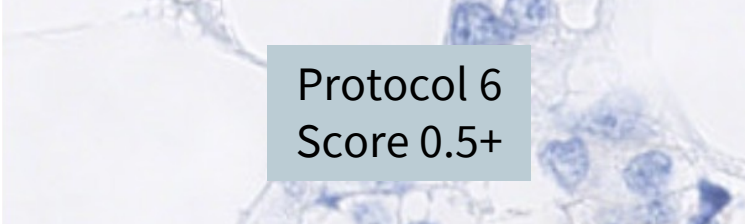




Protocol 1
Score 3+

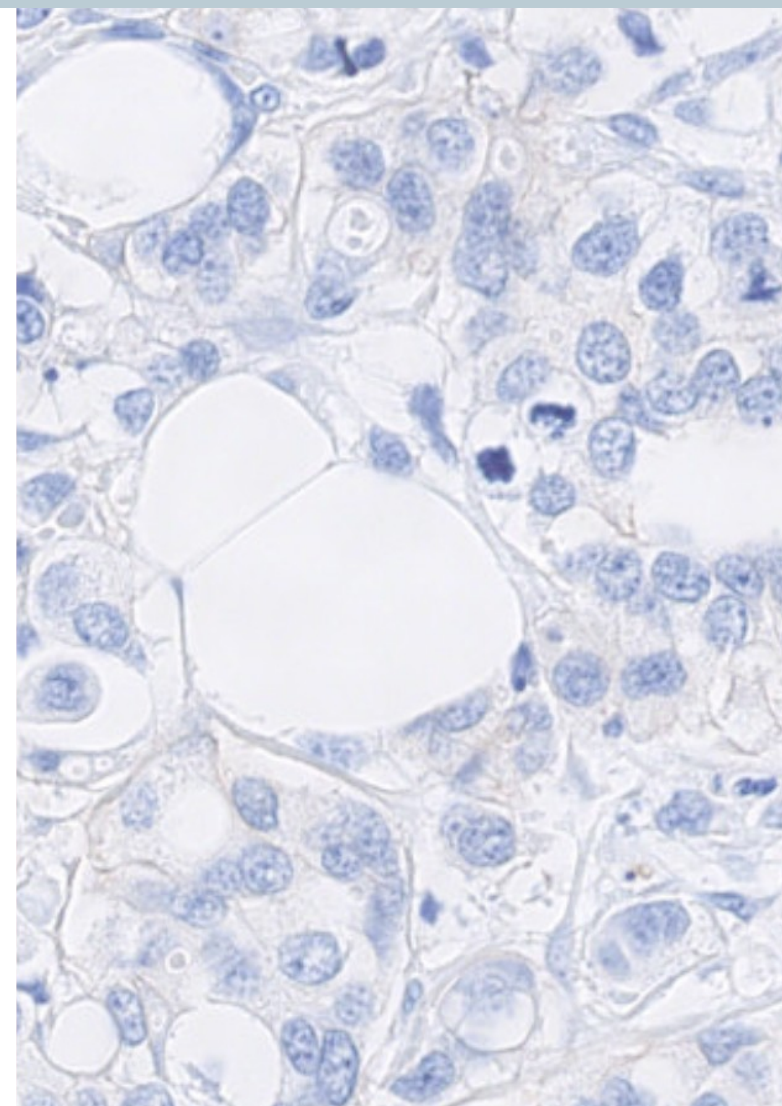
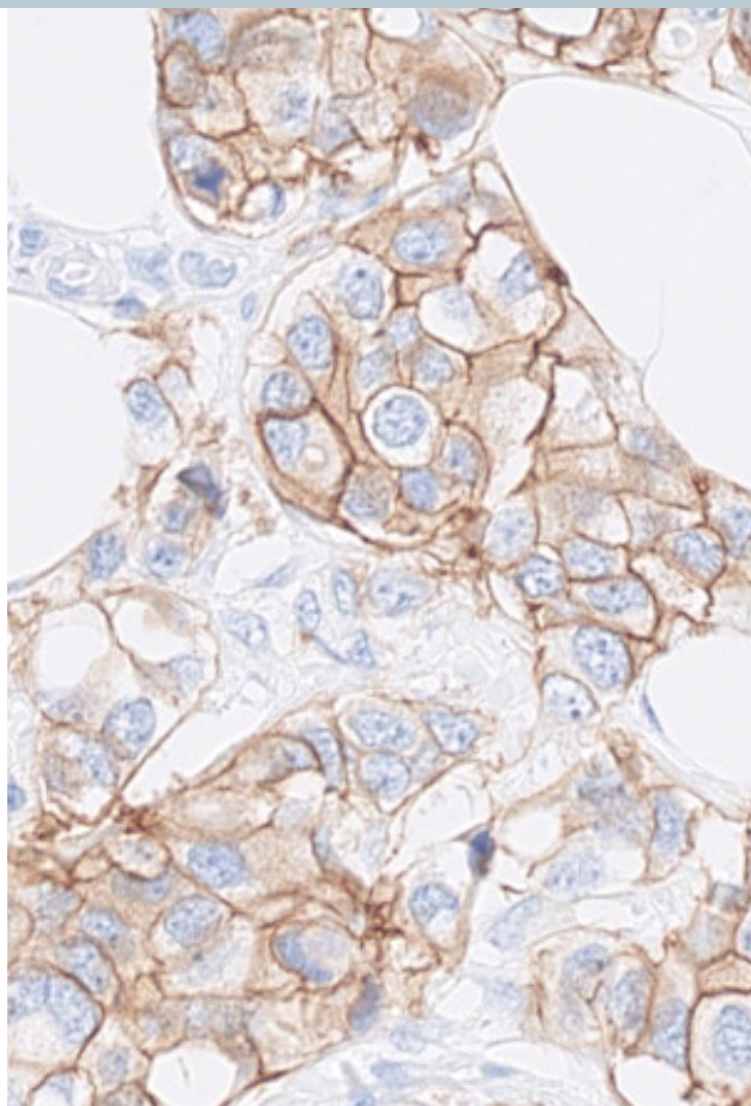
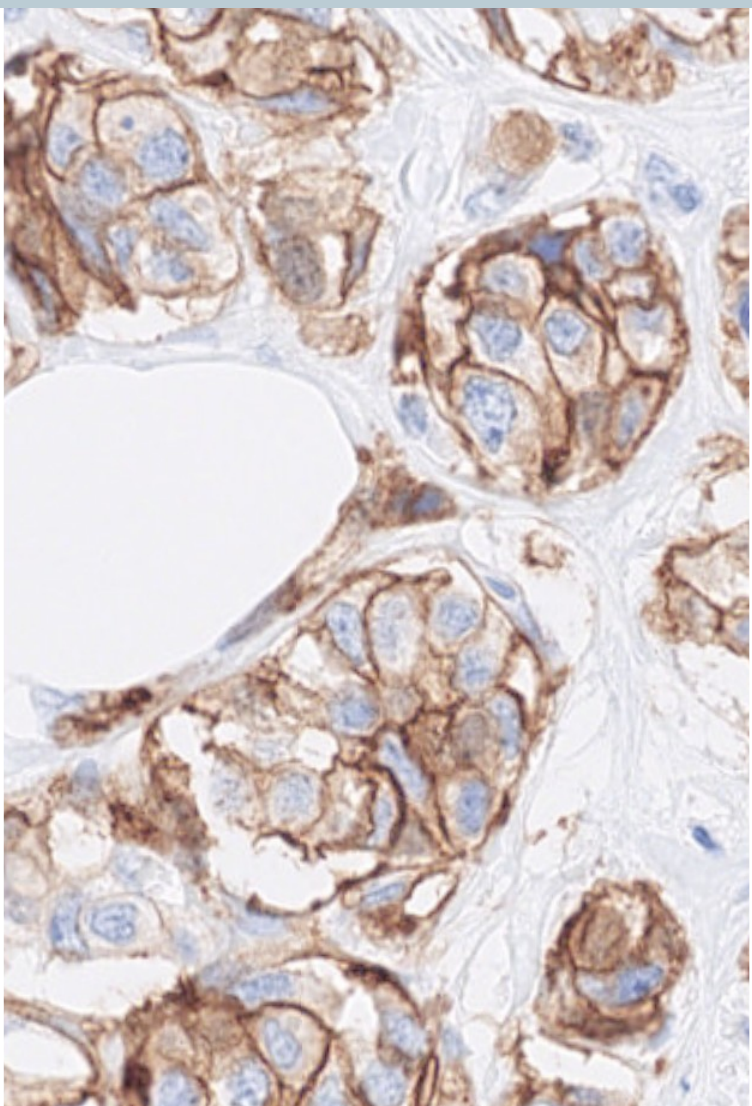


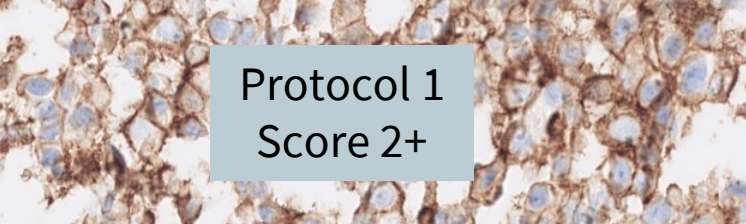
Protocol 4
Score 3+



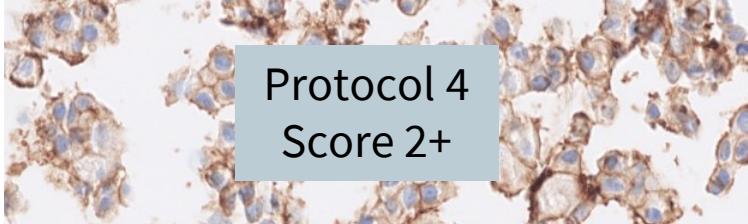
Protocol 6
Score 0.5+

Breast carcinoma **Score 3+**

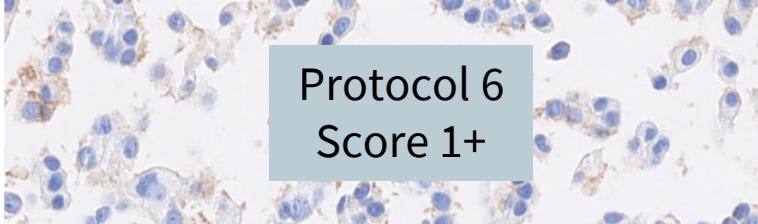




Protocol 1
Score 2+

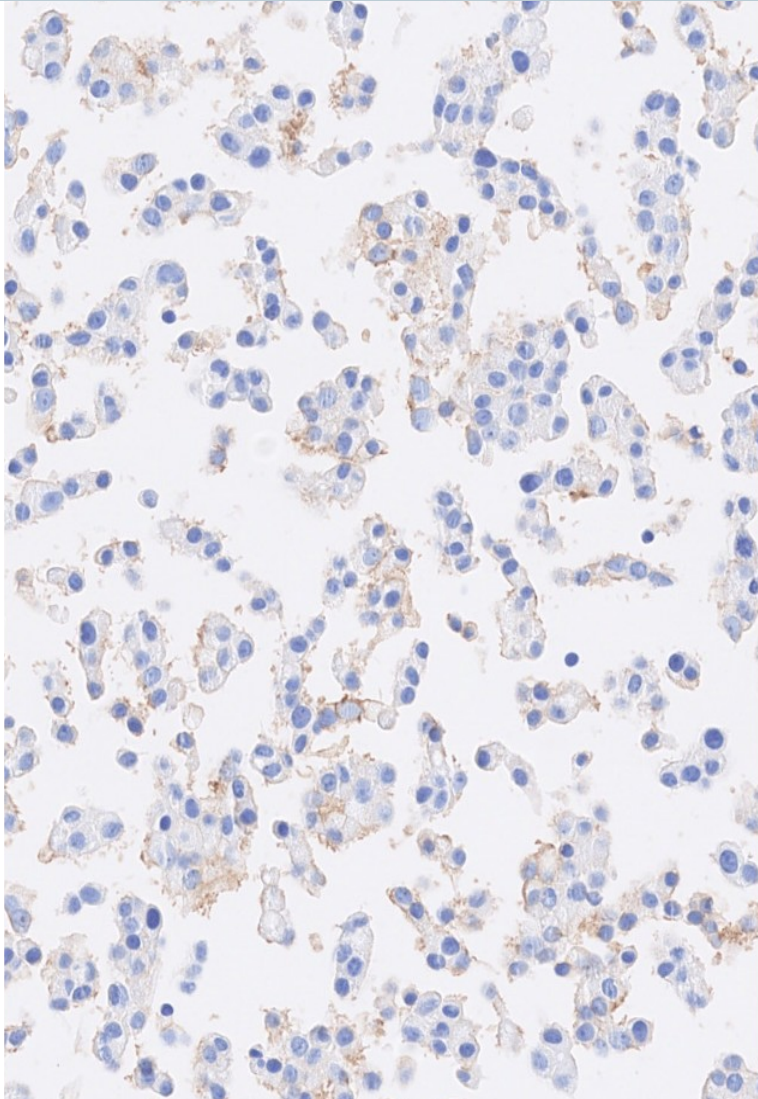
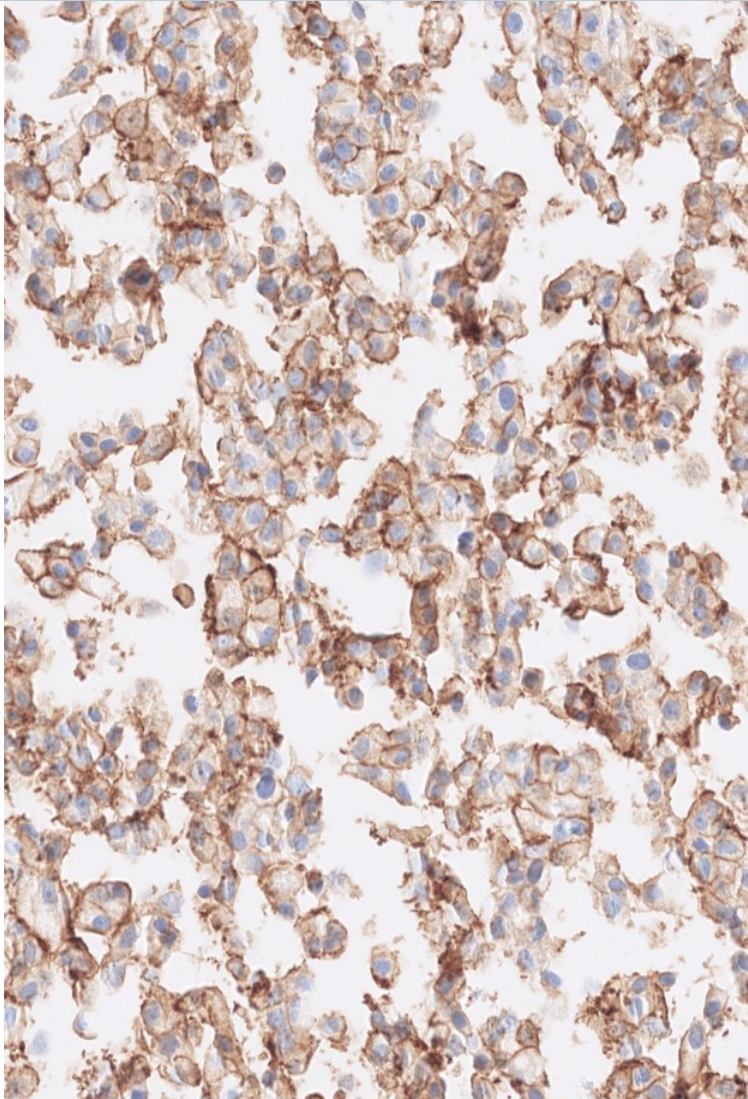
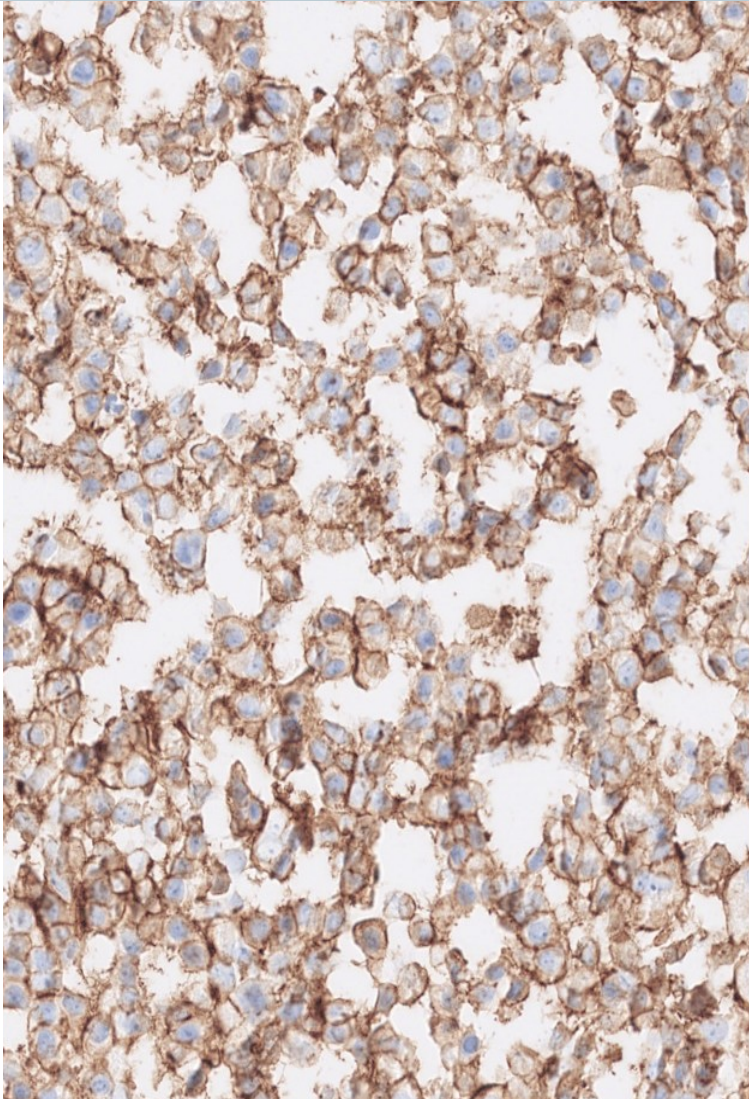


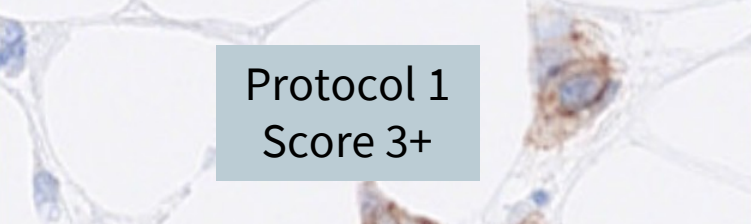
Protocol 4
Score 2+



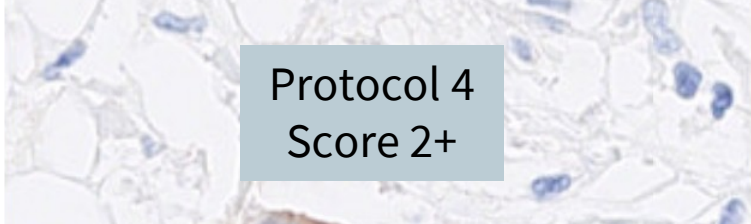
Protocol 6
Score 1+

Cell lines **Core 2+**

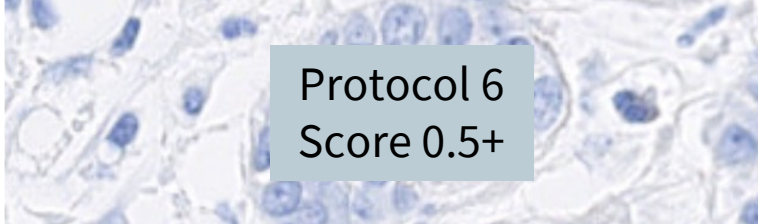




Protocol 1
Score 3+

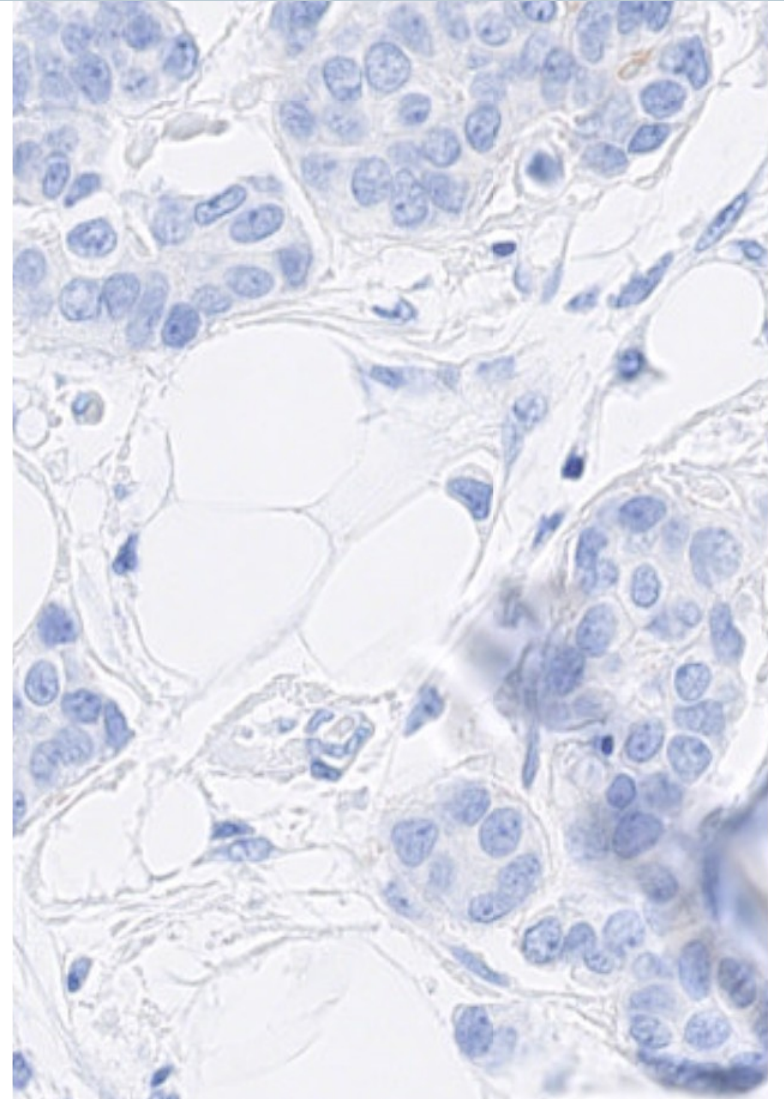
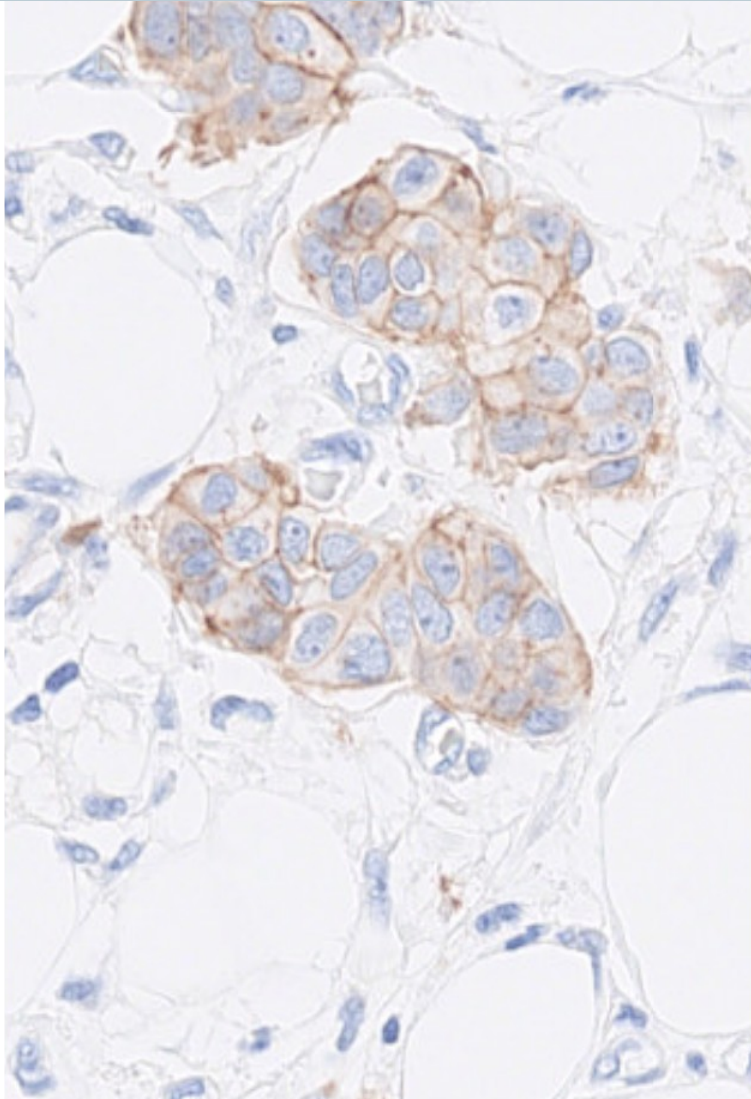
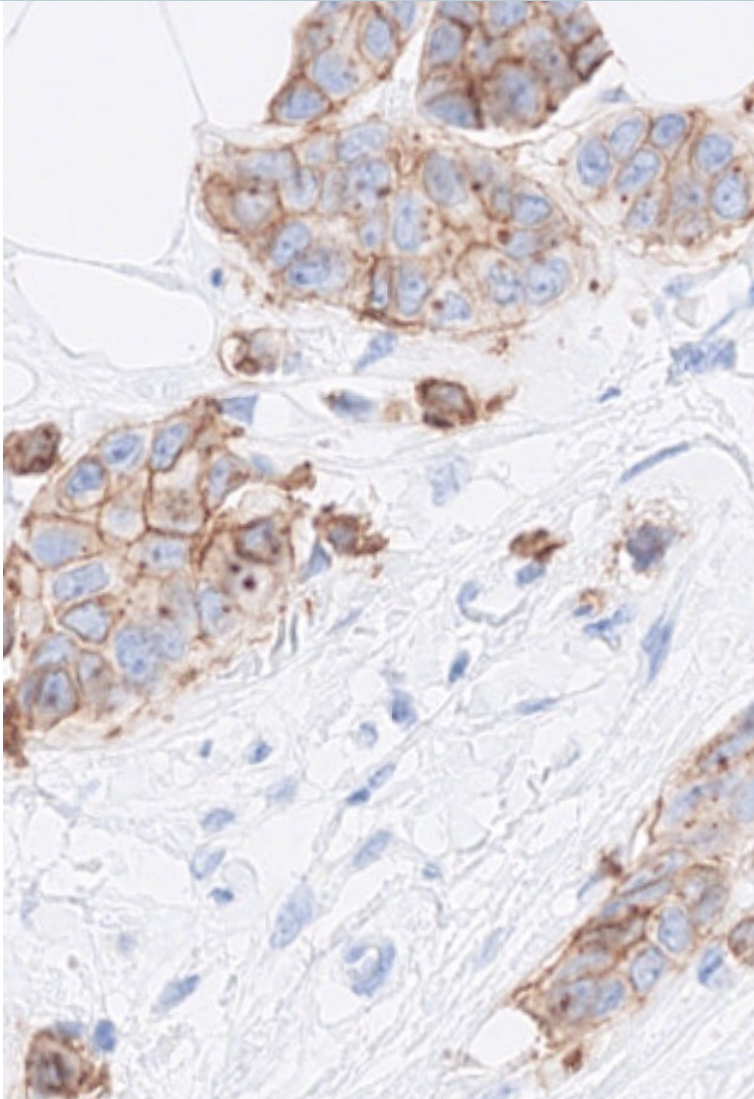


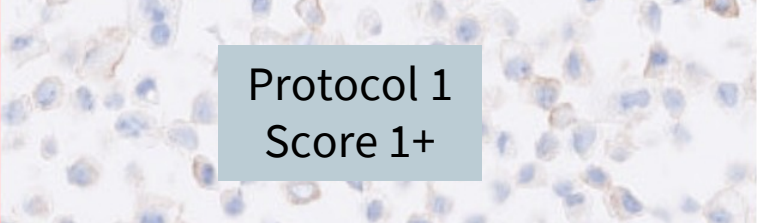
Protocol 4
Score 2+



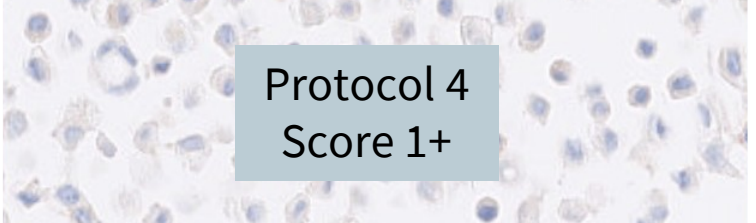
Protocol 6
Score 0.5+

Breast carcinoma **Score 2+**

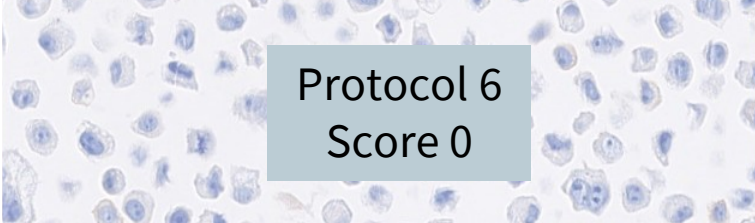




Protocol 1
Score 1+

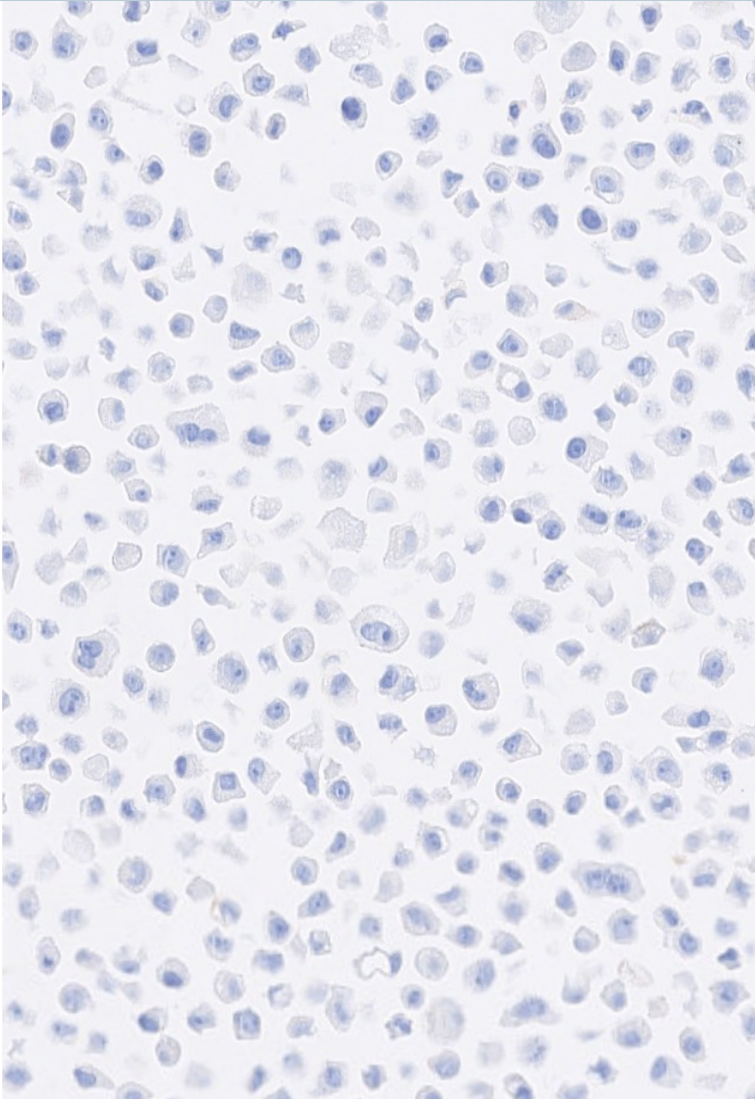
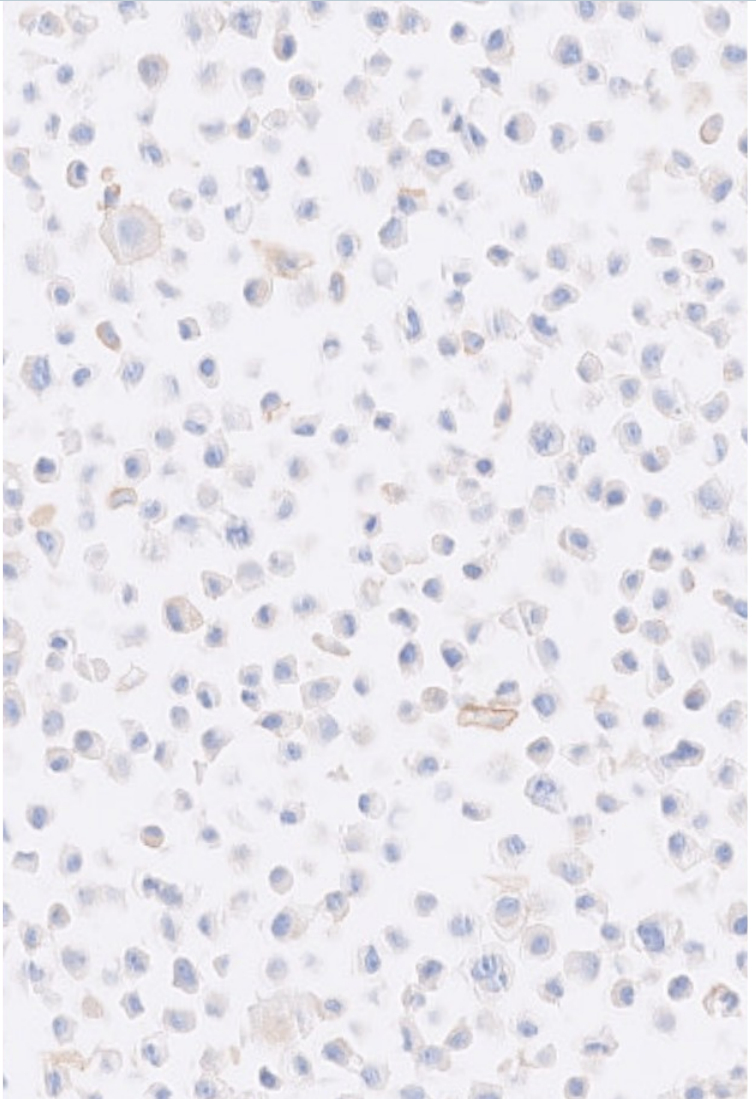
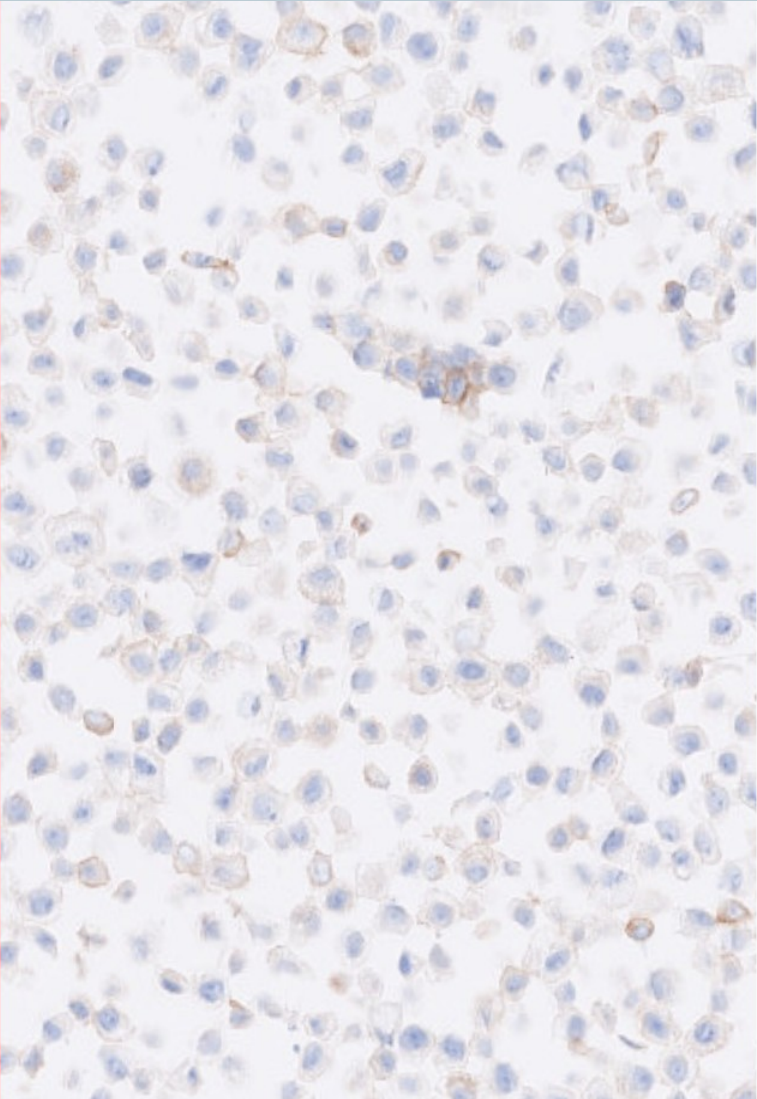


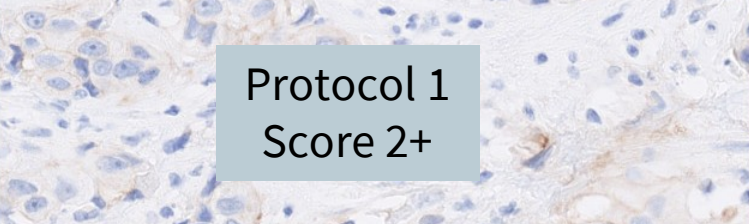
Protocol 4
Score 1+



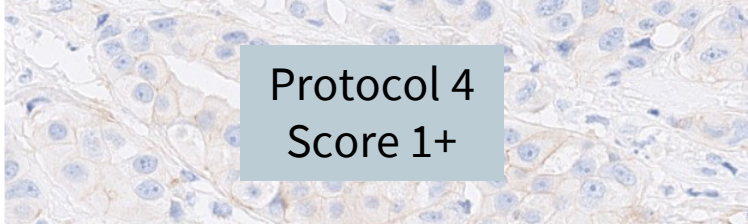
Protocol 6
Score 0

Cell lines **Core 1+**

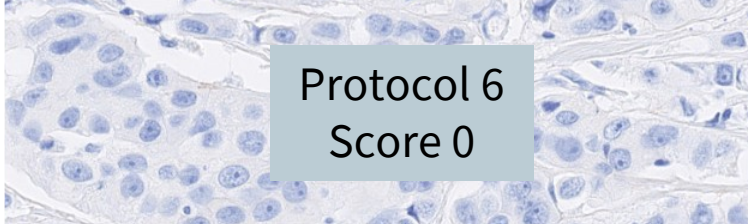




Protocol 1
Score 2+

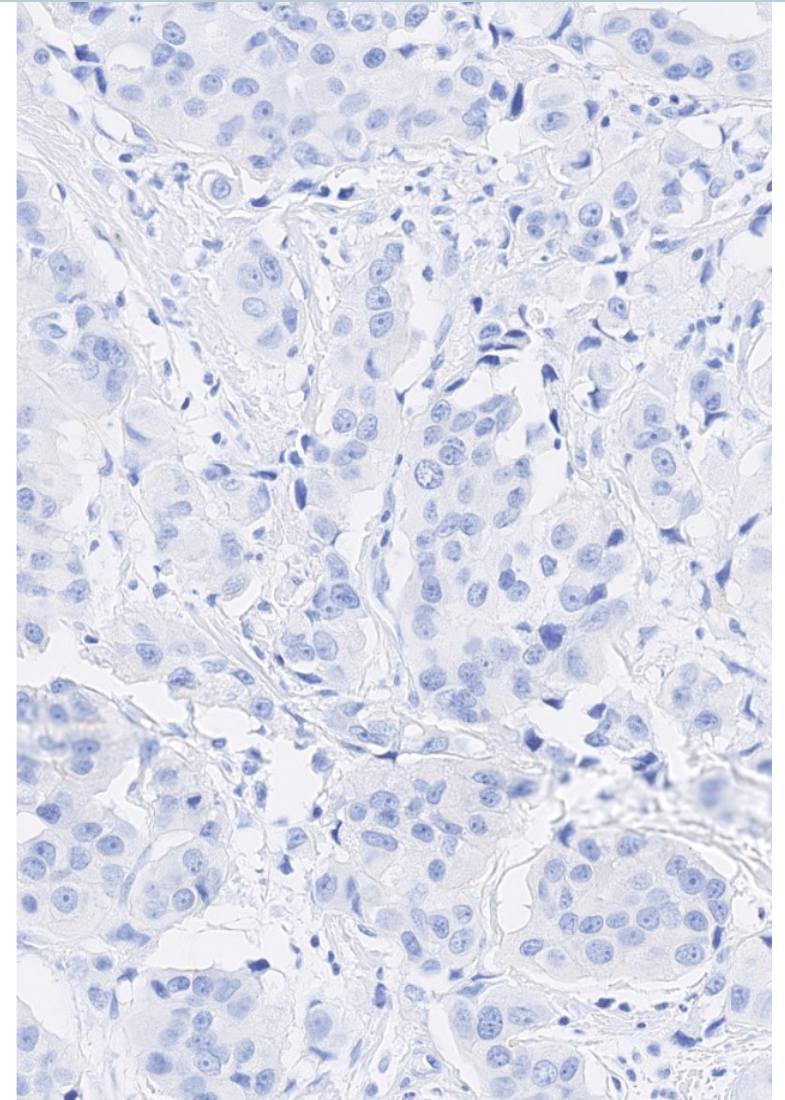
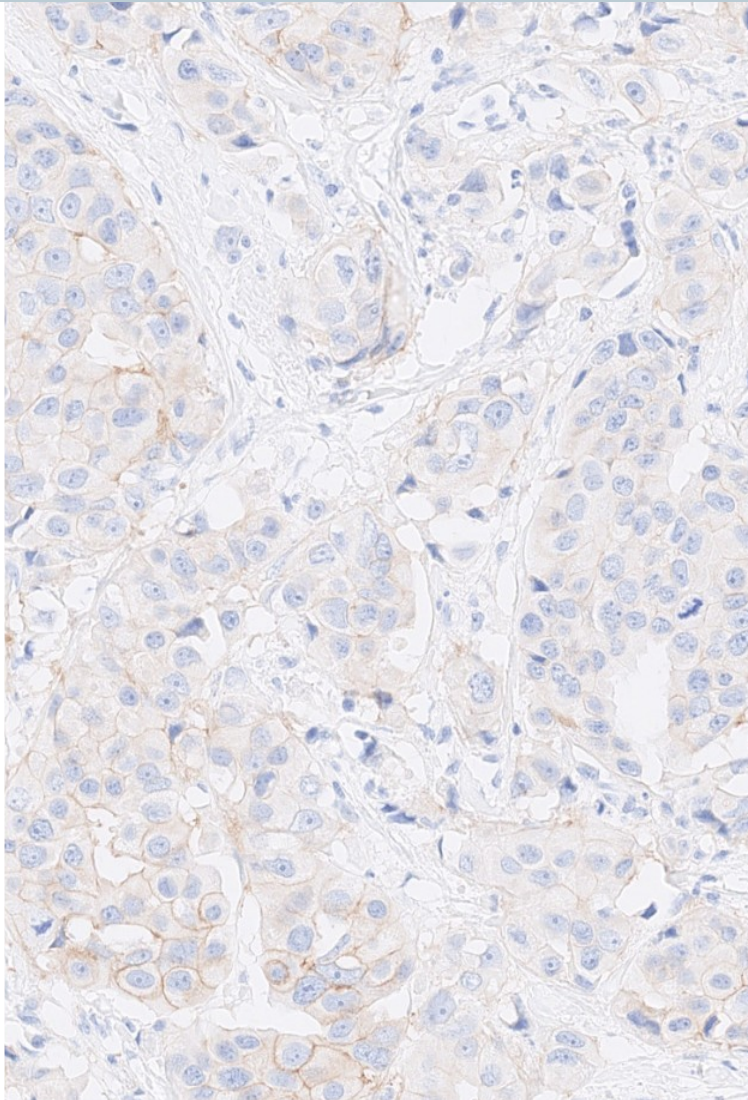
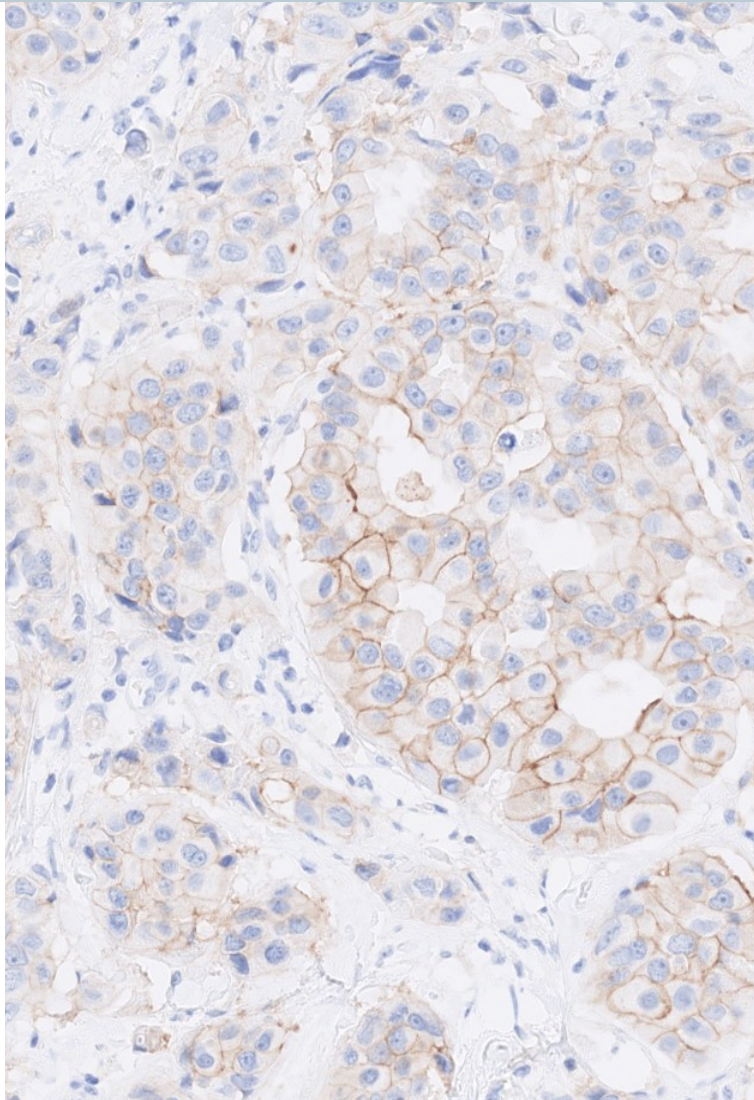


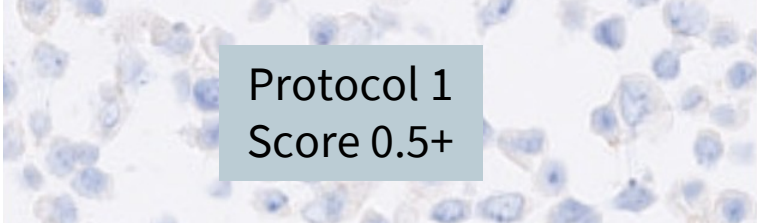
Protocol 4
Score 1+



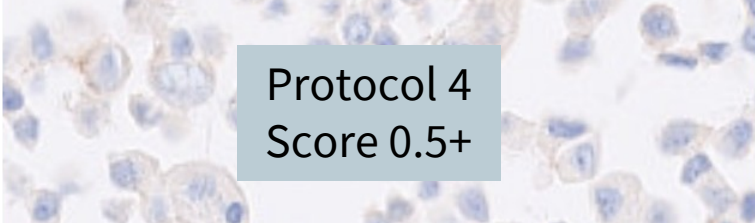
Protocol 6
Score 0

Breast carcinoma **Score 1+**

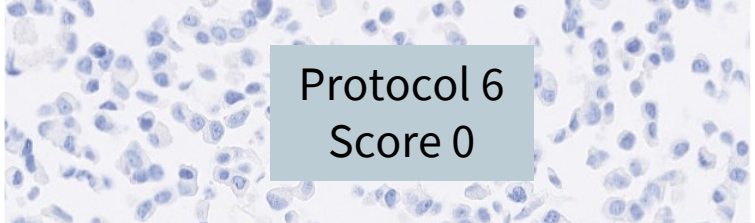




Protocol 1
Score 0.5+

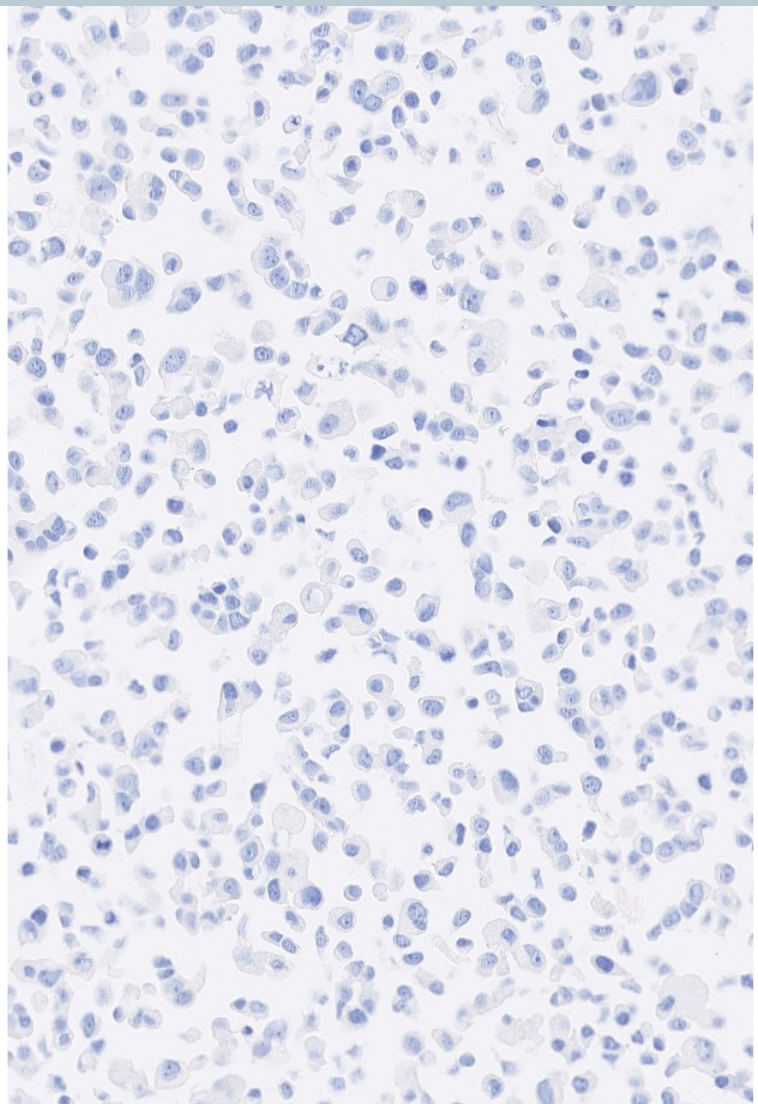
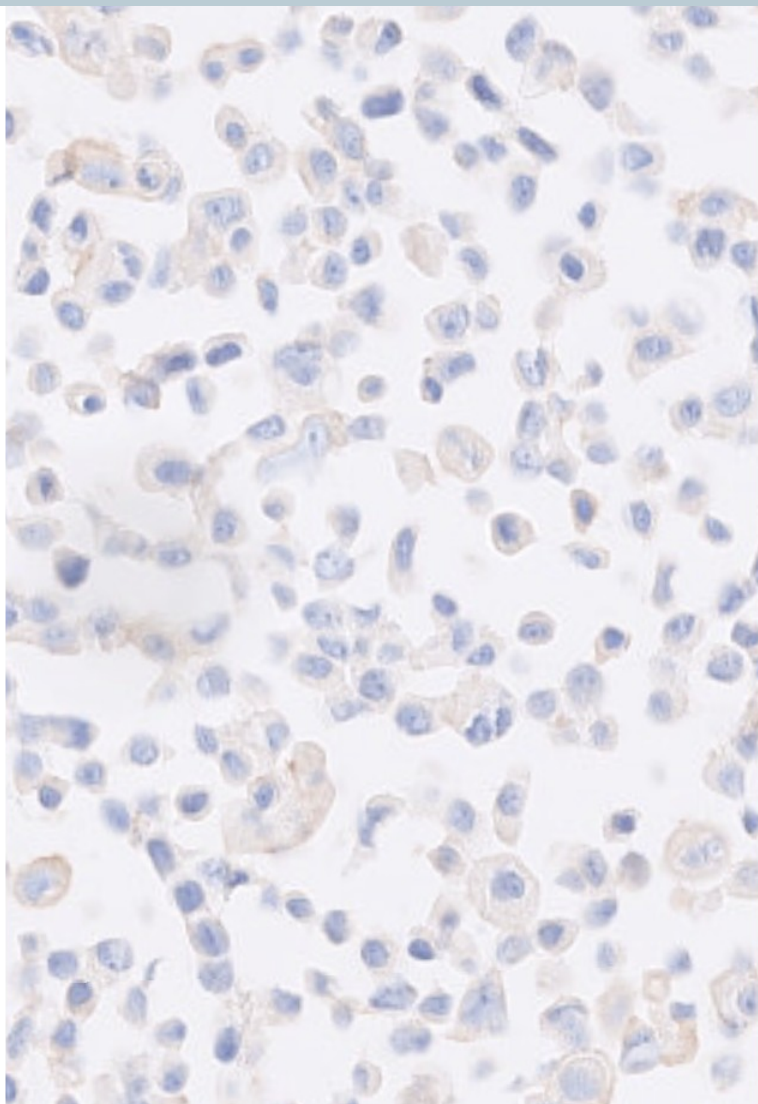
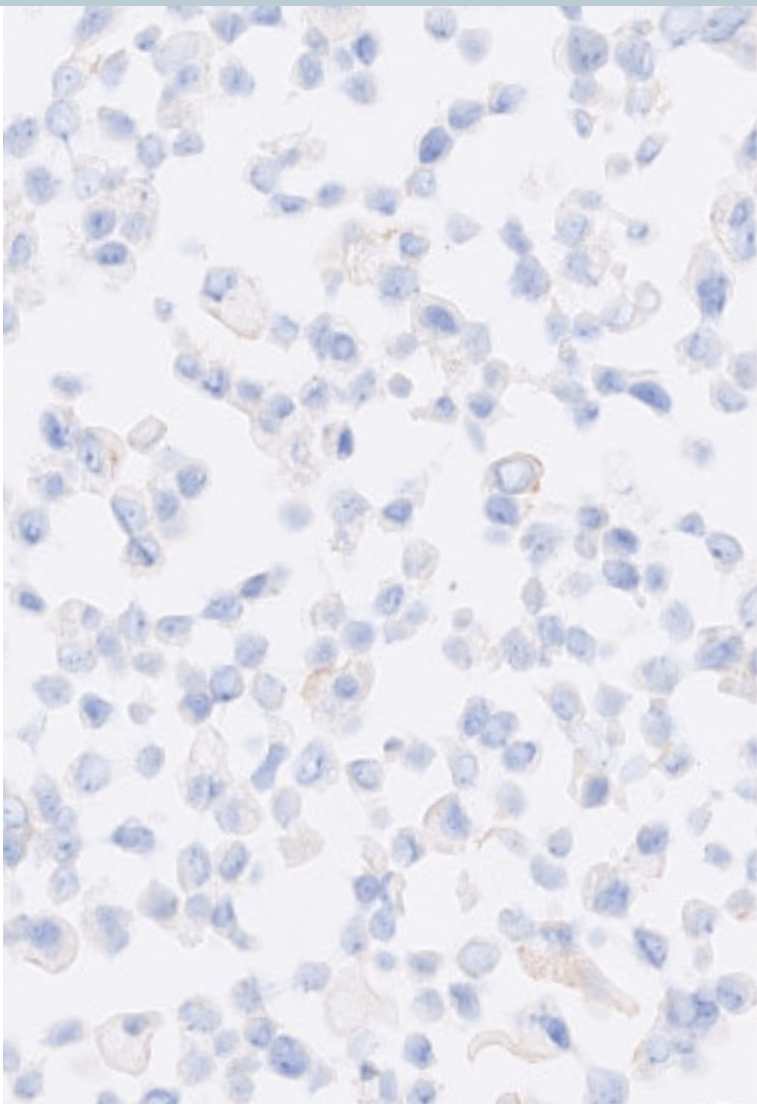


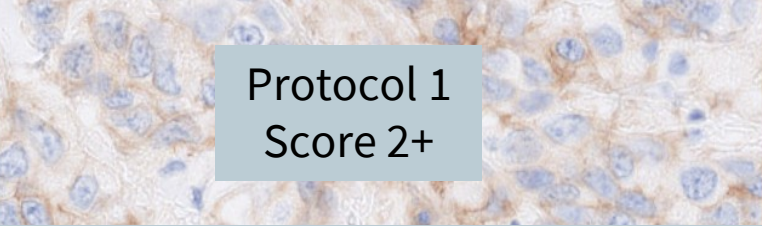
Protocol 4
Score 0.5+



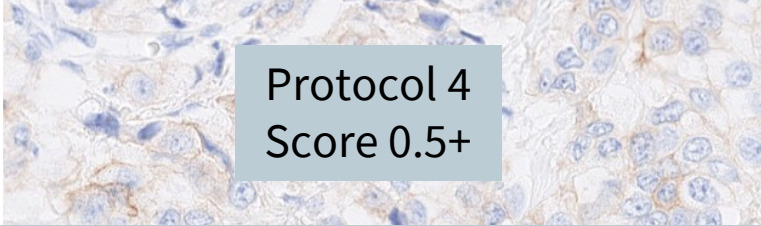
Protocol 6
Score 0

Cell lines **Core 0.5+**

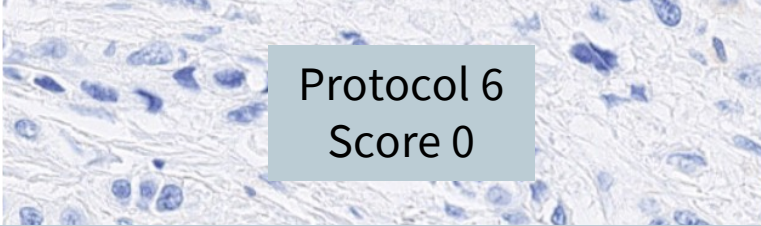




Protocol 1
Score 2+

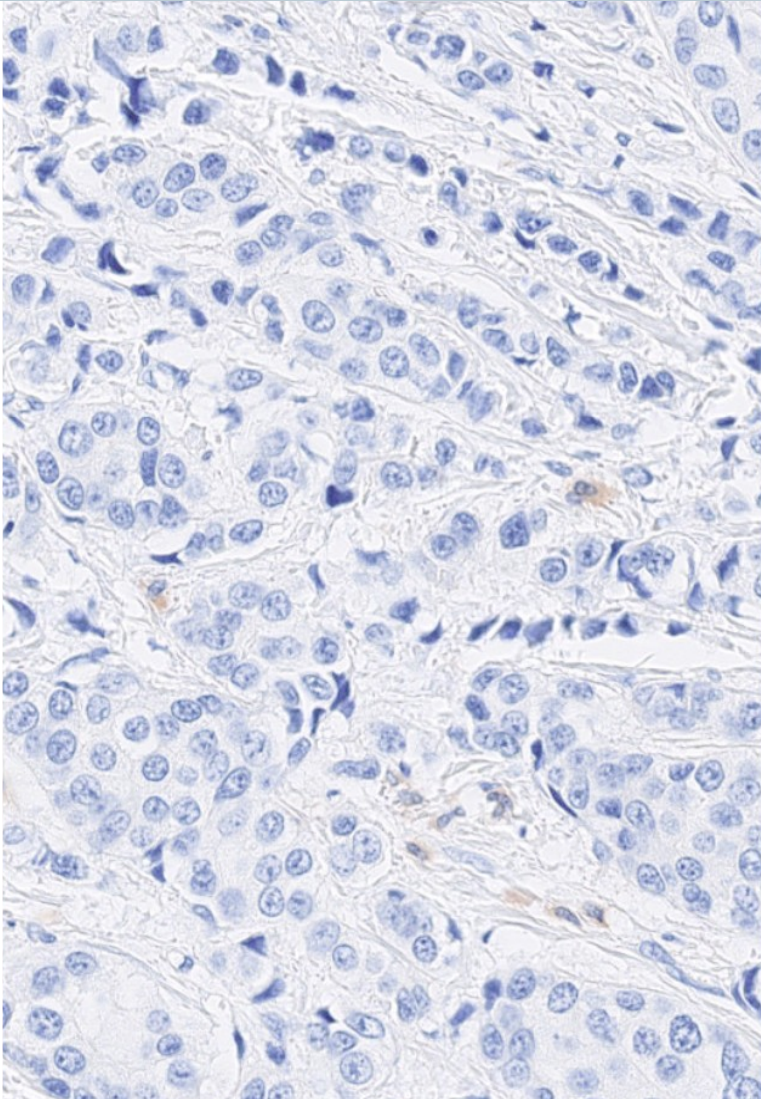
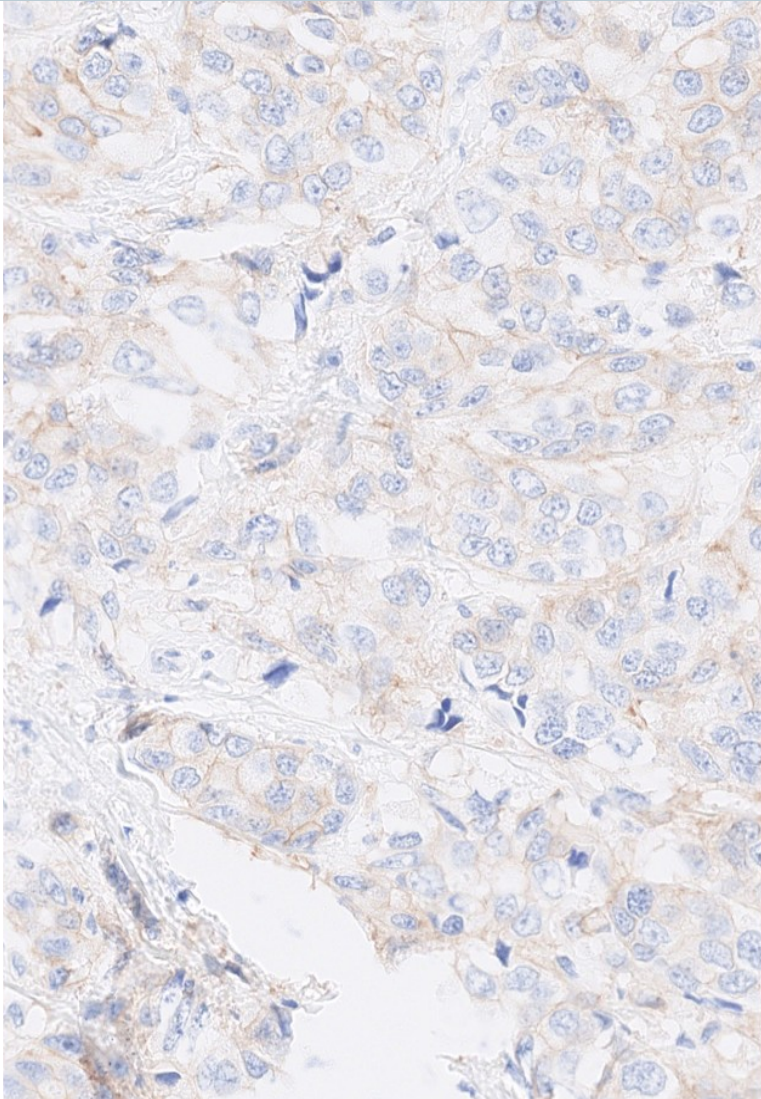
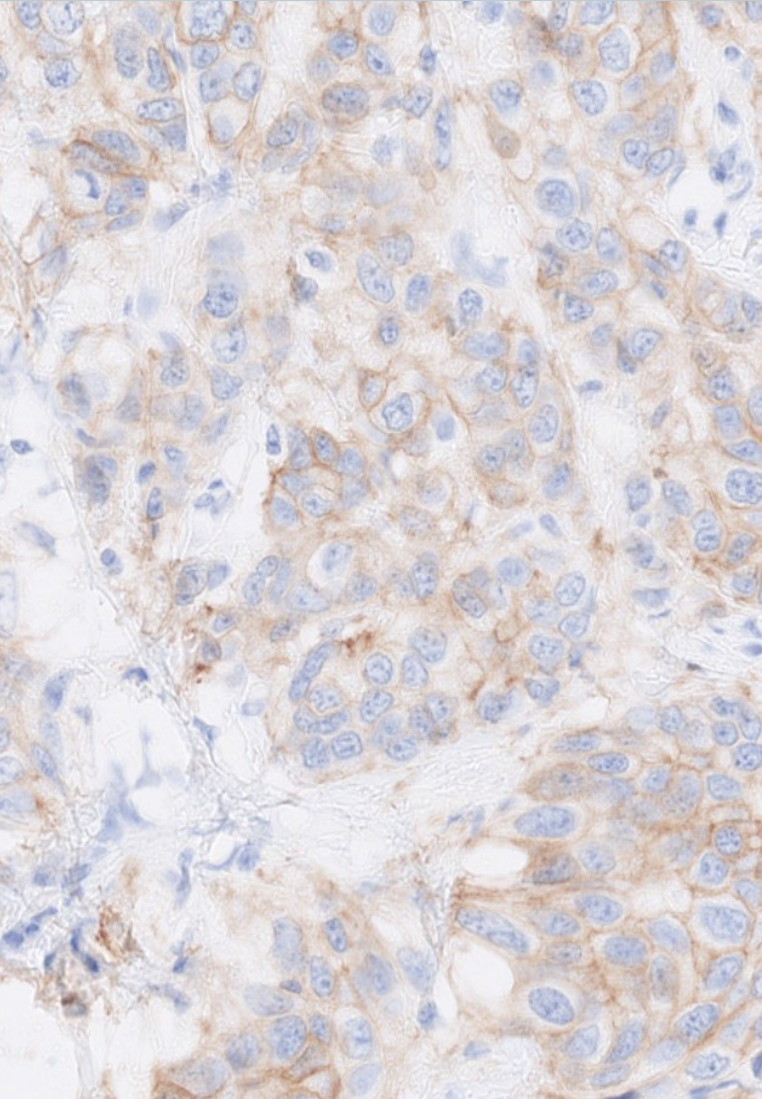


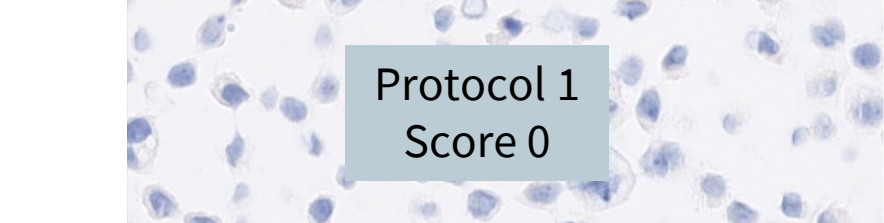
Protocol 4
Score 0.5+



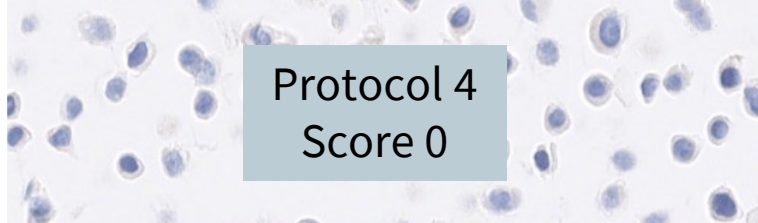
Protocol 6
Score 0

Breast carcinoma **Score 0.5+**

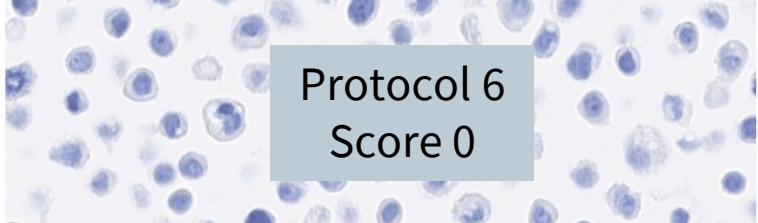




Protocol 1
Score 0

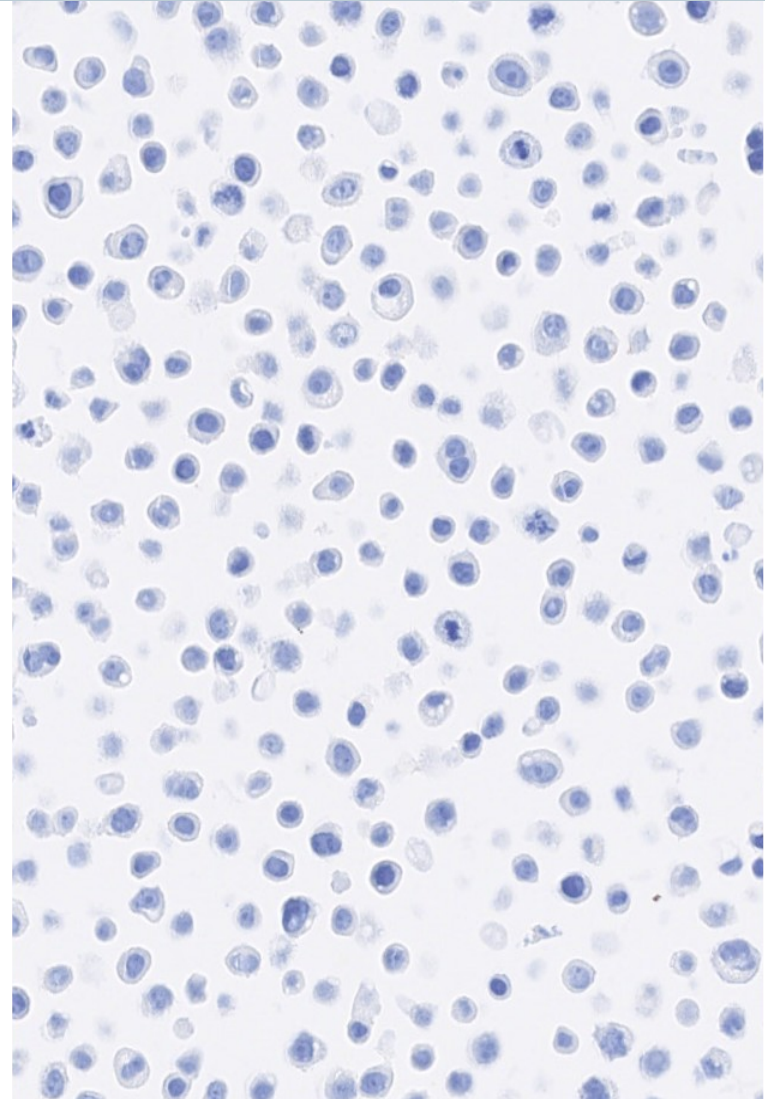
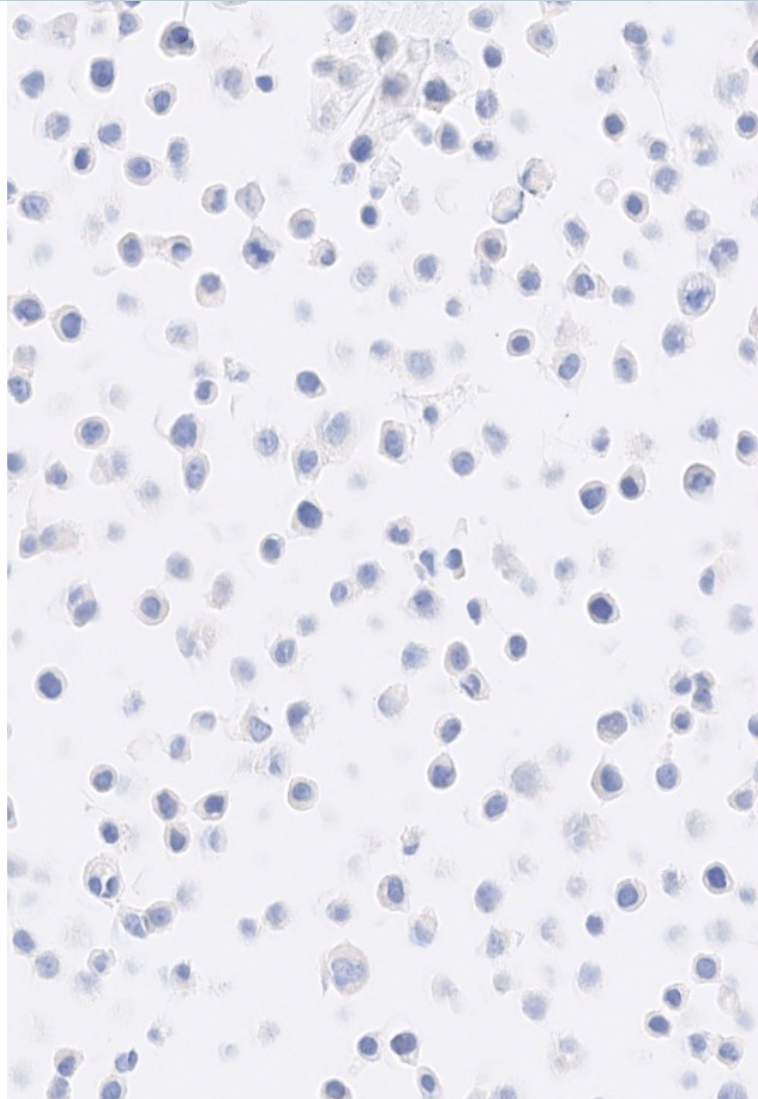
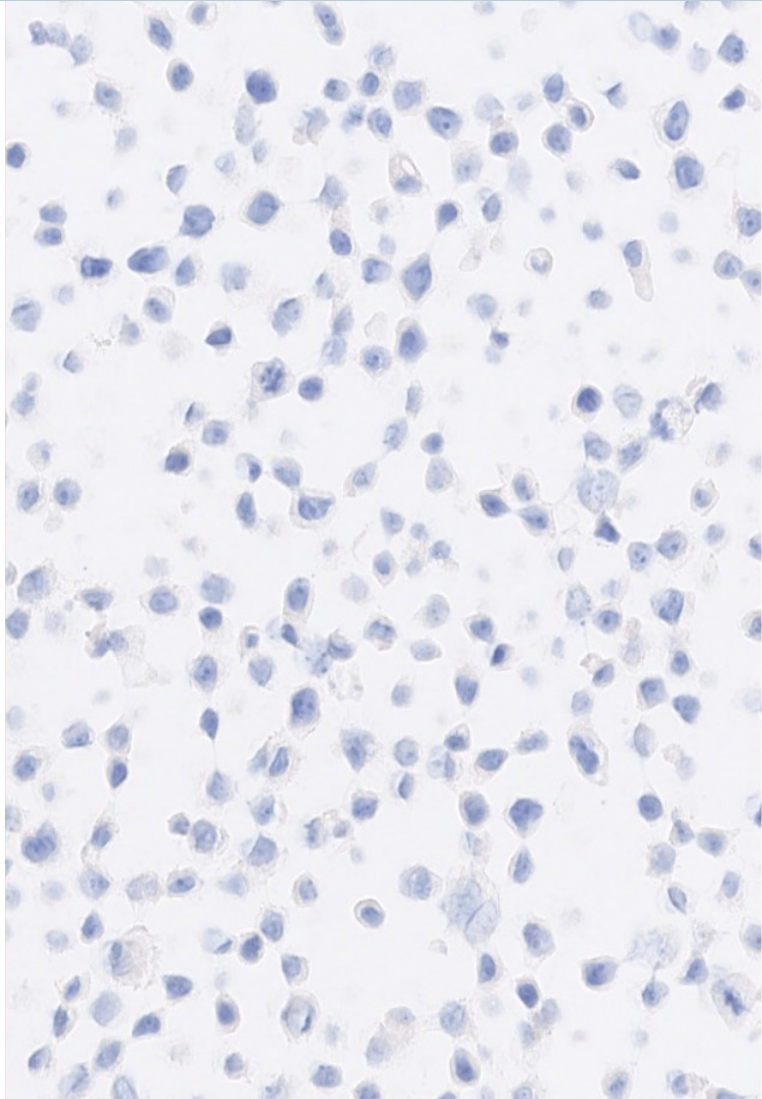


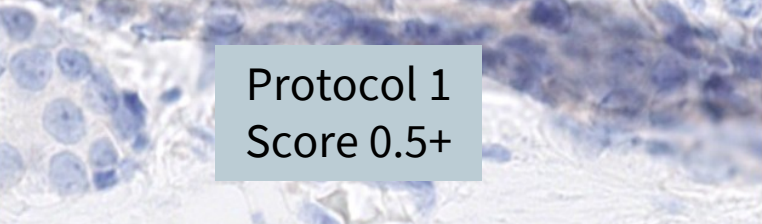
Protocol 4
Score 0



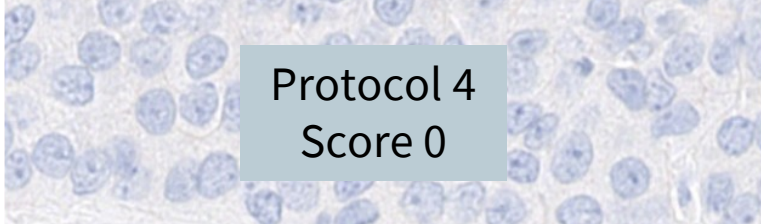
Protocol 6
Score 0

Cell lines **Core 0**

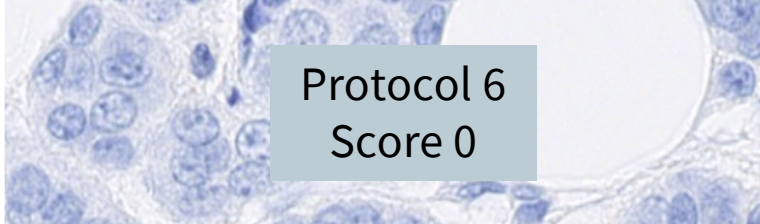




Protocol 1
Score 0.5+

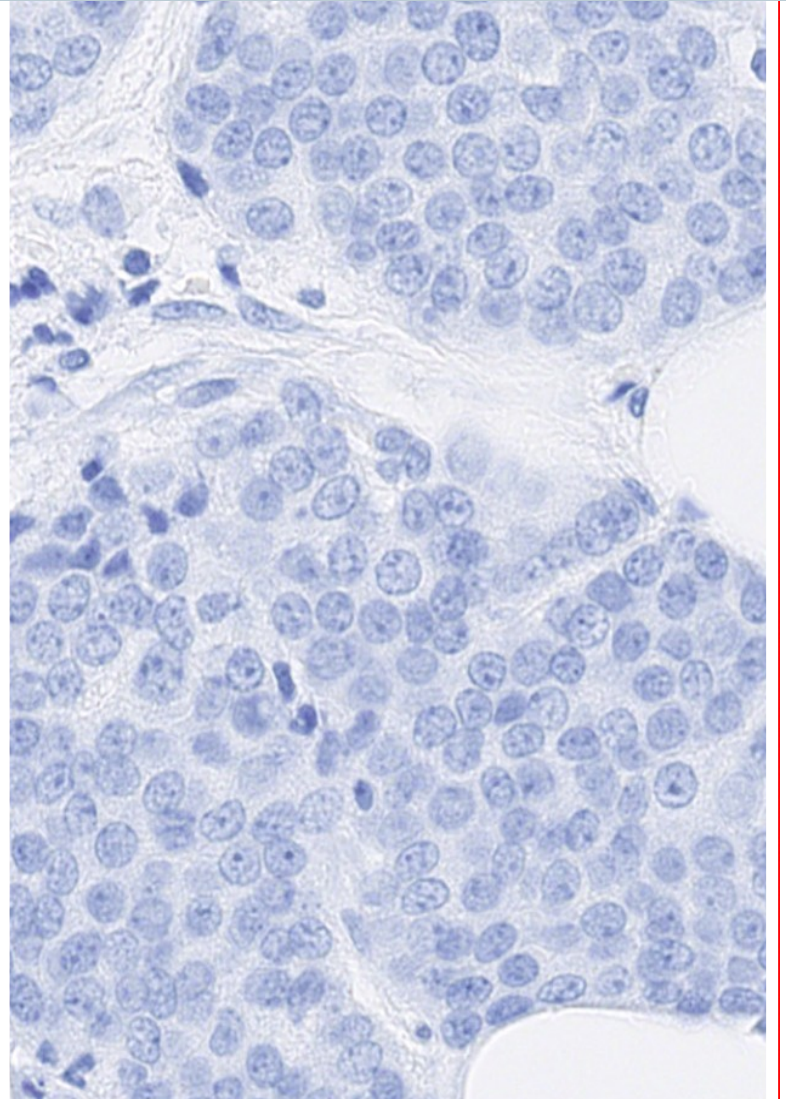
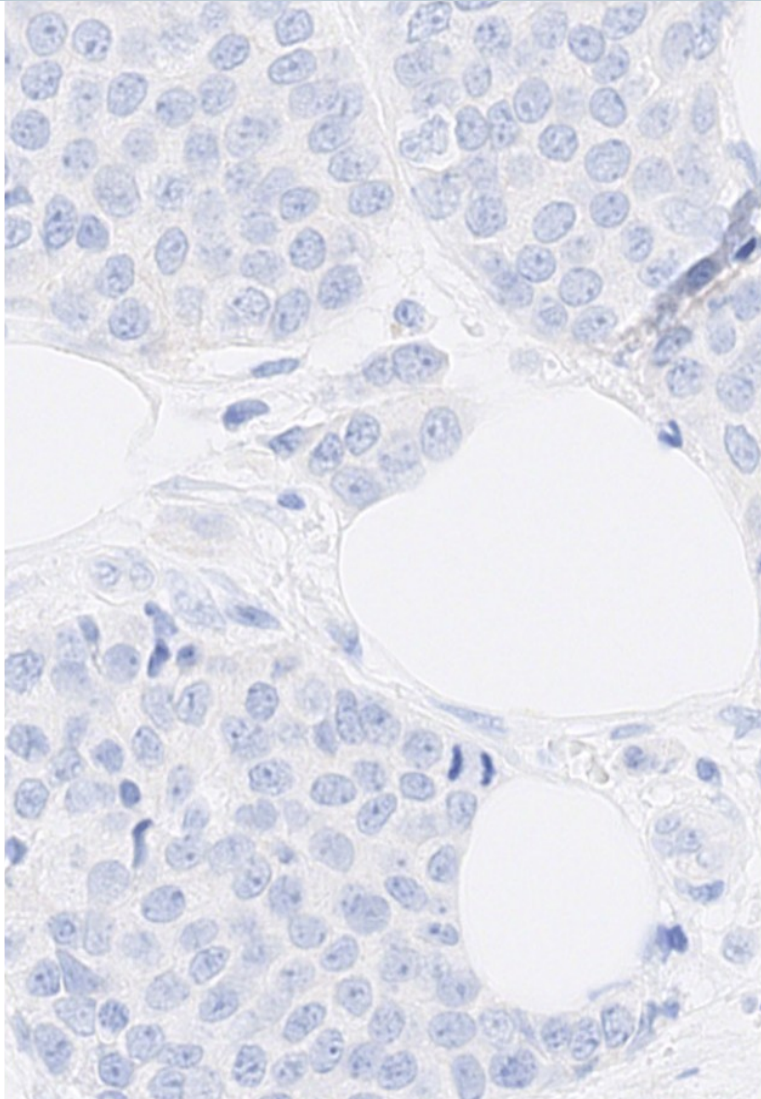
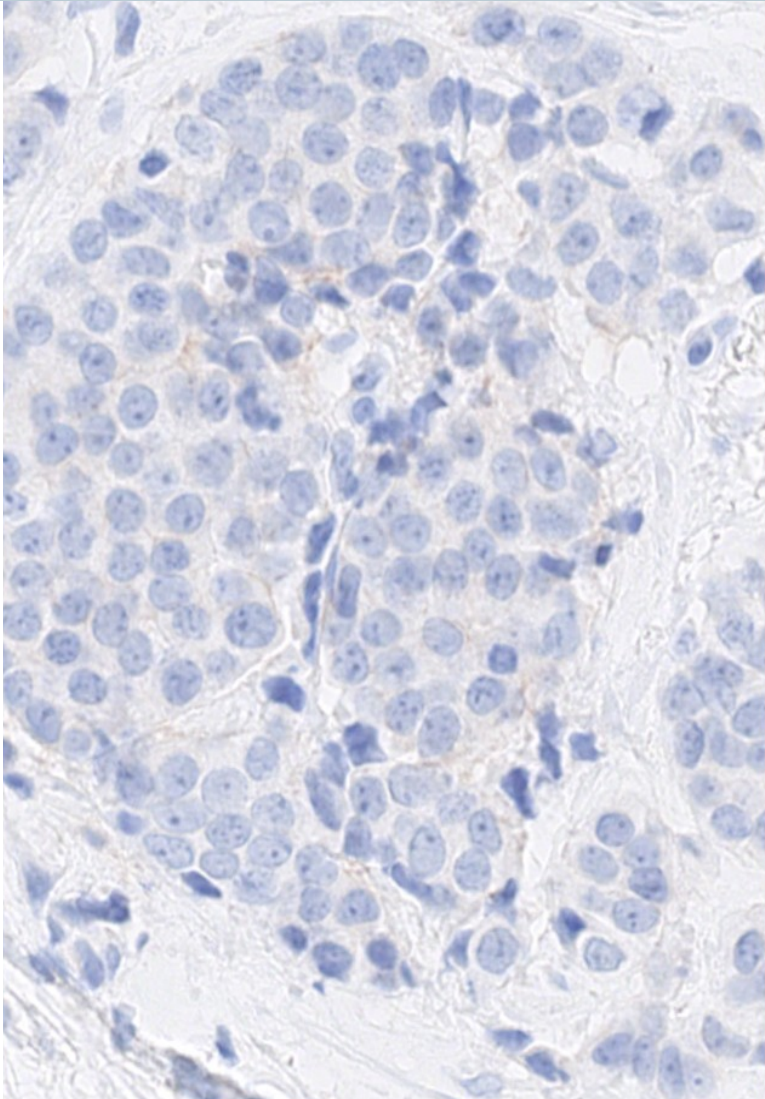


Protocol 4
Score 0



Protocol 6
Score 0

Breast carcinoma **Score 0**



Cell lines covering the full clinically relevant HER2 expression range including Ultralow for the first time as quality tool for IHC assay performance

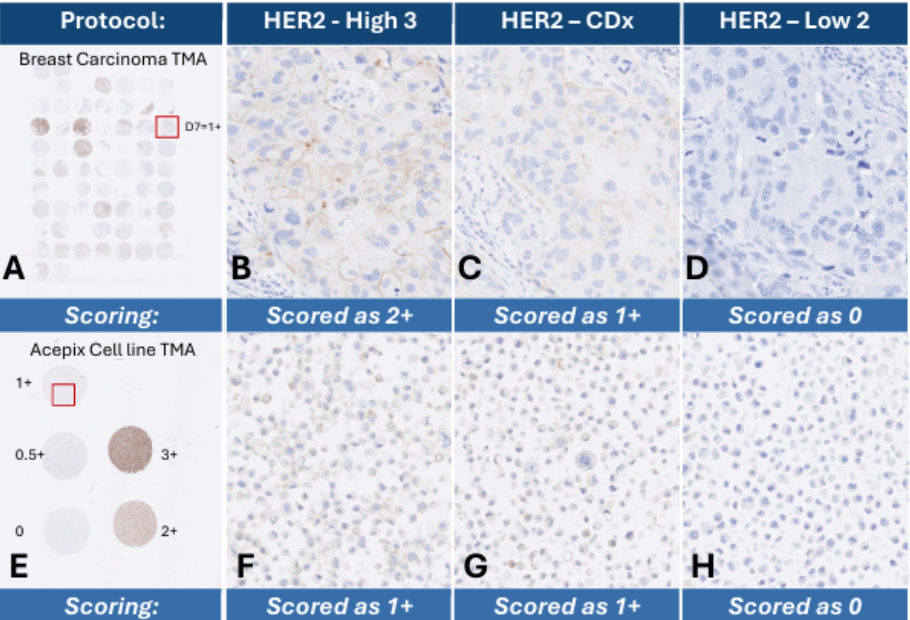
Tanya Julio¹, Søren Nielsen¹, Nan Su², Kuang-Jung (KJ) Chang², Rasmus Røge^{1,3} and Kristi Bøgh Anderson^{1,3}
¹Aalborg University Hospital, Pathology Department / NordiQC, Denmark, ²Acepix Biosciences, USA, ³Department of Clinical Medicine, The Faculty of Medicine, Aalborg University, Denmark.



Background: The requirements for diagnostic accuracy and reproducibility of immunohistochemistry (IHC) for Human epidermal growth factor receptor 2 (HER2) in breast cancer (BC) have increased with inclusion of HER2-low and HER2-ultralow categories. The study evaluated whether cell lines covering the full spectrum of HER2-expression could serve as quality control tools for monitoring reproducibility of diagnostic accuracy of HER2-IHC assays applied for both HER2 classical overexpression and HER2-low/ultralow status.

Methods: In total, six IHC assays with varying analytical sensitivity were performed on 55 BCs and on a cell-line array (SKU 3700, Acepix) containing five cell lines with clinically relevant HER2-expression levels (0, 0.5+, 1+, 2+, 3+). Reference HER2 IHC status was characterized using the Ready-To-Use (RTU) 4B5 IHC-assay (Roche) and gene amplification status in BCs was confirmed by fluorescence hybridization (FISH) analysis (Zytovision). To evaluate whether cell lines could identify IHC-assays with inaccurate results, five additional IHC-assays with either increased or reduced analytical sensitivity were performed on both BCs and cell lines. HER2-expressions were scored manually according to the 2025 ASCO guidelines.

Results: IHC-assays with reduced analytical sensitivity produced more false-negative (FN) results for both HER2 classical overexpression and HER2-low/ultralow tumours. Cell lines with HER2-expression 0.5 and 1+ detected this change. In contrast, assays with increased analytical sensitivity produced false-positive (FP) results as HER2 0 BCs changed status to HER2-low/ultralow. However, no corresponding change was detected in the cell lines. Protocols with increased analytical sensitivity did not affect the accuracy for HER2 classical overexpression.



HER2 IHC diagnostic accuracy - HER2 low and HER2 ultralow					
HER2 High 3	HER2 High 2	HER2 High 1	Reference CDx	HER2 Low 1	HER2 Low 2
OptaView, CC1 64 min, Ab 32 min	UltraView, CC1 64 min, Ab 32 min	UltraView, CC1 36 min, Ab 20 min	UltraView, CC1 36 min, Ab 12 min	UltraView, CC1 20 min, Ab 8 min	UltraView, CC1 20 min, Ab 8 min
OPA 87.9% (29/33)	93.9% (31/33)	100% (33/33)	100% (33/33)	75.8% (25/33)	75.8% (25/33)
PPA 100% (29/29)	100% (29/29)	100% (29/29)	100% (29/29)	72.4% (21/29)	72.4% (21/29)
NPA 0% (0/4)	50% (2/4)	100% (4/4)	100% (4/4)	100% (4/4)	100% (4/4)
HER2 IHC diagnostic accuracy - HER2 classical overexpression					
OPA 100% (55/55)	98.2% (54/55)	98.2% (54/55)	96.4% (53/55)	83.6% (46/55)	83.6% (46/55)
PPA 100% (23/23)	95.7% (22/23)	95.7% (22/23)	91.3% (21/23)	60.9% (14/23)	60.9% (14/23)
NPA 100% (32/32)	100% (32/32)	100% (32/32)	100% (32/32)	100% (32/32)	100% (32/32)
HER2 IHC scores in Acepix cell lines					
3+	3+	3+	3+	3+	3+
2+	2+	2+	2+	2+	2+
1+	1+	1+	1+	1+	0.5+
0.5+	0.5+	0.5+	0.5+	0.5+	0+
0	0+	0+	0+	0+	0+



Standardized controls for Immunohistochemistry

- Precision testing for precision medicine needs precision IHC controls
- At present no "golden standard IHC controls" to fit all IHC biomarkers
- A mixture of carefully selected external tissue controls and non-tissue based controls as cell lines and/or microbeads seem to be best practice
- Cell lines and microbeads have potential to monitor IHC test precision and accuracy, BUT still require extensive documentation and data how to use these

Different performances related to different IHC assays

Different thresholds for adequate vs inadequate result

Software DIA/AI QC-tools to be developed and verified

Tak for jeres opmærksomhed!



Let's connect



Rasmus Røge
Jette Boysen Møller
Heidi Lykke Kristoffersen
Louise Rønnow Holler
Charlotte Papior Carstensen
Tanya Julio
Jacqueline Capion Lemming
Mette Skov Mikkelsen
Lilli Guldager Malaca
Carsten Munck Malmgaard
Jesper Lund Lauridsen
Kristi Bøgh Anderson



AALBORG UNIVERSITY
HOSPITAL DENMARK

With deep appreciation and gratitude to **Søren Nielsen**

