



Seminar in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 17-19 2025

The Tissue Tool Box

-

IHC Critical Assay Performance Controls

*Søren Nielsen,
Director, NordiQC*

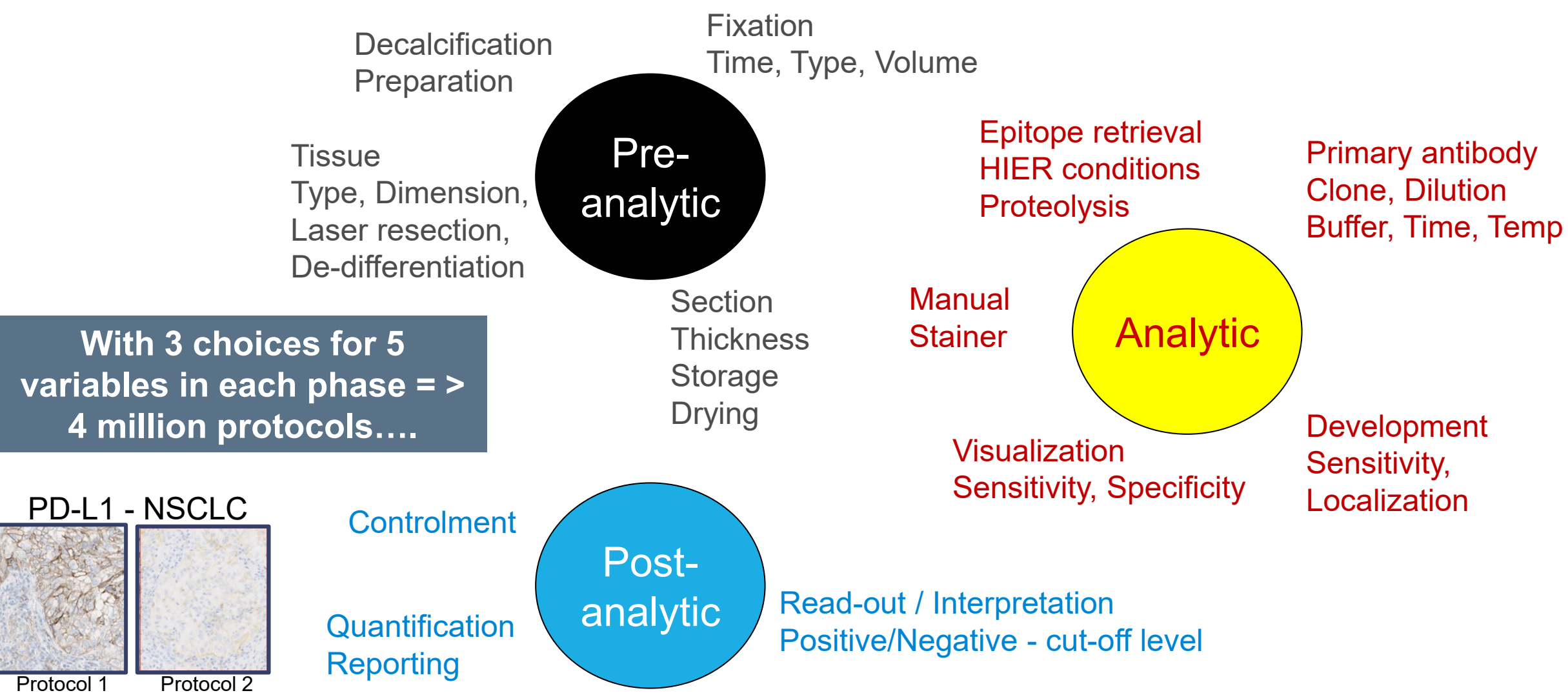


Agenda and focus areas

- What is recommended and best practice for IHC controls in diagnostic IHC?
- What are the potentials and limitations for the use of IHC controls ?
- How can IHC controls be used by laboratories and IHC stakeholders?
 - How to use IHC controls to implement new markers.
 - How to use IHC controls to monitor IHC assay consistency.
 - How to use IHC controls to adress inter and intra test accuracy (e.g. EQA).

The role and concept behind ICAPCs -
IHC Critical Assay Performance Controls

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



The right control material will expose right or wrong choices

Importance of IHC controls have been neglected....

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 Gladv

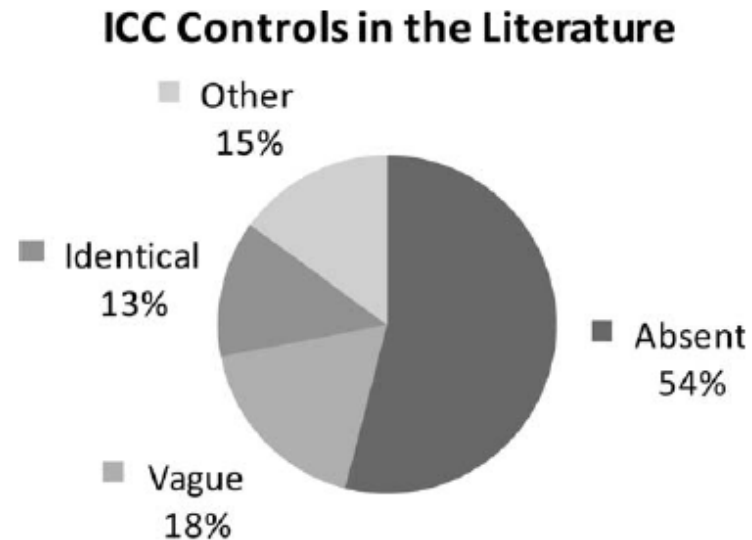


Fig. 1. Description of immunocytochemistry controls in articles reviewed.

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.

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

Level same in 2025....

IHC controls to guide reliability of data...

PAX8 expression in breast cancer – true or false...?

Central for subtyping of unknown primary carcinoma; Ovary, uterine, kidney...

But....

Can PAX8 expression be seen in breast carcinoma??

Right choice, right use and results reported in positive and negative
IHC control tissues needed to verify data

IHC controls to guide reliability of data...

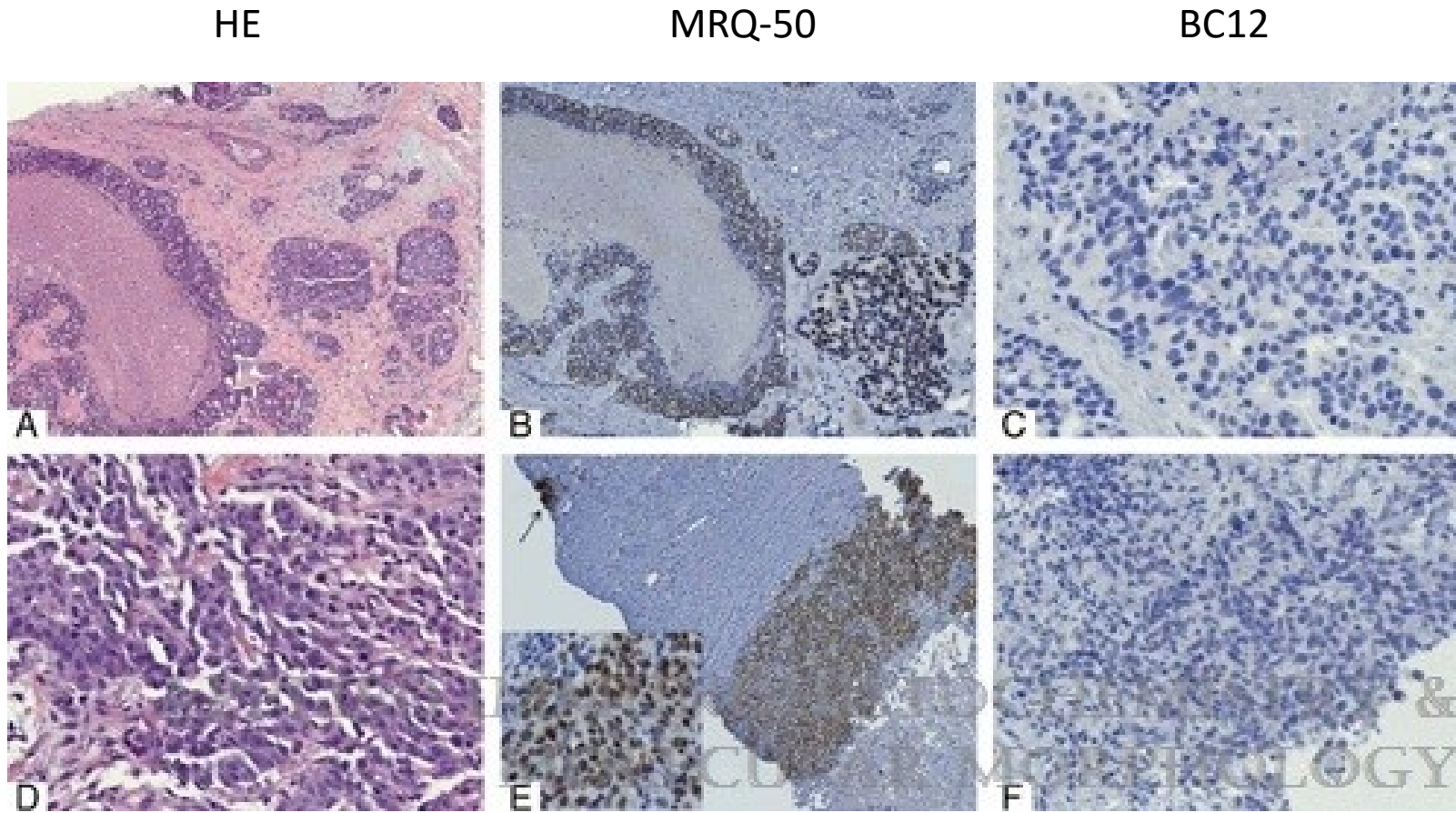


FIGURE 1

Aberrant Immunostaining of Breast Carcinoma by MRQ-50 PAX8 Antibody

Singh, Kamaljeet; Hansen, Katrine; Quddus, M. Ruhul

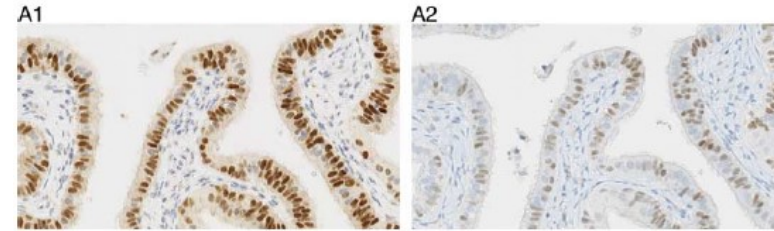
Applied Immunohistochemistry & Molecular Morphology 28(4):e37-e38, April 2020.

doi: 10.1097/PAI.0000000000000682

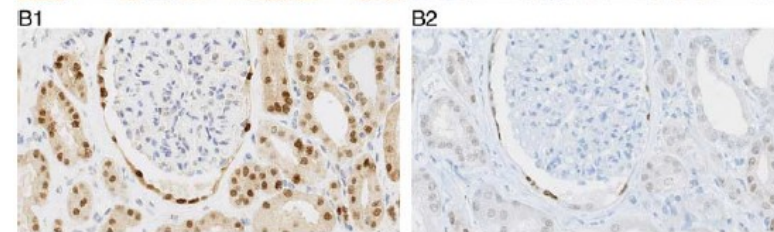
Photomicrographs from 2 breast carcinomas with aberrant PAX8 expression by MRQ-50 clone. On staining with hematoxylin and eosin (A, D) both tumors were high grade with necrosis. Immunohistochemistry for PAX8 with MRQ-50 antibody (B, E) showed nuclear positivity in tumor cells and lymphocytes (arrow).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

IHC controls to guide reliability of data...

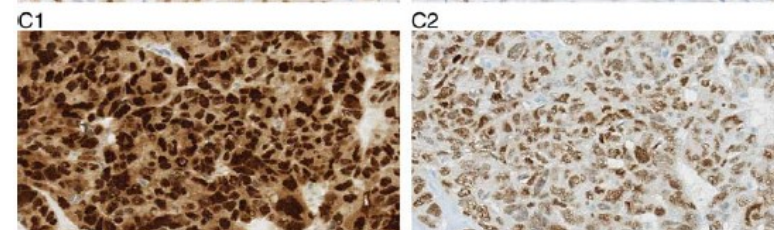
Positive tissue control 1
Fallopian tuba



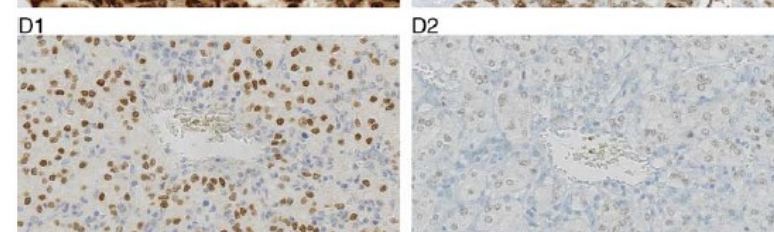
Positive tissue control 2
Kidney



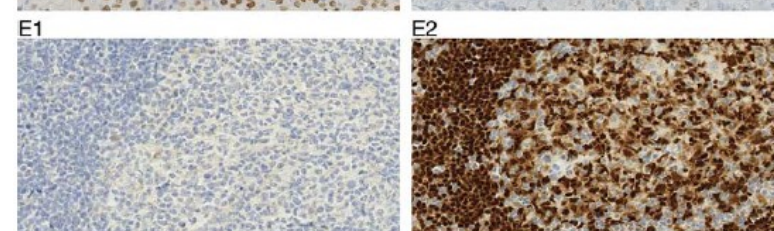
Tumour type 1
Ovarian carc.



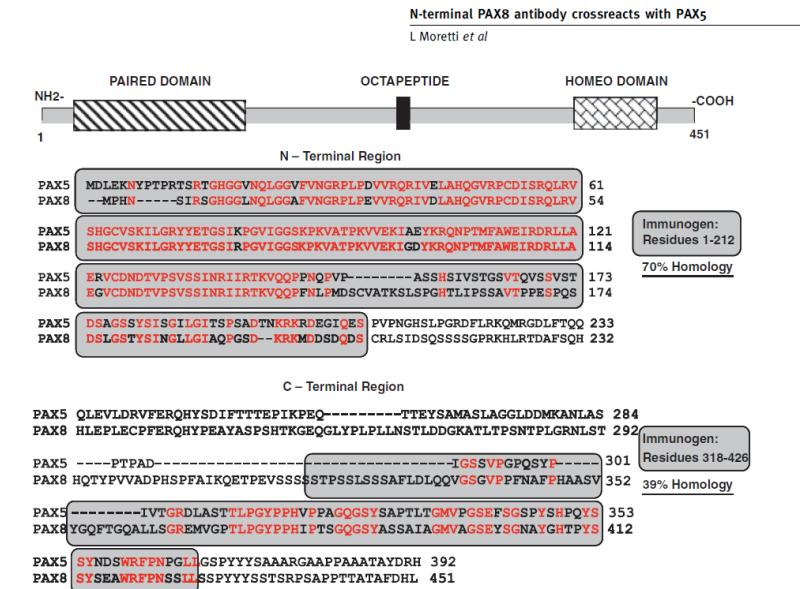
Tumour type 2
Renal cell carc.



Negative tissue control 1
Tonsil



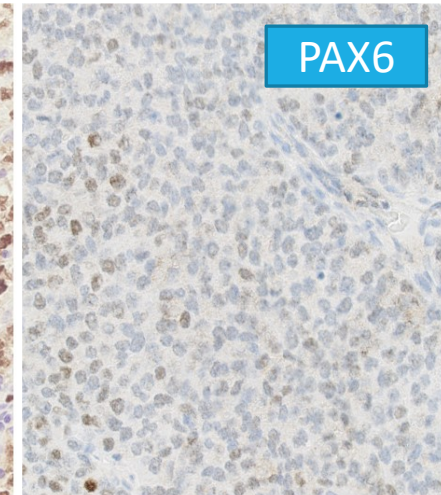
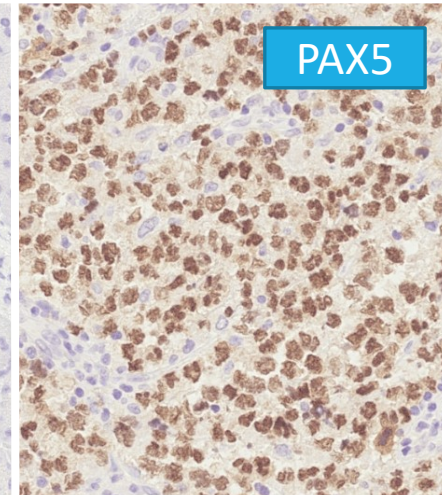
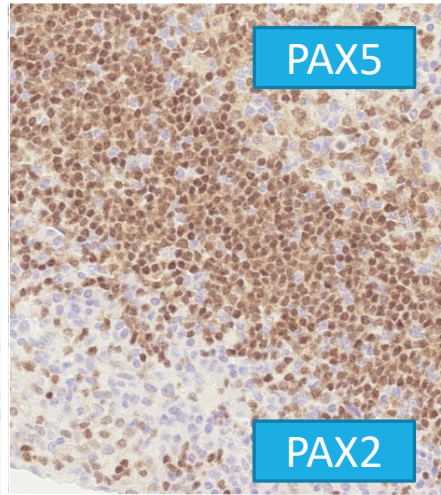
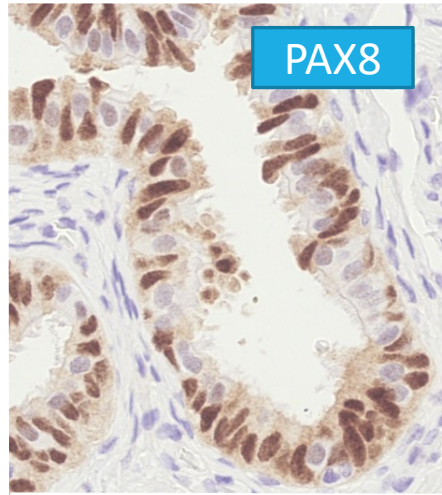
Level of analytical sensitivity



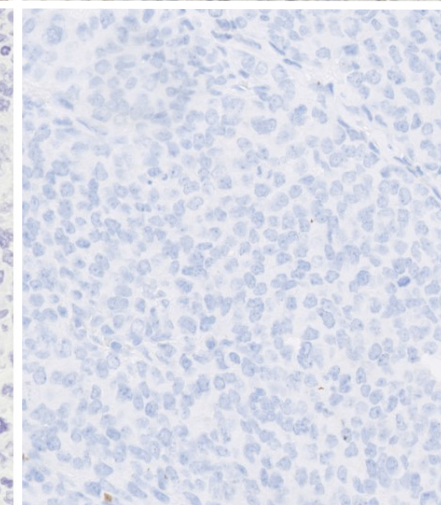
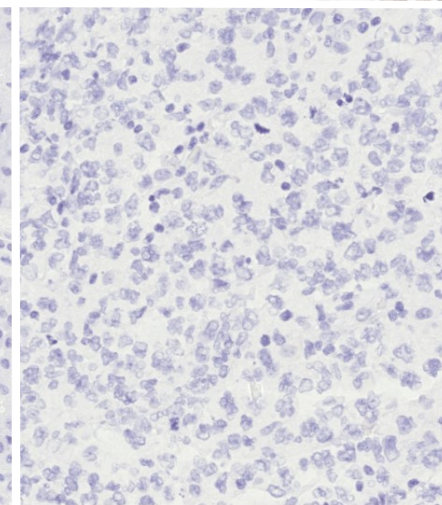
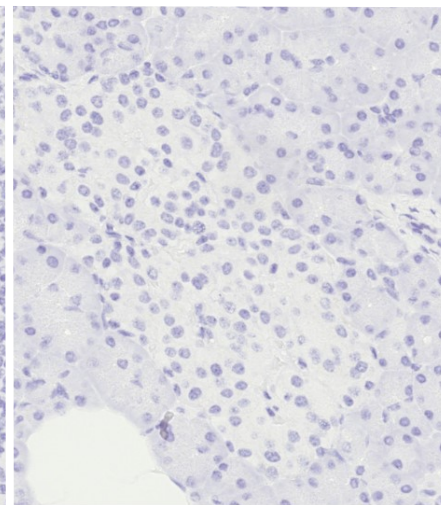
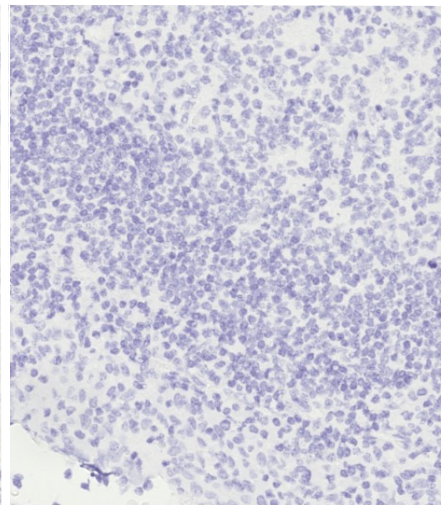
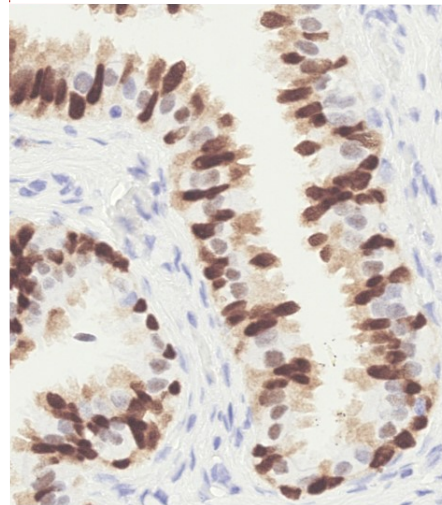
Level of analytical specificity

IHC controls to guide reliability of data...

MRQ-50
(& pAb)



SP348
(& BC12)



Fal. Tube

Tonsil

Pancreas

DLBCL

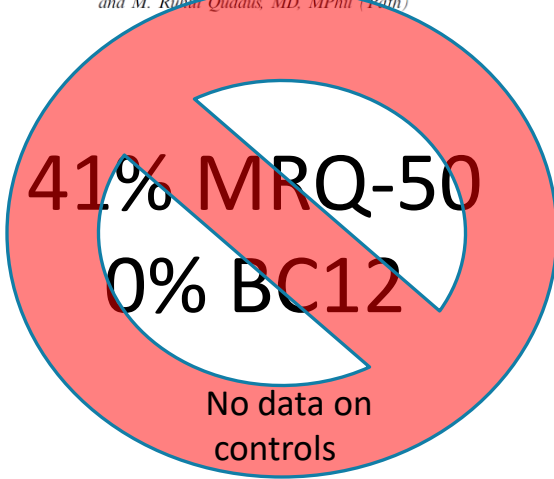
Breast carc.

IHC controls to guide reliability of data...

PAX8 expression in breast cancer – true or false...?

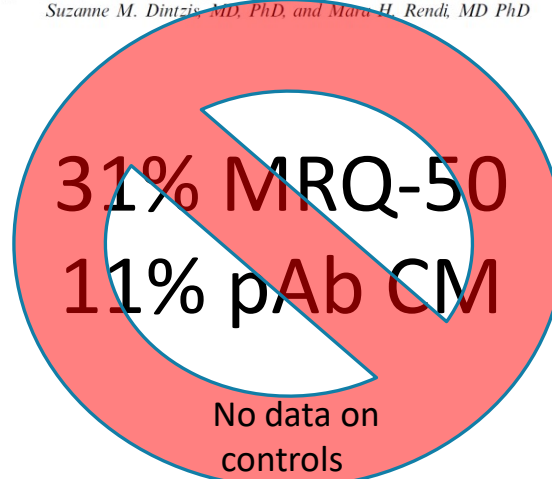
Comparison of PAX8 Expression in Breast Carcinoma Using MRQ50 and BC12 Monoclonal Antibodies

Kamaljeet Singh, MD, Linda C. Hanley, MD, C. James Sung, MD, and M. Ruben Qaddus, MD, MPhil (Path)



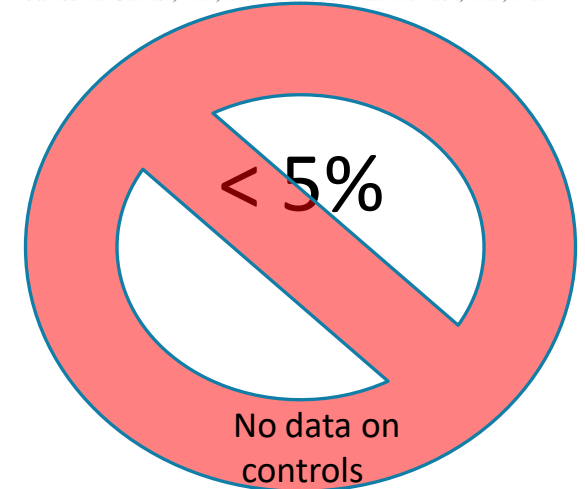
Unexpected PAX8 Immunoreactivity in Metastatic High-grade Breast Cancer

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



Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

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



Right choice, right use and results reported in positive and negative IHC control tissues needed to verify data

NordiQC data – PAX8

Graph 1. Proportion of sufficient results for PAX8 in the eight NordiQC runs performed

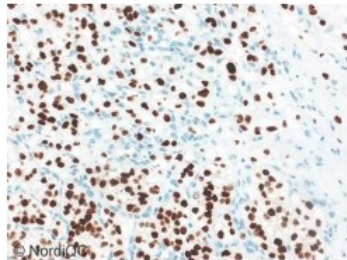
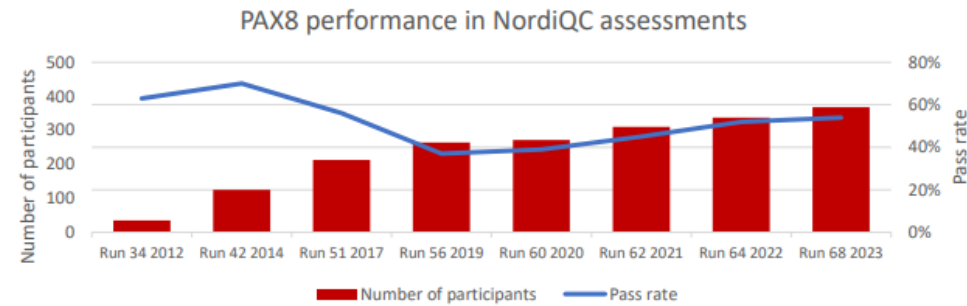


Fig. 4a x200
Optimal PAX8 staining of the RCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig. 4b.

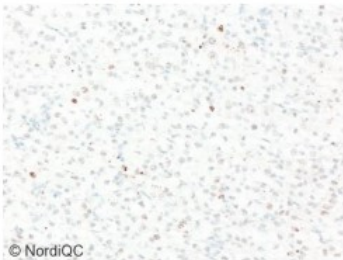


Fig. 4b x200
Insufficient PAX8 staining of the RCC using the same protocol as in Figs. 1b-3b. Only a faint nuclear staining is seen in the vast majority of neoplastic cells. Compare to Fig. 4a.

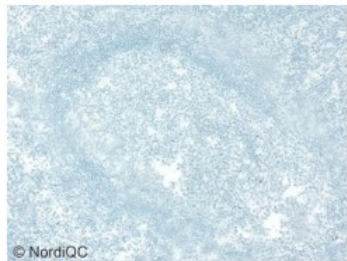


Fig. 5a x100
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

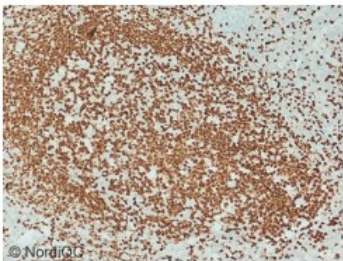


Fig. 5b x100
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.

Table 1. Antibodies and assessment marks for PAX8, run 68							
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹ OR ²
mAb clone BC12*	9	Biocare Zytomed Systems	-	3	7	3	23%
mAb clone MRQ-50	16	Cell Marque	-	8	6	2	50%
mAb clone PAX8R1	1	Abcam	-	-	1	-	-
mAb clone ZM28	1	Zeta Corporation	-	1	-	-	-
rmAb clone EP298 ⁵ *	1	Epitomics ⁵	-	1	-	-	-
rmAb clone EP331*	10	Cell Marque Epitomics	-	5	8	1	36%
rmAb clone SP348*	146	Abcam Gennova Spring Bioscience	102	31	9	4	91%
rmAb clone ZR-1*	2	Zeta Corporation BioSite	1	-	2	1	-
rmAb clone BP6157*	2	Biolyx	-	1	1	-	-
rmAb clone QR016*	7	Quartett	3	3	1	-	86%
pAb, 10336-1-AP	11	Proteintech	-	1	3	7	9%
pAb, 363A-15	1	Cell Marque	-	-	1	-	-
pAb, CP379 AK	3	Biocare	-	-	1	2	-
pAb, RBK047	3	Zytomed Systems Diagonics	-	-	3	-	-
Conc total	223		106	54	43	20	72%
Ready-To-Use antibodies							Suff. ¹ OR. ²
mAb clone MRQ-50, 760-4618 (VRPS) ³	6	Ventana/Roche	-	-	-	6	0%
mAb clone MRQ-50, 760-4618 (LMPS) ⁴	49	Ventana/Roche	-	3	34	12	6%
rmAb clone, EP331* 760-6077(VRPS) ³	3	Ventana/Cell Marque	-	1	2	-	-
rmAb clone, EP331* 760-6077(LMPS) ⁴	11	Ventana/Cell Marque	-	4	6	1	36%
mAb clone, BC12* API438	6	Biocare Medical	-	2	4	-	33%
mAb clone IHC008 PII177R06	3	DCS	-	-	3	-	-
rmAb clone ZR-1* Z2202	2	Zeta corporation	-	-	1	1	-
rmAb clone SP348* M6481	3	Spring Bioscience	2	1	-	-	-
rmAb clone 2774R ANB31	1	Biogenex	-	-	1	-	-
rmAb clone GR002* GT210202	1	GeneTech	1	-	-	-	-
rmAb clone QR016* P-P008	2	Quartett	1	1	-	-	-
rmAb clone EP331* 363M/AC0338	12	Cell Marque	-	3	7	2	25%
rmAb clone SP348* 363R-38	4	Cell Marque	2	1	1	-	-
mAb clone MRQ-50, 363M-10/17/18	24	Cell Marque	-	5	13	6	21%
pAb clone 363A-17/18 363A17/18	4	Cell Marque	-	-	3	1	-
mAb clone MRQ-50, MAD-000550QD	6	Master Diagnostica	-	4	1	1	67%
rmAb clone RM436* 8257-C010	2	Sakura Finetek	1	1	-	-	-
rmAb clone IHC048*	1	GenomeMe	-	-	1	-	-
mAb clone C12A32	1	Celnovte	-	1	-	-	-
Clone MXR013* RMA-1024	2	Fuzhou Maixin	2	-	-	-	-
Clone H5A8 DTBL0220101	1	DaTe Bioengineering Technology	1	-	-	-	-
Unknown	1		-	-	-	1	-
RTU total	145		10	27	77	31	26%
Total	368		116	81	120	51	
Proportion			32%	22%	32%	14%	54%

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Ab terminated by vendor.
*Clones that do not show cross reactivity with PAX5.

PAX8 – to be in breast or not to be in breast...

RESEARCH ARTICLE

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

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*

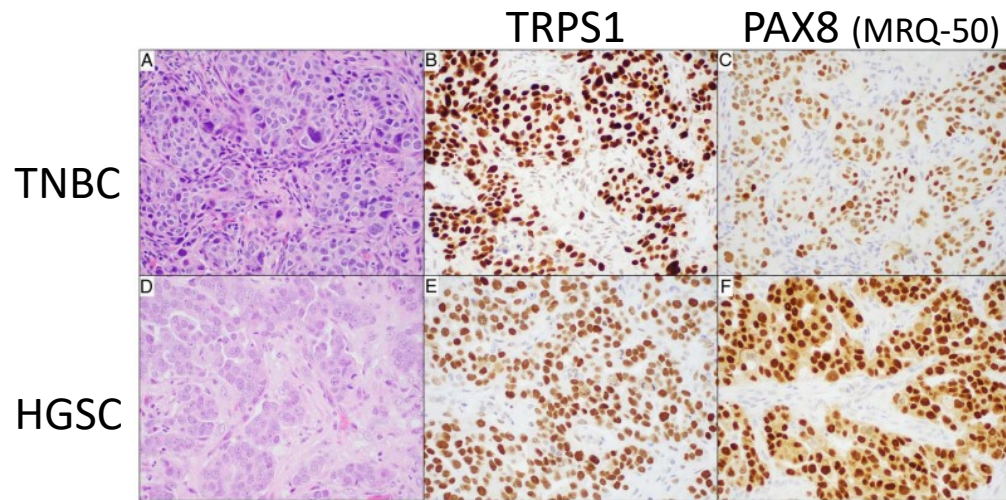


FIGURE 1. A–C, An example case of a triple-negative invasive ductal carcinoma (A) with diffuse, strong nuclear TRPS1 expression, with background breast stromal cells showing weak, nuclear TRPS1 staining (B); breast carcinoma cells also show diffuse, moderate to strong nuclear staining with PAX8 via the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

References central for the area of IHC controls

The "Kick-off" phase for

"Standardization of IHC controls"

Definitions and requirements

Usage

Potentials / Limitations

Perspectives

REVIEW ARTICLE

Appl Immunohistochem Mol Morphol . Volume 22, Number 4, October 2014

Standardization of Negative Controls in Diagnostic Immunohistochemistry: Recommendations From the International Ad Hoc Expert Panel

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

REVIEW ARTICLE

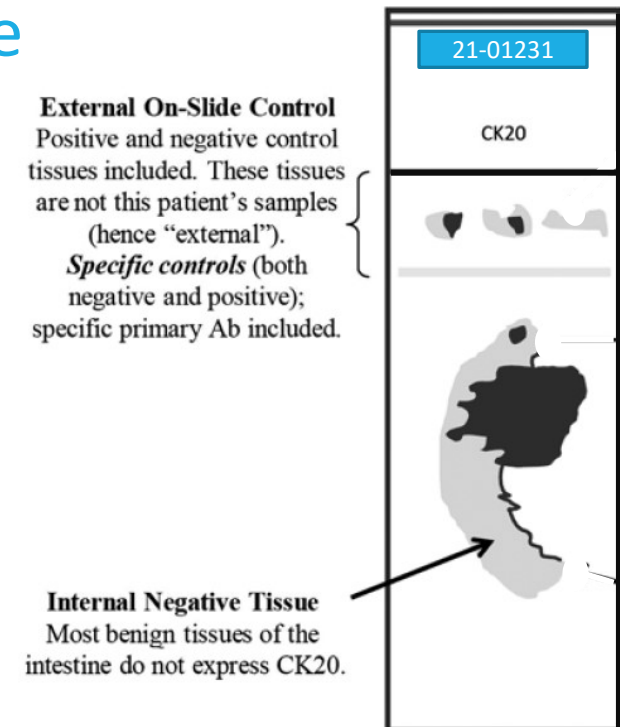
Appl Immunohistochem Mol Morphol • Volume 23, Number 1, January 2015

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‡§*

Tissue controls

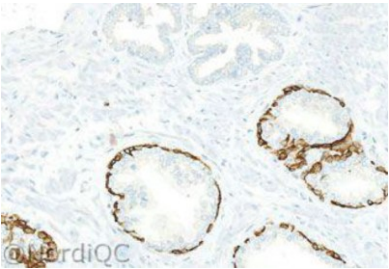
- Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.
- Tissue controls are the most valuable tools to monitor the analytical specificity and sensitivity for IHC
 - Internal positive and negative tissue control
 - Cells/structures within the patient material
 - External positive and negative tissue control
 - Slide next to patient material



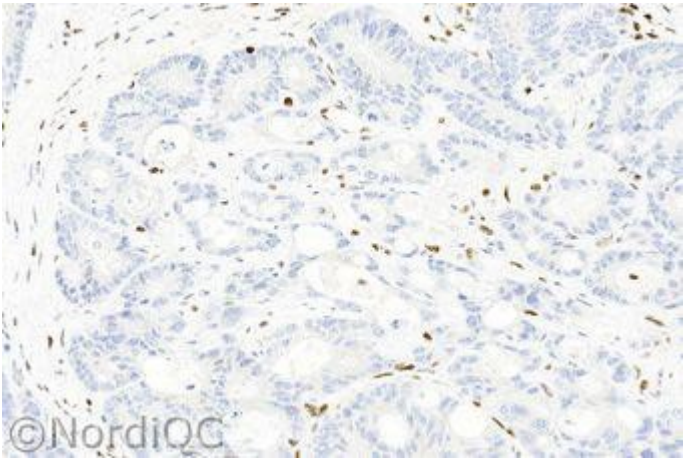
How to use internal tissue controls

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



Internal positive tissue controls;
Principally ideal as processed identically to relevant patient material / target evaluated



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



If internal positive control is neg or dubious – test is repeated.

Limitations of internal tissue controls

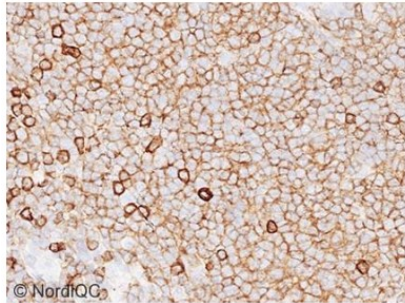


Fig. 2a
Optimal staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

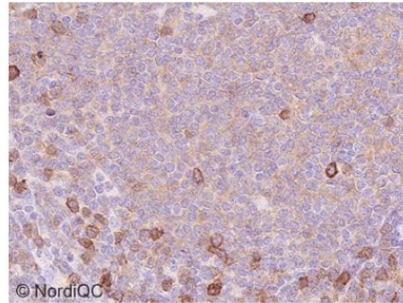


Fig. 2b
Insufficient staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1b. The intensity of the neoplastic cells is significantly reduced and displays an indistinct staining reaction pattern - compare with Fig. 2a (same field). T-cells intermingling with the neoplastic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

CD5

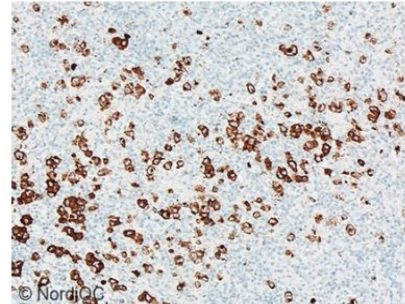


Fig. 5a (x100)
Optimal CD15 staining of the Hodgkin lymphoma using same protocol as in Fig. 4a. The Reed-Sternberg and Hodgkin cells show a moderate membranous staining reaction and a strong dot-like positivity. Also, compare with Fig. 5b.

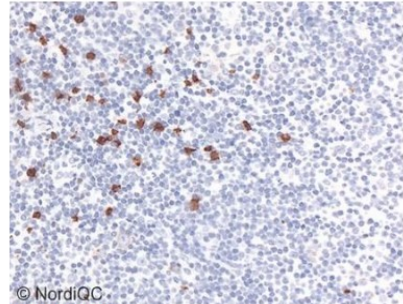


Fig. 5b (x200)
Insufficient CD15 staining of the Hodgkin lymphoma using same protocol as in Fig. 4b. The vast majority of Reed-Sternberg cells and Hodgkin cells are false negative and only the neutrophil granulocytes are demonstrated. Also, compare with Fig. 5a.

CD15

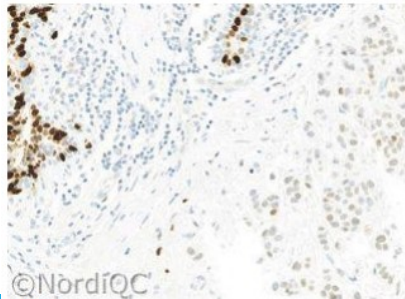


Fig. 3a
Optimal ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

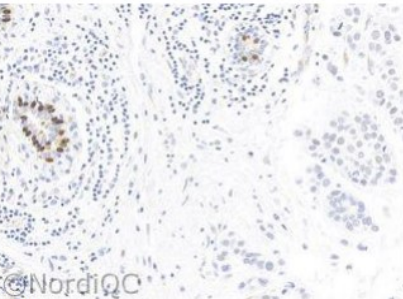


Fig. 3b
Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. Only dispersed neoplastic cells show an equivocal staining reaction.

ER

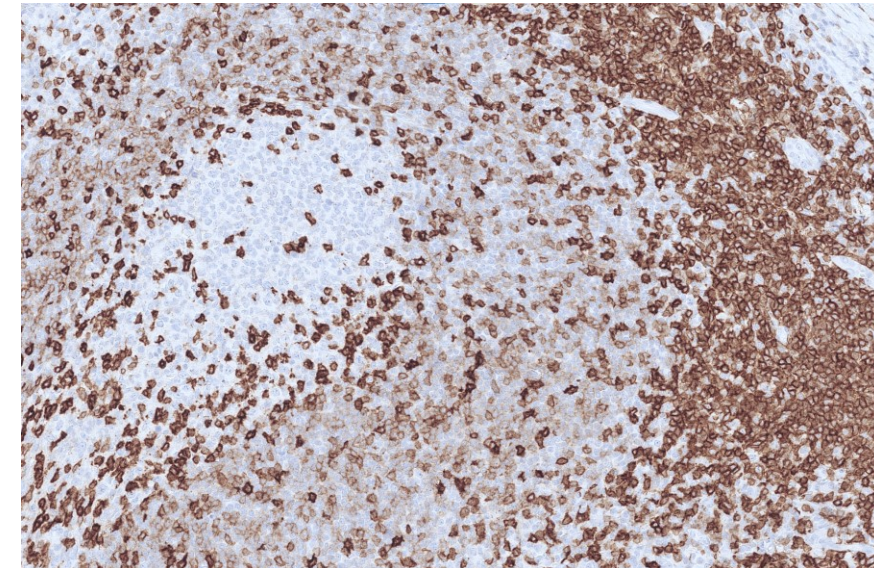
Internal positive tissue controls;

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

e.g. CD5, CD15,, CD45, CD56, CDX2, CGA, DES, ER, PD-L1 etc

CD5;
Tonsil
Mantle zone
B-cells

Critical
control



Critical tissue controls = ICAPCs

IHC Critical Assay Performance Controls (ICAPCs)

are basically human positive control tissues with

- clinical relevant range of target analyte (antigen) – especially with low limit detection
- well characterized expression pattern – preferable normal tissues
- predictable levels and specified cellular and architectural localization

	High expression	Low expression	No expression
Purpose	Right antibody	Right analytical sensitivity	Basic right specificity

REVIEW ARTICLE

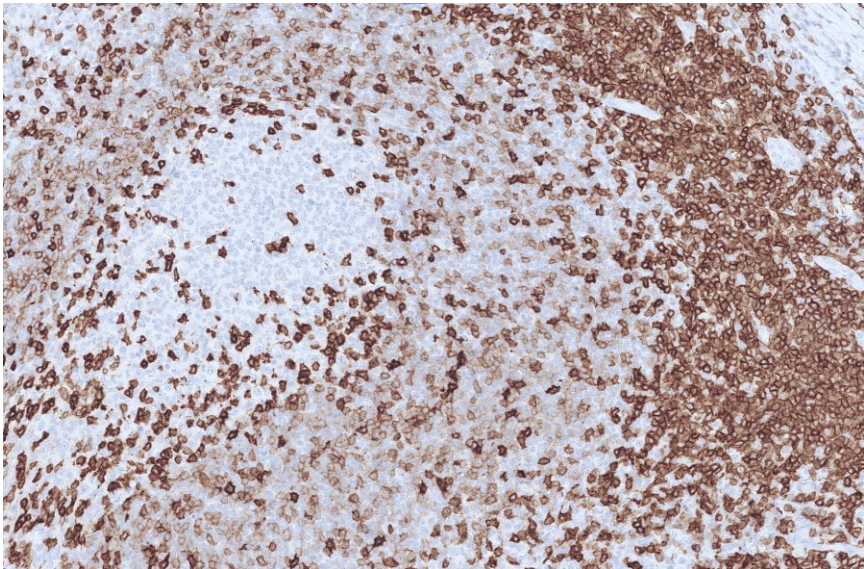
Appl Immunohistochem Mol Morphol • Volume 23, Number 1, January 2015

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, FFS (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,‡§*

CD5;
Tonsil
Mantle zone
B-cells

Critical
control



Main elements to develop, verify & validate IHC assays

The journey from an antibody to a diagnostic IHC assay with a specific purpose

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of analytical and diagnostic sensitivity / specificity
4. Identification of IHC performance controls providing information that the established level of detection is obtained in each test performed in daily practice.

Based on selection and use of appropriate external tissue controls

External tissue control tool box

E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification	Range of relevant expression levels With expression No expression	Range of relevant expression levels High expression Low expression No expression	Reproducibility Method of transfer proof
High expression Low expression No expression	With expression No expression		10/20 of each Type I/II IHC	+ relevant cut-off	

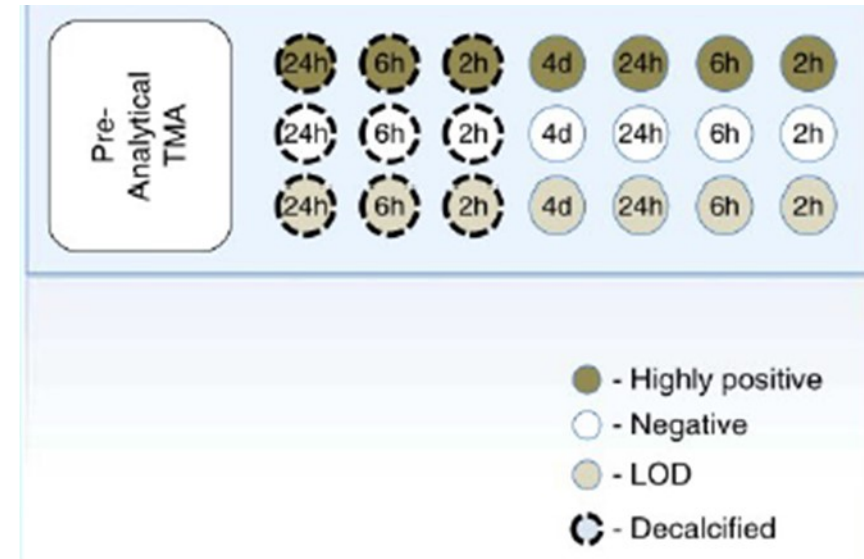


*Immunohistochemical critical assay performance controls

External tissue control tool box

E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

Calibration TMA's		
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification
High expression Low expression No expression	With expression No expression	



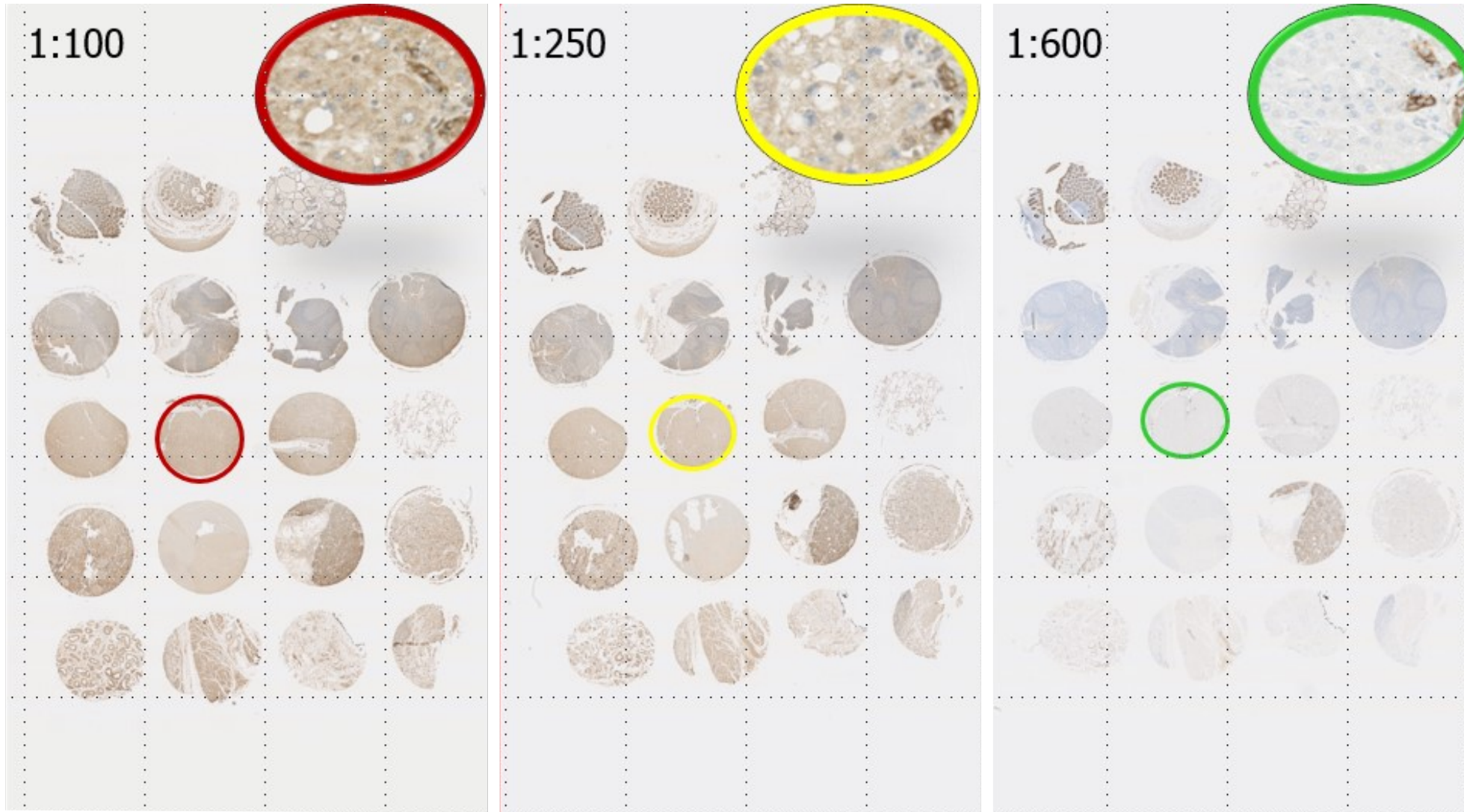
"Poor mans" specificity and pre-analytical TMAs



Inspirational set-up to address issue of specificity and impact on pre-analytics

Source:
NordiQC and Aalborg University Hospital

"Poor mans" specificity and pre-analytical TMAs

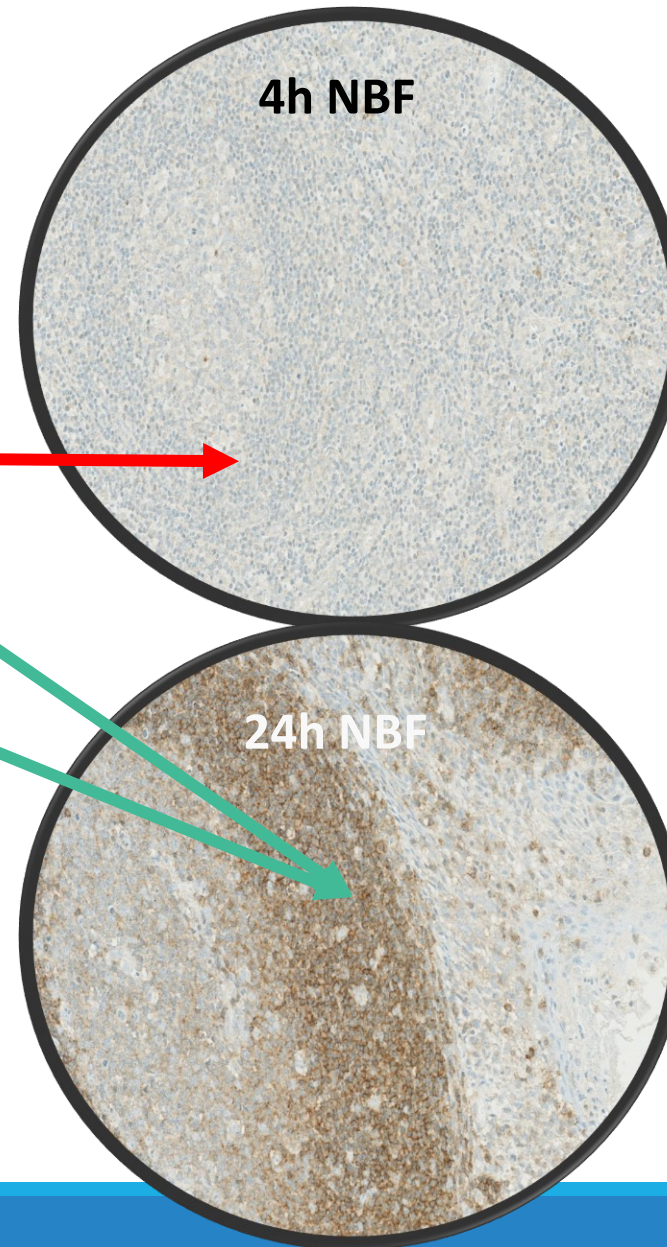
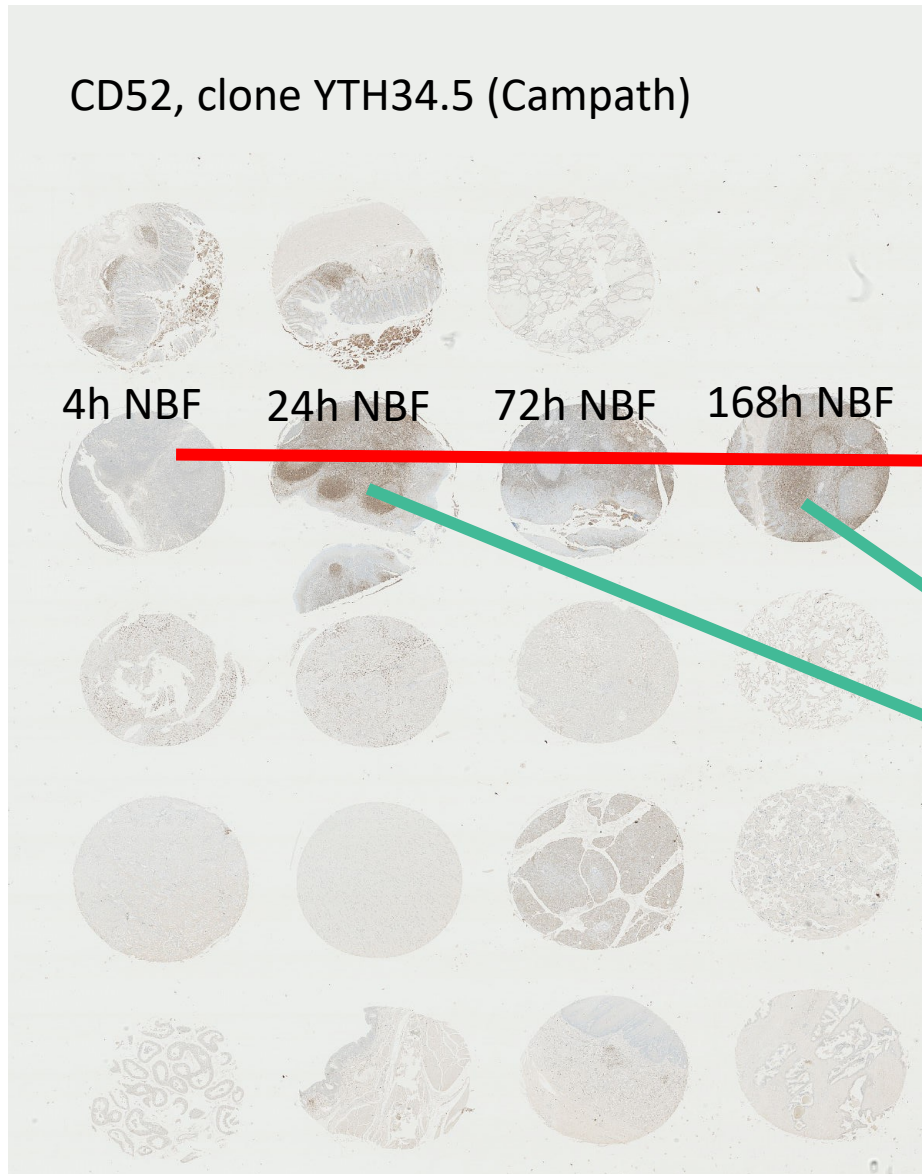


EPCAM calibration

Tissue cores are used to identify best practice protocol providing highest signal-to-noise ratio for qualitative IHC markers

Source:
NordiQC and Aalborg
University Hospital

"Poor mans" specificity and pre-analytical TMAs



CD52 calibration

Tissue cores are used to identify best practice protocol providing impact on on pre-analytics

Source:
NordiQC and Aalborg
University Hospital

Main elements to develop & validate IHC assays

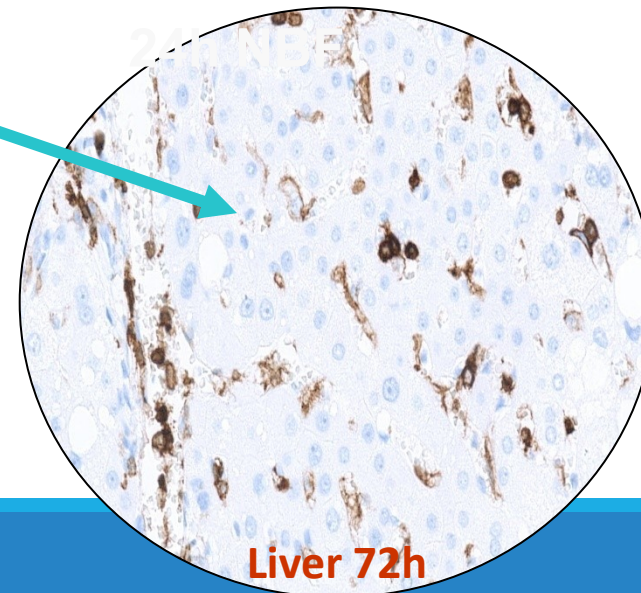
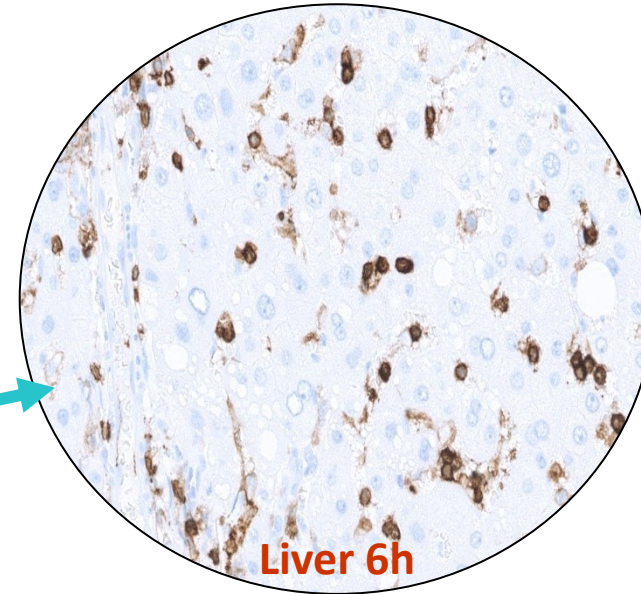
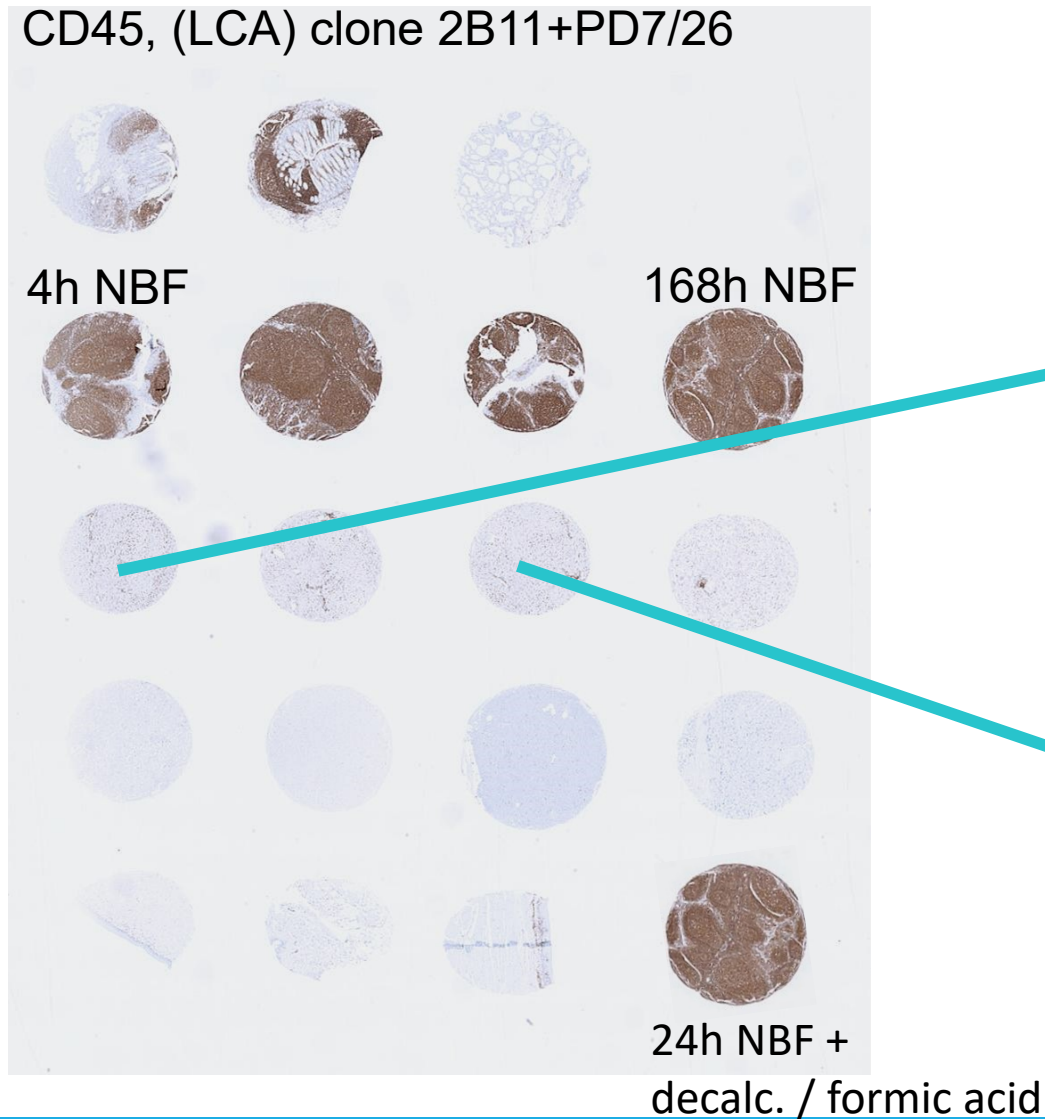
The journey from an antibody to a diagnostic IHC assay with a specific purpose

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of **analytical** and diagnostic sensitivity/specificity
4. Identification of IHC performance controls providing information that the established level of detection is obtained in each test performed in daily practice.

Based on selection and use of appropriate external tissue controls

"Poor mans" specificity and pre-analytical TMAs

CD45, (LCA) clone 2B11+PD7/26



CD45 calibration

Tissue cores are used to identify best practice protocol providing impact on on pre-analytics

1. Not affected by pre-analytics
2. IHC protocol found
3. *Liver and tonsil as Controls.....???*

Which reaction pattern indicates optimal result?

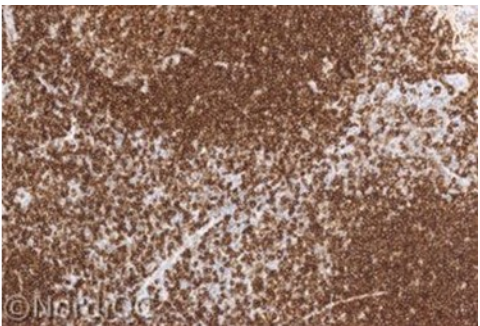
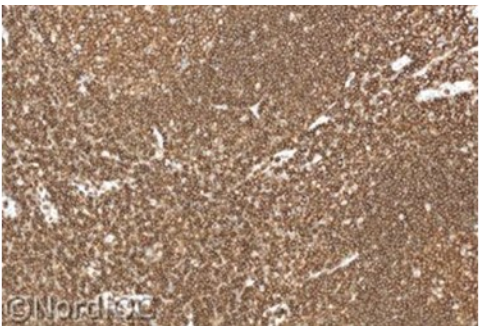
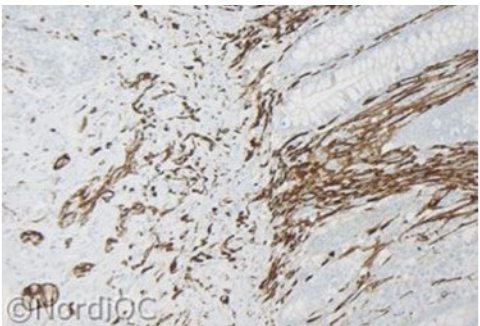
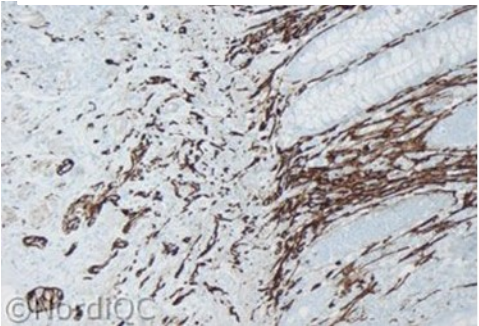
Source:
NordiQC and Aalborg University Hospital

Fit For Purpose; the selection....

CD56

CD45

Colon/
Appendix



Tonsil

Test A

Test B

Test A

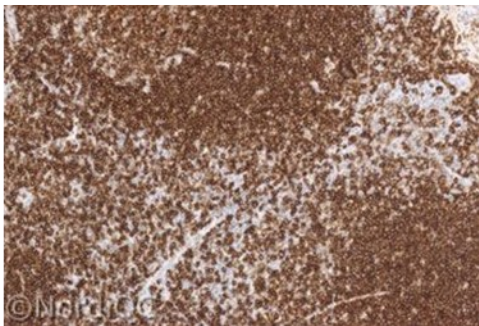
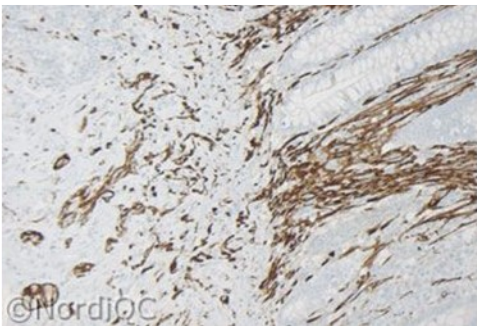
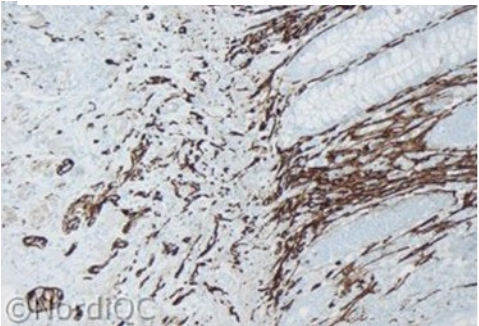
Test B

Fit For Purpose; the selection....

CD56

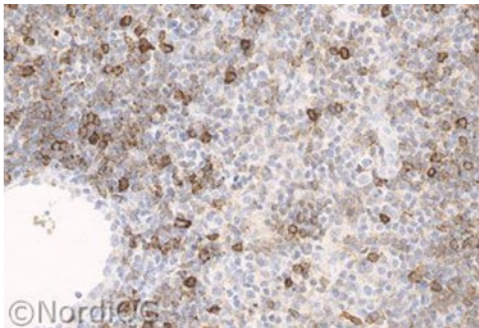
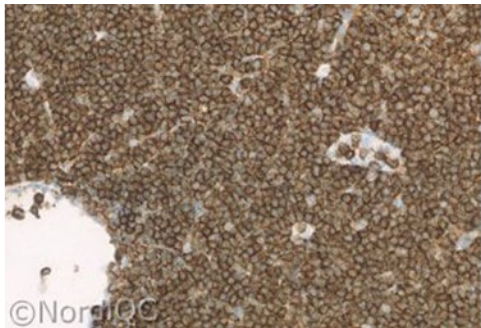
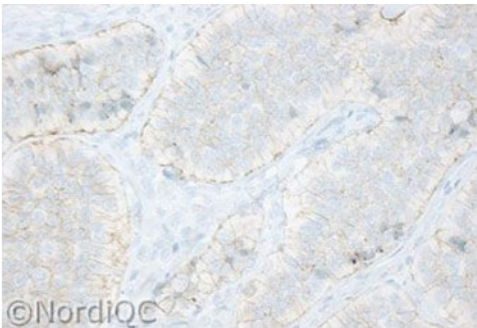
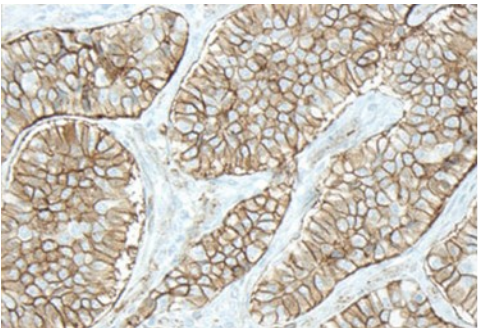
CD45

Colon/
Appendix



Tonsil

NET



B-CLL

Test A

Test B

Test A

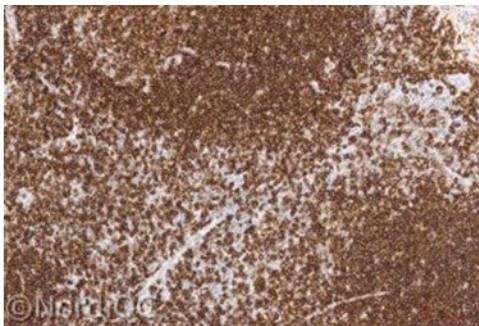
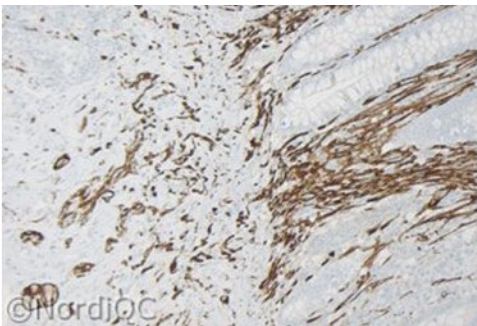
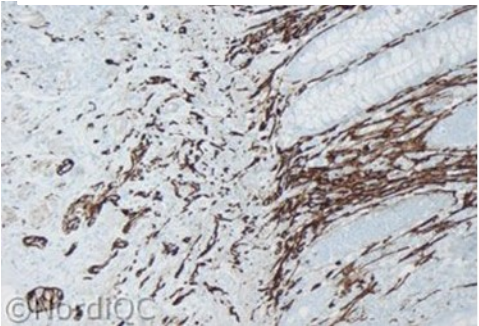
Test B

Fit For Purpose; the selection....

CD56

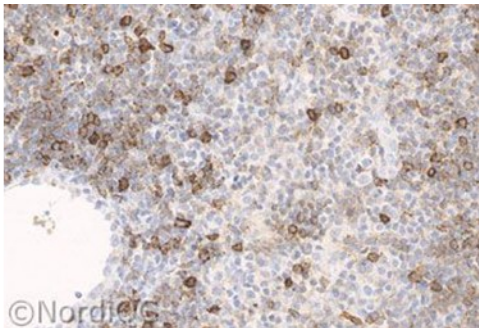
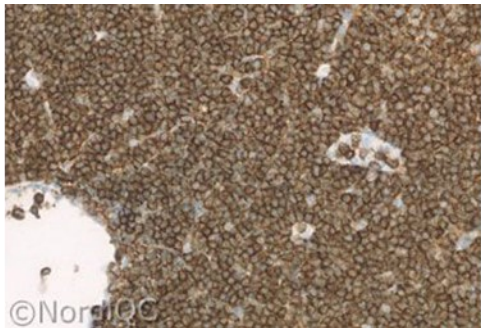
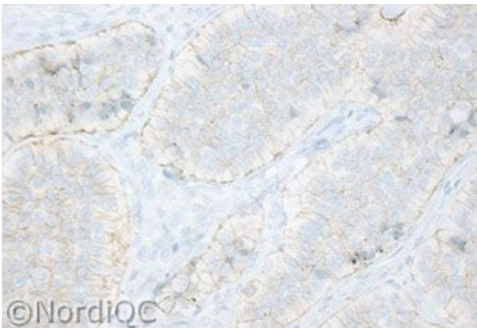
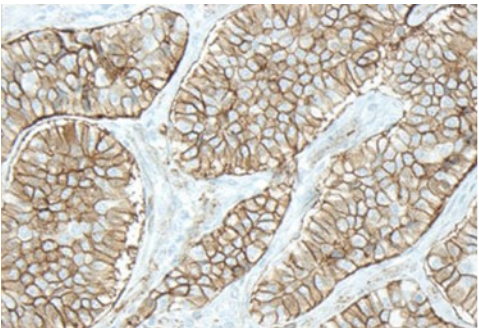
CD45

Colon/
Appendix



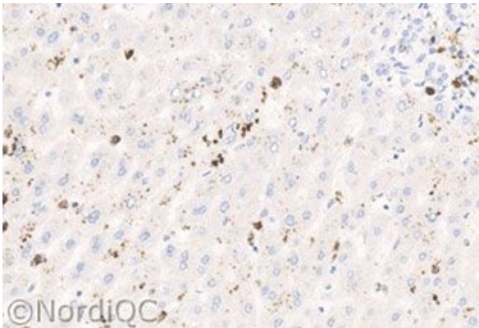
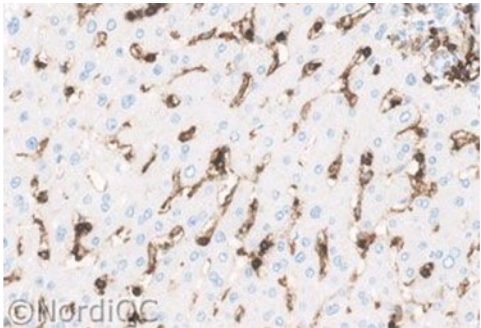
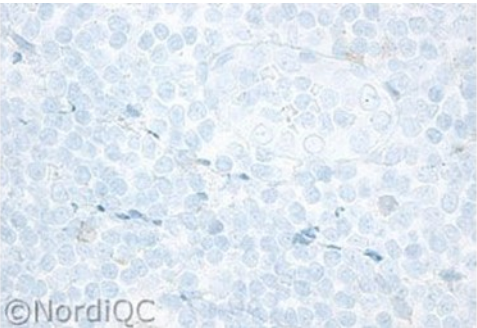
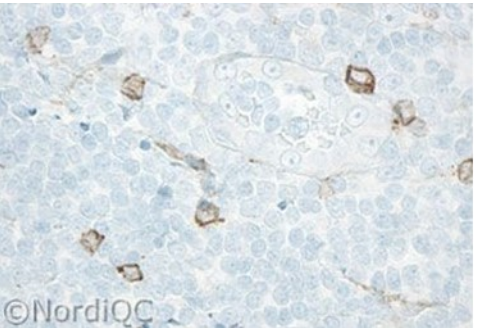
Tonsil

NET



B-CLL

Tonsil



Liver

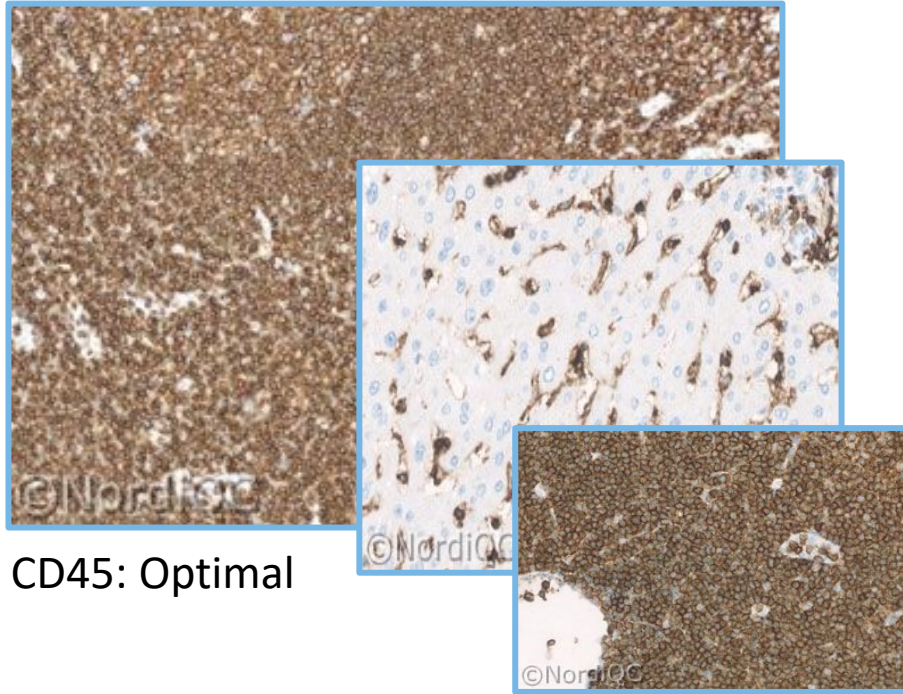
Test A

Test B

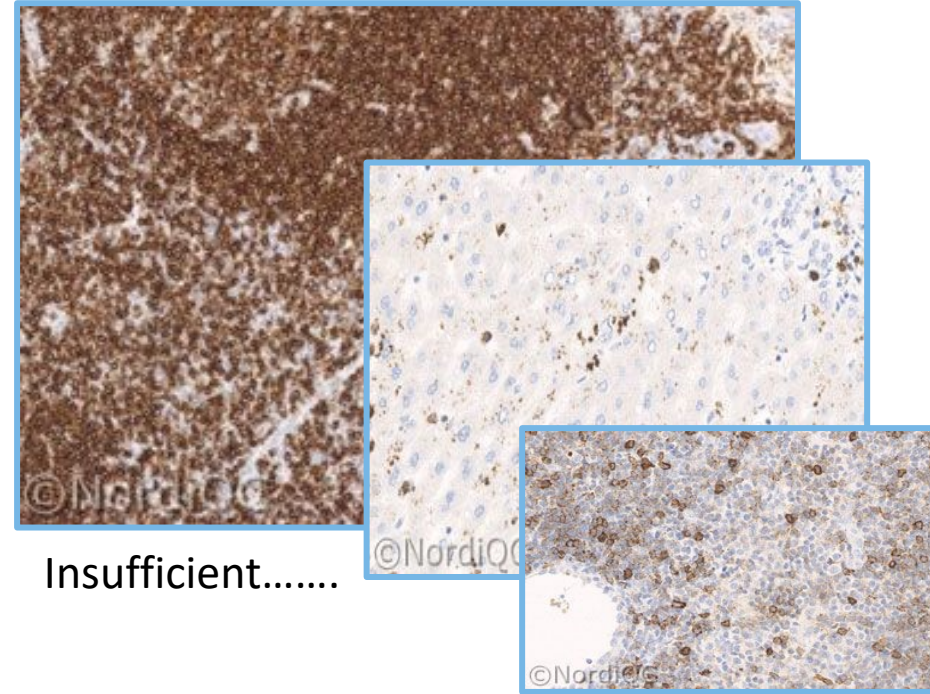
Test A

Test B

Fit For Purpose; the selection....



CD45: Optimal



Insufficient.....

Tissues/cells with only high expression will not identify:

1. A poorly calibrated IHC assay
2. A reduced analytical sensitivity of an optimally calibrated IHC assay – assay reproducibility/consistency
3. A reduced expression level of changed tissue processing settings

If an IHC test is used to identify the target antigen being expressed at different levels, controls must reflect this!

iCAPCs - concept

IHC Critical Assay Performance Controls (iCAPCs)

Which tissues are recommended ?

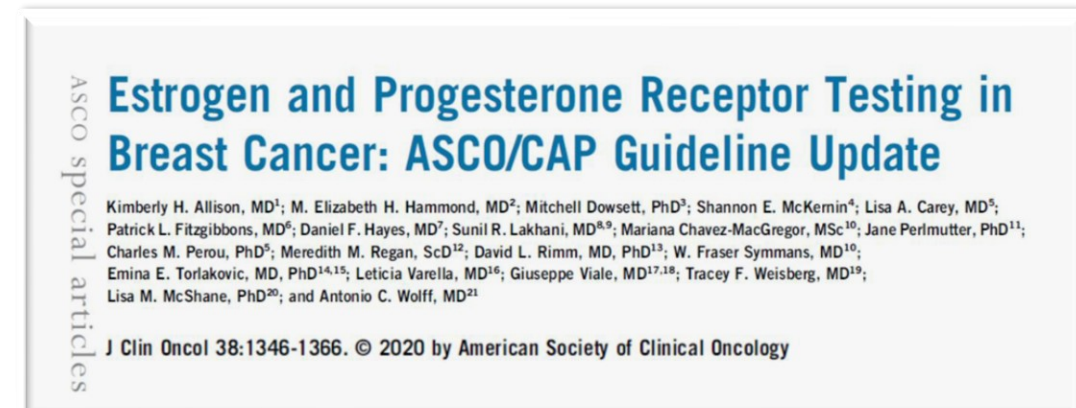
What is the expected staining pattern ?

Which tissues / cells are critical ?

Right antibody

Appropriate level of sensitivity

Guidance level of specificity



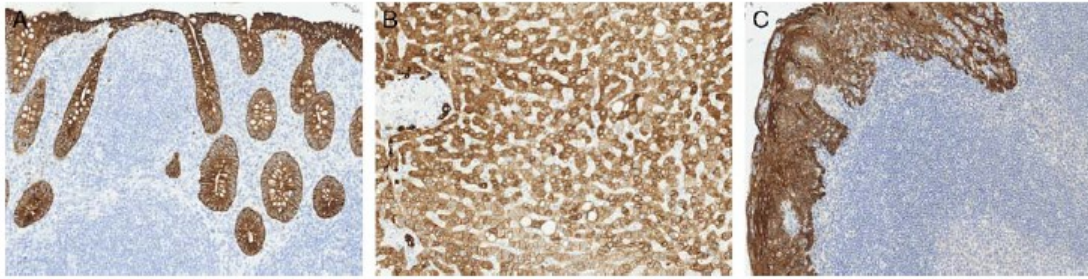


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining reaction with a membranous accentuation (LLOD). C, Tonsil: all squamous epithelial cells must show a moderate to strong cytoplasmic staining reaction. Cytokeratin (CK)-positive interstitial reticulum cells (CIRCs) with dendritic/reticular pattern can show a weak to moderate cytoplasmic staining reaction (LLOD). iCAPC indicates immunohistochemistry critical assay performance controls; LLOD, low limit of detection.

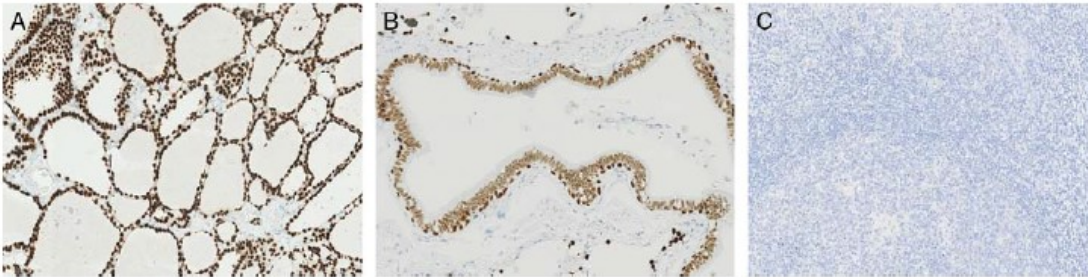


FIGURE 7. TTF-1 iCAPC. A, Thyroid: virtually all epithelial cells must show a strong nuclear staining reaction. B, Lung: virtually all pneumocytes and basal cells of terminal bronchi must show a moderate to strong nuclear staining reaction. Columnar epithelial cells of terminal bronchi must show an at least weak nuclear staining reaction (LLOD). C, Tonsil: no staining reaction must be seen. iCAPC indicates immunohistochemistry critical assay performance controls; LLOD, low limit of detection.

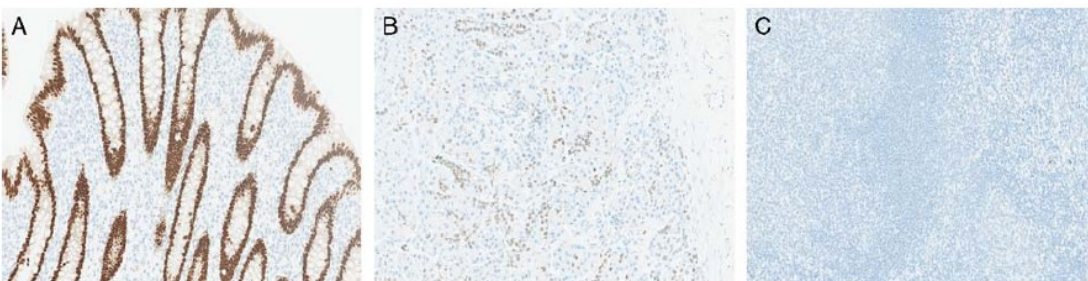


FIGURE 8. CDX-2 iCAPC. A, Appendix: virtually all epithelial cells must show a strong nuclear staining reaction. A weak cytoplasmic staining reaction in addition to strong nuclear staining is often present. B, Pancreas: the majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction (LLOD). C, Tonsil: no staining reaction must be seen. iCAPC indicates immunohistochemistry critical assay performance controls; LLOD, low limit of detection.

Examples for 17 markers

General expected patterns

High expression
(Right antibody)

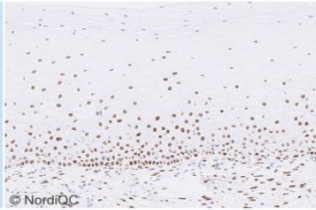
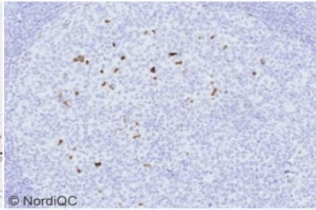
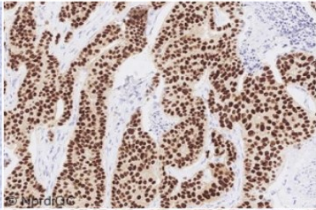
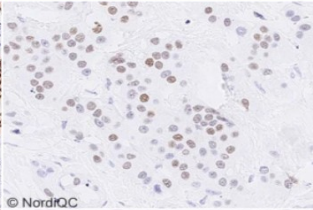
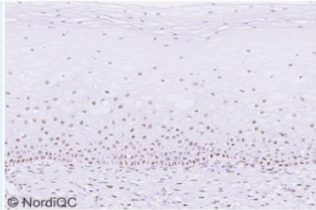
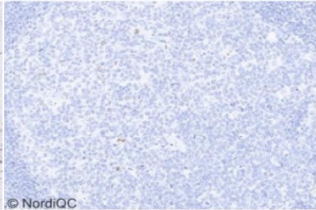
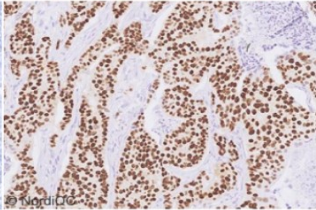
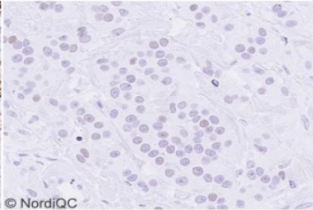
Low expression
(Appropriate sensitivity)

No expression
(Appropriate specificity)

Which tissue
Which cells
Which extension
Which intensity

iCAPCs – ASCO/CAP



	Uterine cervix Right IHC test	Tonsil High analytical sen. High analytical spec.	IHC result in Breast carc. I ER High	IHC result in Breast carc. II ER Low
IHC protocol I				
IHC protocol II				

Recommendation 1.5

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

On-slide controls are recommended.

ascospecialarticles

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 Varella, 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

NordiQC IHC tissue control atlas



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

Recommended controls

Search:

Epitope ▲	Tissues ◆	Actions ◆
ALK (lung)	Appendix/colon, Tonsil	See controls
AMACR	Kidney, Prostate	See controls
ASMA	Appendix/colon, Liver	See controls
Bcl-2	Tonsil	See controls
Bcl-6	Tonsil	See controls
BSAP	Hodgkin lymphoma, Tonsil	See controls
C-MYC	Appendix/colon, Tonsil	See controls
CD3	Appendix/colon, Tonsil	See controls
CD4	Liver, Tonsil	See controls
CD5	Tonsil	See controls
CD8	Appendix/colon, Tonsil	See controls
CD10	Kidney, Tonsil	See controls
CD15	Kidney, Tonsil	See controls
CD19	Appendix/colon, Tonsil	See controls
CD20	Appendix/colon, Tonsil	See controls
CD23	Tonsil	See controls
CD30	Tonsil	See controls
CD31	Appendix/colon, Liver, Tonsil	See controls

Available for NordiQC participants

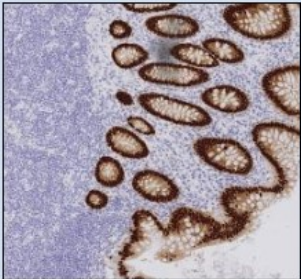
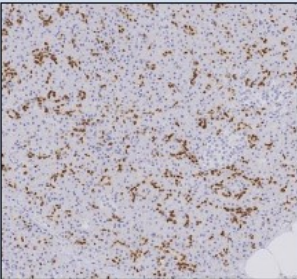
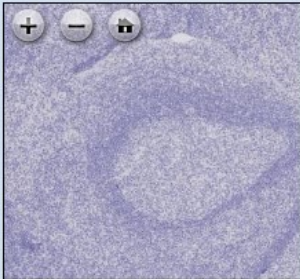
Tissues

Purpose

Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas

 Info ▾ Modules ▾ Assessments Protocols Controls Events ▾ SN			
CDX2 - CDX2			
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Pancreas	Tonsil
Description	All epithelial cells must show a strong nuclear staining reaction. <i>Note, a weak cytoplasmic staining reaction in CDX2 positive cells can be seen and should be accepted if signal-to-noise ratio otherwise is acceptable.</i>	The vast majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction.	No staining reaction should be seen. <i>Note, dispersed lymphocytes can show a faint nuclear staining reaction.</i>
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge
Back			

Available for NordiQC participants

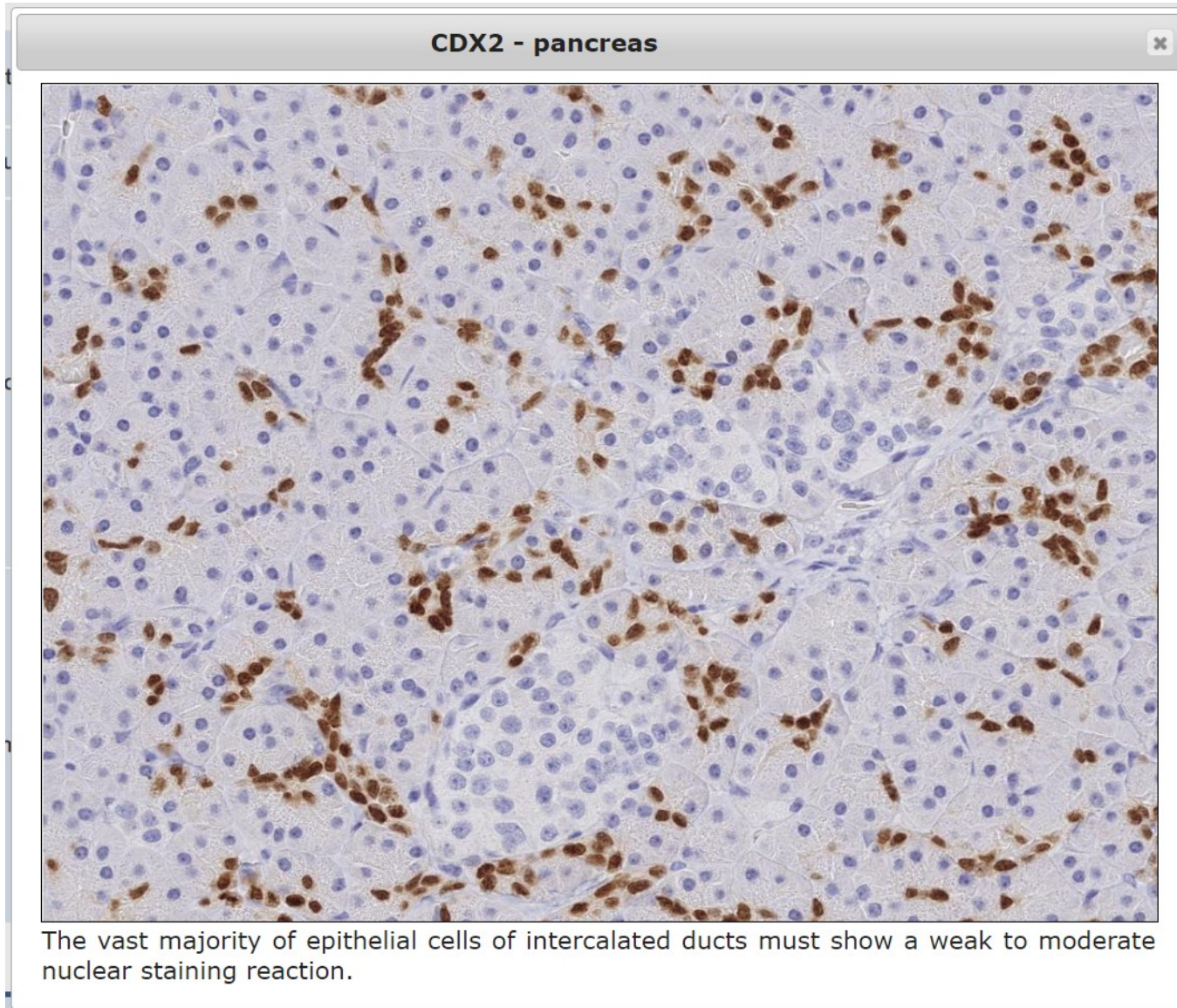
Tissues

Purpose

Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas



Available for NordiQC participants

Tissues

Purpose

Reaction patterns

Online scans accessible



Main elements to develop & validate IHC assays

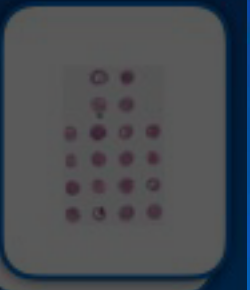
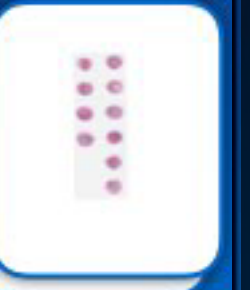
The journey from an antibody to a diagnostic IHC assay with a specific purpose

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of analytical and **diagnostic** sensitivity/specificity
4. Identification of IHC performance controls providing information that the established level of detection is obtained in each test performed in daily practice.

Based on selection and use of appropriate external tissue controls

External tissue control tool box

E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility
High expression Low expression No expression	With expression No expression		With expression No expression	High expression Low expression No expression	Method of transfer proof
			10/20 of each Type I/II IHC	+ relevant cut-off	



*Immunohistochemical critical assay performance controls

CAP* 2024 guidelines for IHC assay validation/verification



Principles of Analytic Validation of Immunohistochemical Assays: Guideline Update

Statements and Strengths of Recommendations

SUMMARY OF STATEMENTS	
Guideline Statement	Category
1. Laboratories must analytically validate all laboratory developed immunohistochemistry (IHC) assays and verify all FDA-cleared IHC assays before reporting results on patient tissues. <i>Note:</i> A validation study design may include but is not necessarily limited to, such means as the following: • Comparing the new assay's results with the expected architectural and subcellular localization of the antigen • Comparing the new assay's results with the results of prior testing of the same tissues with a validated/verified assay in the same laboratory • Comparing the new assay's results with the results of testing the same tissues in another laboratory using a validated/verified assay • Comparing the new assay's results with results of a non-immunohistochemical method • Comparing the new assay's results with the results from testing the same tissues in a laboratory that performed testing for a clinical trial • Comparing the new assay's results against percent positive rates documented in published clinical trials • Comparing the new assay's results to IHC results from cell lines that contain known amounts of protein • Comparing previously graded tissue challenges from a formal proficiency testing program (if available) with the graded responses	Good Practice Statement
2. For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least 90% overall concordance between the new assay and the comparator assay or expected results.	Strong Recommendation
3. For initial analytic validation of nonpredictive laboratory-developed assays, laboratories should test a minimum of 10 positive and 10 negative tissues. When the laboratory medical director determines that fewer than 20 validation cases are sufficient for a specific marker (eg, rare antigen), the rationale for that decision needs to be documented. <i>Note:</i> The validation set should include high and low expressors for positive cases when appropriate and should span the expected range of clinical results (expression levels) for markers that are reported using either a semiquantitative or numerical scoring system.	Good Practice Statement

© 2024 College of American Pathologists. All rights reserved.

Arch Pathol Lab Med—Vol 148, June 2024

Principles of Analytic Validation of Immunohistochemical Assays

Guideline Update

Jeffrey D. Goldsmith, MD; Megan L. Troxell, MD, PhD; Sinchita Roy-Chowdhuri, MD, PhD; Carol F. Colasacco, MLIS, SCT(ASCP); Mary Elizabeth Edgerton, MD, PhD; Patrick L. Fitzgibbons, MD; Regan Fulton, MD, PhD; Thomas Haas, DO; Patricia L. Kandalafi, MD; Tanja Kalicanin, MLS(ASCP)^{cm}; Christina Lacchetti, MHSc; Patti Loykasek, HTL(ASCP); Nicole E. Thomas, MPH, CT(ASCP)^{cm}; Paul E. Swanson, MD; Andrew M. Bellizzi, MD

Guideline Revision

This guideline will be reviewed every 4 years, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. The status of the guideline can be found on www.cap.org.

Sample set to validate/verify IHC assays – CAP 2024 guidelines

■ Diagnostic validation

- Laboratory developed tests (concentrates and RTU's being applied modified to official protocol*)
 - Type I non-predictive markers (CD5, PAX8, SOX10, TTF1....)
 - 10 positive, 10 negative samples (minimum)
 - Type II Predictive markers (CLDN18.2, ER, HER2, PD-L1....)
 - 20 positive, 20 negative samples (minimum)

The validation set should include:

- **Positive, negative, and low-positive tissues** that are **tailored** to the intended clinical **use of the assay**.
- Should not be all normal tissues.
- Positive and negative cells on the same section could be scored as separate challenges

CAP* 2024 guidelines for IHC assay validation/verification

For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least **90% overall concordance between the new assay and the comparator assay or expected results.**

	Comparator old IHC assay	Comparator other test	Expected reaction pattern
Tissue/sample 1	+/-	Flow/ISH/NGS..	Descriptive – cells +/-
Tissue/sample 2	CD5 Old Conc. 4C7 New RTU SP19	CD5 Flow CD5 IHC CD5	CD5 Publications Data sheet Antibodypedia Human protein atlas IHC CD5
Tissue/sample 3			
Tissue/sample 4			
Tissue/sample 5			
Tissue/sample 6			
Tissue/sample 7		HER2 ISH HER2/Chr17 IHC HER2	
Tissue/sample 8			
Tissue/sample 9			
Tissue/sample 10			

* College of American Pathologists

CAP* 2024 guidelines for IHC assay validation/verification

The validation set should include:

- **Positive, negative, and low-positive tissues** that are **tailored** to the intended clinical **use of the assay**.
- Should not be all normal tissues.
- Positive and negative cells on the same section could be scored as separate challenges

For initial analytic validation of **non-predictive laboratory developed assays**¹, laboratories should test a minimum of **10 positive and 10 negative tissues**.

For initial analytic validation of **predictive laboratory developed assays**¹, laboratories should test a minimum of **20 positive and 20 negative tissues for each scoring system** (e.g. PD-L1 and HER2 (classical and low)).

For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least **90% overall concordance between the new assay and the comparator assay or expected results**.

¹ Concentrated primary antibodies or RTU's performed by significantly modified protocols.

* College of American Pathologists

NordiQC working method for IHC assay validation/verification

For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least **90% overall concordance between the new assay and the comparator assay or expected results.**

NordiQC, Aalborg Universitetshospital

Validation of IHC assay

1.1 Protocol settings

Marker	PAX8	Clone	SP348 (rabbit mono)	Titer / Ready-To-Use	1:400
Prod. Id., Comp.	Ref. M6484	Lot no.	GR3386977-2	Diluent	Renoir red
Platform	Omnis	Protocol no.	-	Protocol settings	Ab R, H2O, 30-10-30
Biomedical sc.	Louise Rønnow Holler	Scientific lead.	Søren Nielsen	Medical Lead	Rasmus Røge
Date	31.03.2023	Test Id no.	23-400090	Localization of marker	Nuclear

2.1 NordiQC run and purpose of the IHC assay for PAX8

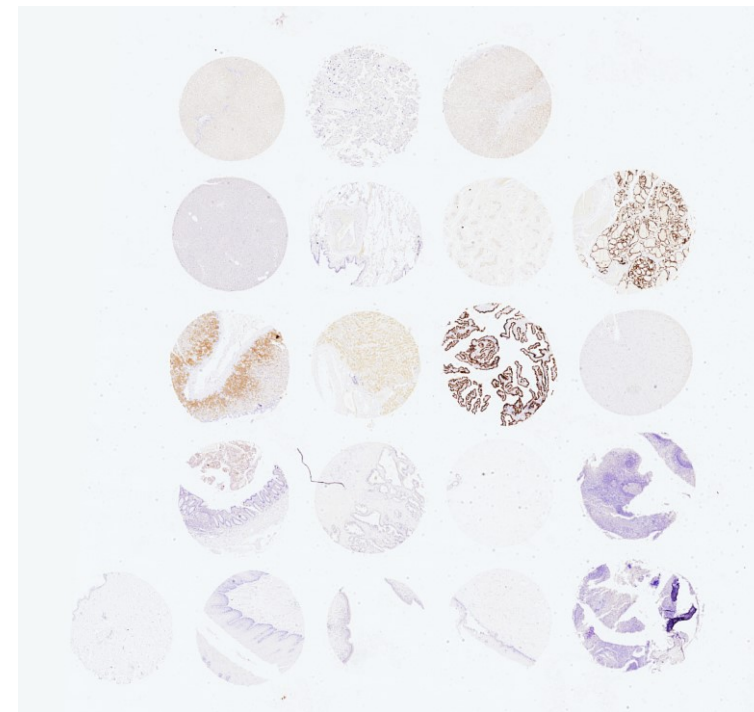
Run 68	1. Identification renal cell carcinoma.
	2. Identification of gynecological carcinoma.

3.1 Material – TMA Normal;

TMA normal	Expected result*		Obtained result**	Comments
Tissue	Pos. (cell type)	Neg.		
Thyroidea	Virtually epithelial cells 3+		Acceptable	
Brain		X	Acceptable	
Tonsil 1		X	Acceptable	
Tonsil 2		X	Acceptable	
Spleen		X	Acceptable	
Adrenal gland		X	Acceptable	
Testis		X	Acceptable	
Tuba	Virtually all secretory cells 3+, ciliated cells 1-2+		Acceptable	
Mamma		X	Acceptable	
Cervix uteri		X	Acceptable	
Placenta		X	Acceptable	
Lung		X	Acceptable	
Kidney	Virtually all epithelial cells in tubules and capsule 2-3+		Acceptable	Areas neg - necrosis
Prostate		X	Acceptable	
Bladder		X	Acceptable	
Liver		X	Acceptable	
Pancreas		X	Acceptable	
Stomach		X	Acceptable	Diffuse cytoplasmic reaction in fundus cells
Colon/Appendix		X	Acceptable	
Esophagus		X	Acceptable	
Skin		X	Acceptable	

* Descriptive concerning negative or positive (if pos; which cells, intensity and extent 1+ weak, 2+ moderate, 3+ strong)

** Acceptable / Uacceptable



NordiQC working method for IHC assay validation/verification

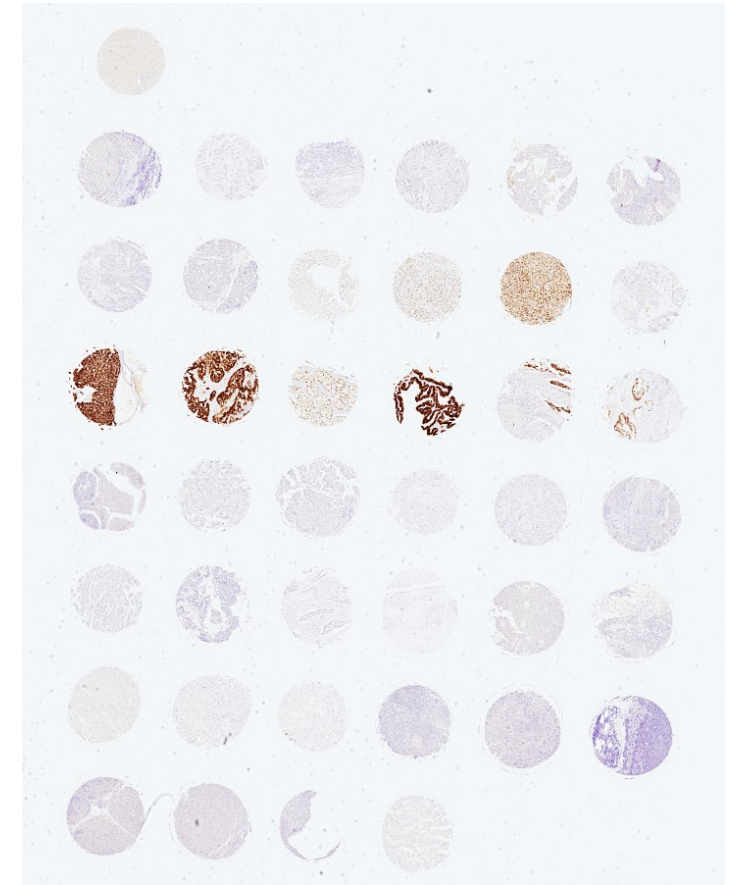
For initial analytic validation/verification of every assay used clinically, laboratories should achieve at least **90% overall concordance between the new assay and the comparator assay or expected results.**

3.2 Material – TMA neoplasia

TMA neoplasia	Expected result*		Obtained result**	Comments
Tissue	Pos.	Neg.		
Mamma duc. carc.		X	Acceptable	
Mamma duc. carc.		X	Acceptable	
Mamma lob. carc.		X	Acceptable	
Lung ad. carc.		X	Acceptable	
Lung ad. carc.		X	Acceptable	
Lung pl. carc.		X	Acceptable	
Colon ad. carc.		X	Acceptable	
Colon ad. carc.		X	Acceptable	
Renal cl. cell carc.	+ / ++ (80-95% RCC are expected pos.)		Acceptable	
Renal cl. cell carc.	+ / ++ (80-95% RCC are expected pos.)		Acceptable	Weak but distinct in >50% of tumour cells
Thyr. fol. carc.	++		Acceptable	
Thyr. med. carc.		X	Acceptable	
Serous ovarie carc.	++		Acceptable	
Serous ovarie carc.	++		Acceptable	
Clear cell ov. carc.	++		Acceptable	
End. ovarie carc.	++		Acceptable	
Uterin endom. carc.	++		Acceptable	
Uterin endoc. carc.	++		Acceptable	
Seminoma		X	Acceptable	
Seminoma		X	Acceptable	
Prostate ad. carc.		X	Acceptable	
Prostate ad. carc.		X	Acceptable	
Sq. cel. cervix carc.		X	Acceptable	
Inst. neu.end. carc.		X	Acceptable	
La.cel.neu.end.carc.		X	Acceptable	
Pancreas ad. carc.		X	Acceptable	
Pancreas ad. carc.		X	Acceptable	
Urothelial carc.		X	Acceptable	
Urothelial carc.		X	Acceptable	
GIST		X	Acceptable	
Leiomyosarcoma		X	Acceptable	
Rhabdomyosarcoma		X	Acceptable	
Hodgkin, classical		X	Acceptable	
Hodgkin, classical		X	Acceptable	
B-CLL		X	Acceptable	
DLBCL		X	Acceptable	
DLBCL		X	Acceptable	
Melanoma		X	Acceptable	
Melanoma		X	Acceptable	

* Neg.; <1%, Pos. + ≥1%-9%, Pos. ++ ≥10% - if any cut-off as semiquantitative threshold for pos.,neg. this is used and indicated

Verification and validation of IHC assays applied as NordiQC reference standard assays - 23-10-2023



CAP* 2024 guidelines for IHC assay validation/verification

Assay type

RTU
Type 1

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test are invalid.

e for this antibody is tonsil.

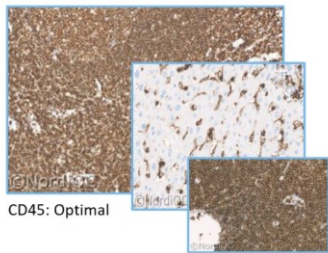
Plug and play

Verification as indicated in package insert

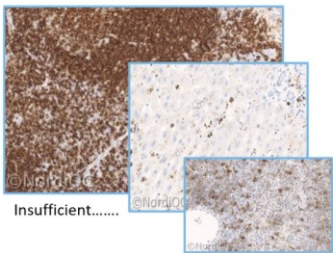
Verification of CD45 IHC assay;

Example from a RTU system provider;

Fit For Purpose; the selection....



CD45: Optimal



Insufficient.....

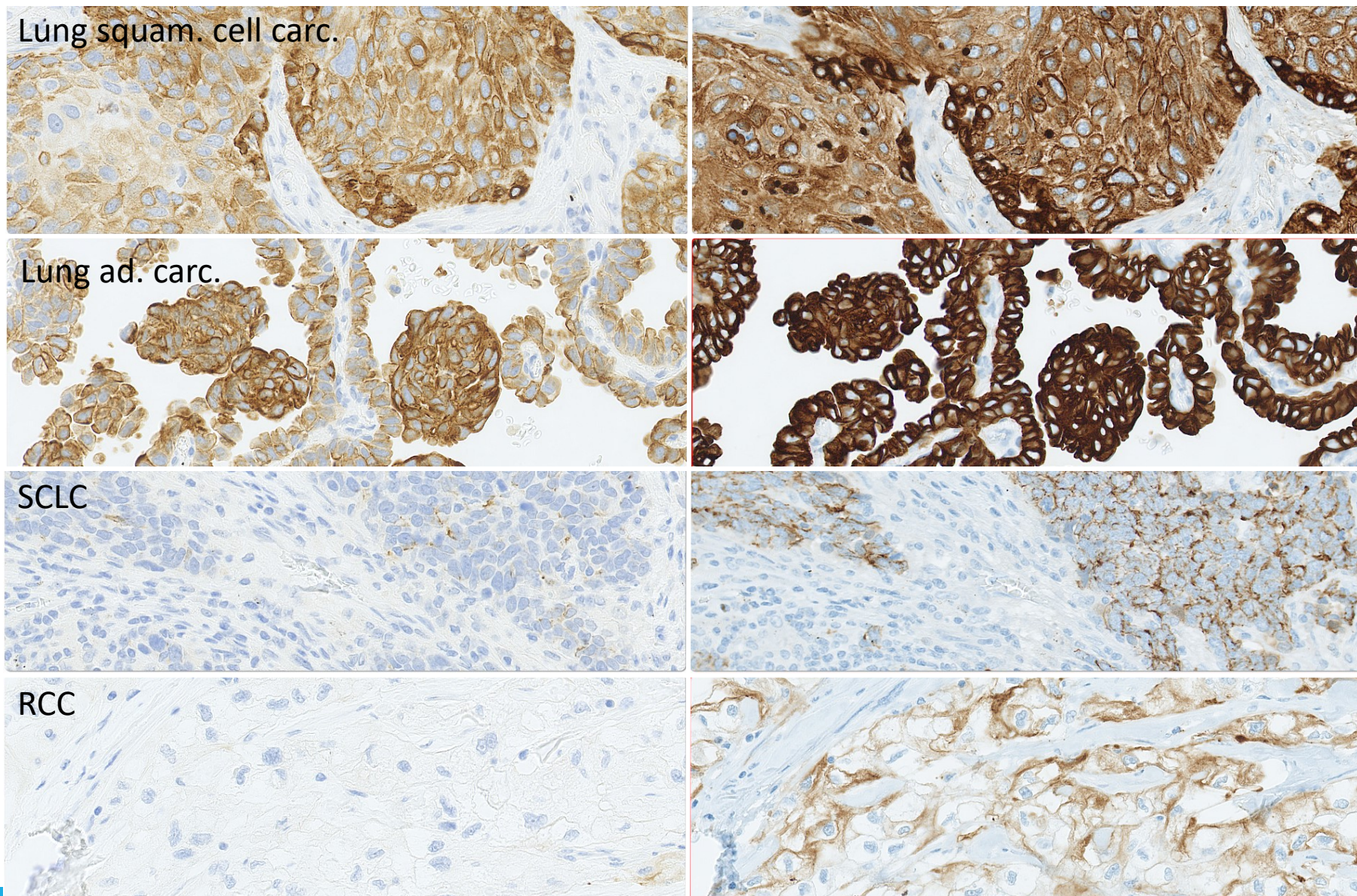
Tissues/cells with only high expression will not identify:

1. A poorly calibrated IHC assay
2. A reduced analytical sensitivity of an optimally calibrated IHC assay – assay reproducibility/consistency
3. A reduced expression level of changed tissue processing settings

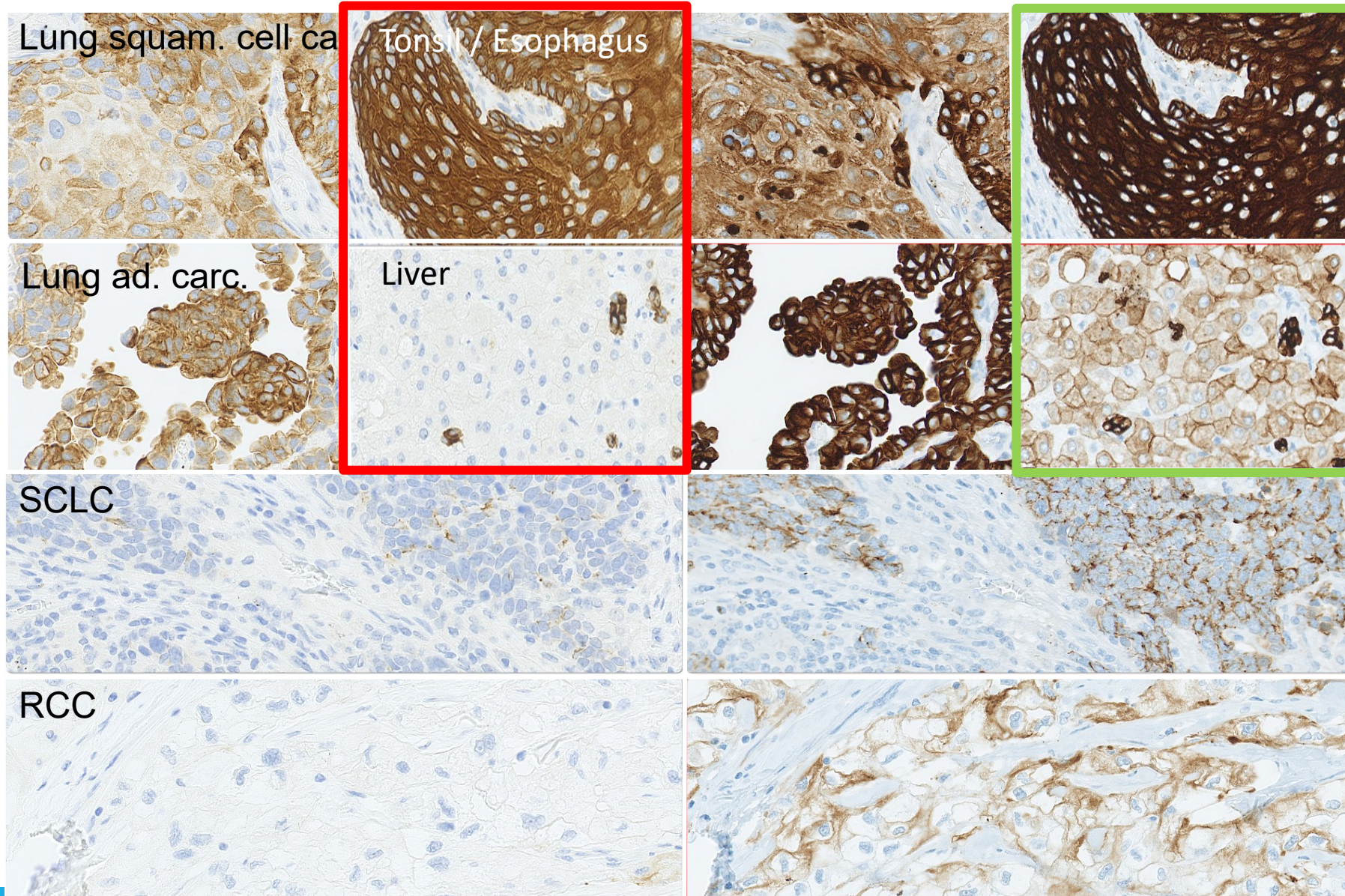
If an IHC test is used to identify the target antigen being expressed at different levels, controls must reflect this!

* College of American Pathologists

Use of samples for diagnostic / analytical validation of IHC



Use of samples for diagnostic / analytical validation of IHC



If access to ICAPCs these must be included to validate/verify IHC test performance

If dynamic range is known, this must be encountered when test material is selected

IHC tests must be fit-for-purpose....

An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

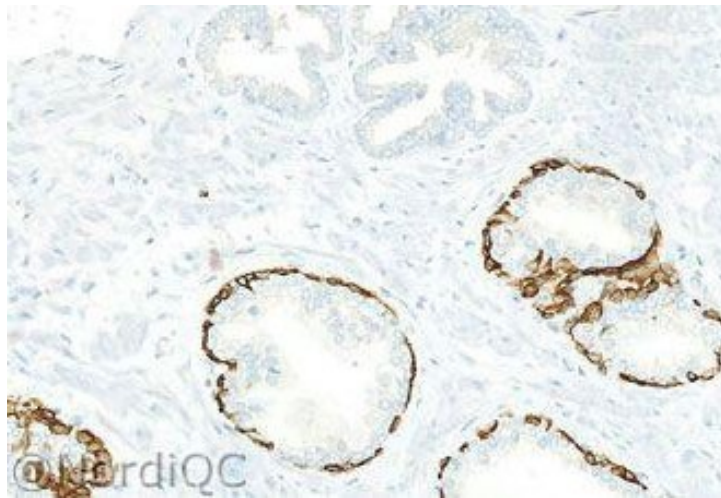
	Purpose* I	Purpose II	Comments
ALK	ALCL	Lung adenocarcinoma with ALK mutation	
CD34	Dermatofibrosarcoma protuberans	Stem cells / leukemia	Different pre-anal
CD56	Neuroendocrine differentiation	Lymphoma classification	
CD117	GIST	Stem cells / leukemia	Different pre-anal
CK5	PIN versus prostate cancer	Lung squamous cell carc vs adenocarcinoma	
CK-PAN	CUP*	Sentinel node status - carcinoma	For CUP a range of expr.
GATA3	Breast carcinoma – CUP	Urothelial carcinoma - CUP	
IgK / IgL	Clonality myeloma (Cytopl)	Clonality lymphoma (Membrane)	
Melan A	Melanoma	Sex cord tumours [‡]	[‡] mAb A103 only
PAX5	B-cell lineage marker (Lymphoma)	Hodgkin	
SOX10	Melanoma - CUP	TNBC - CUP	
TTF1	Lung ad. carc. - CUP	Lung squamous cell carc vs adenocarcinoma	

Identification of purpose of the test

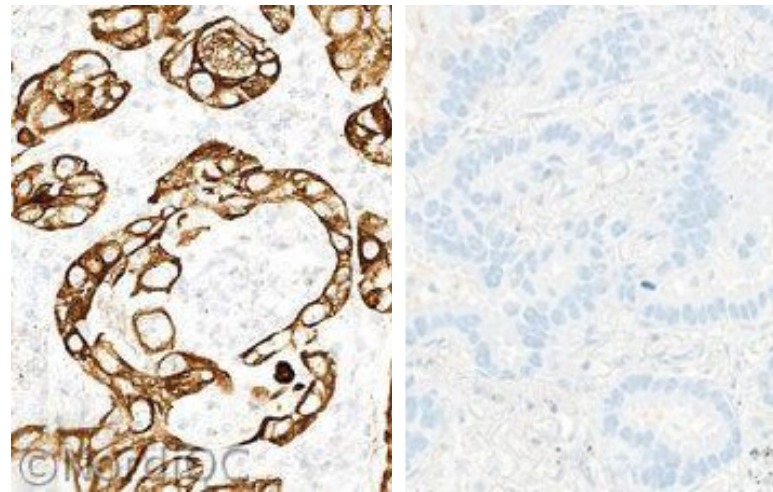
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

IHC for CK5

1. To differentiate prostate gland hyperplasia/PIN from prostate adenocarcinoma
2. Identify squamous cell differentiation in lung carcinomas
3.



Prostate sample



Lung sample

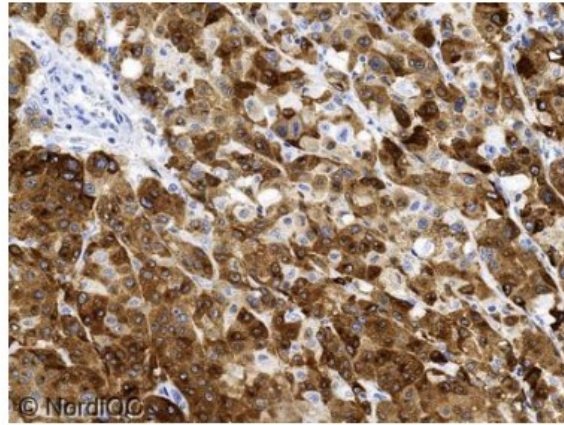
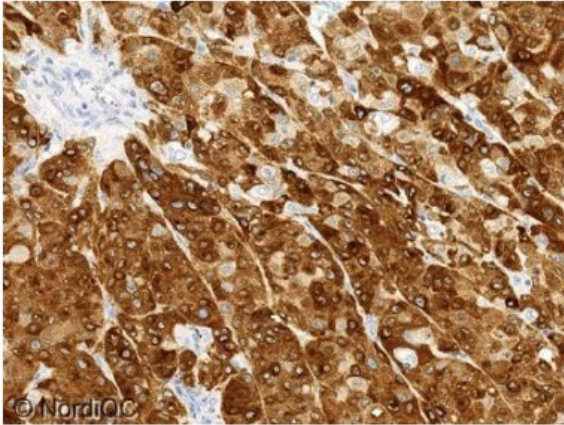
Same protocol applied for two different purposes and meeting the requirements

(source; www.nordiqc.org)

Identification of purpose of the test

E Torlakovic et al. AIMM 2017;25:4-11
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 1

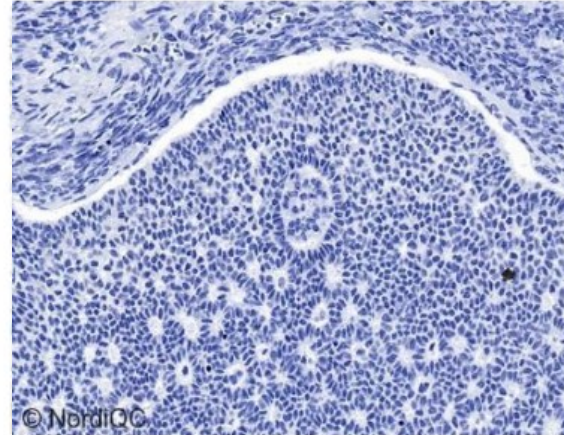
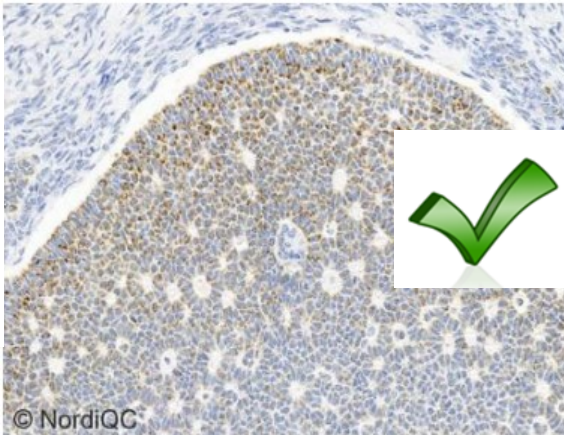
Melanoma



Purpose 1

Typically **mod.- high**
antigen expression
level

Sex cord
tumour



Purpose 2

Typically **low – mod.**
antigen expression
level and only for
clone A103

IHC method 1

IHC method 2

IHC for Melan A

Challenges for diagnostic / analytical validation of IHC

1. Limited access to relevant tissues – rare incidences
 - ALK (lung), ROS1, Myogenin..
2. New markers not described in details – limited data on test performance characteristics
 - SATB2, Claudin-4, Claudin-18, PRAME, TRPS1....
3. Limited access to reference material and/or critical expression levels
 - PD-L1, HER2, Claudin-18.2...



Where to start – how to end.....

The challenge to set-up and implement a "new biomarker" in the lab.

TABLE 5. New Biomarkers Introduced in NordiQC, 2017-2021

Target	Pass Rate (%)	Participants
BRAF	72	135
C-MYC	57	172
ERG	67	130
NKX3.1	65	49
SATB2	58	105
SMAD4	42	52
URO II/III	45	66

Which antibody to select?

How to optimize?

Reaction patterns?

Recommended tissue controls?

How to verify/validate?

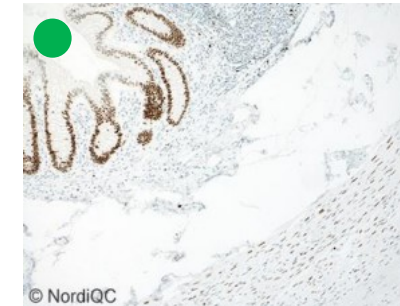
Clone EP281



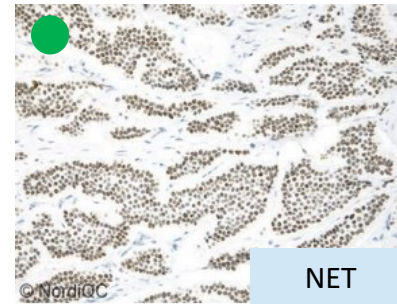
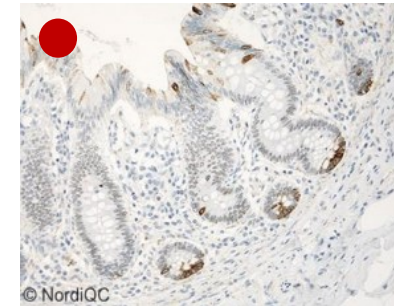
Colon

SATB2 run 58

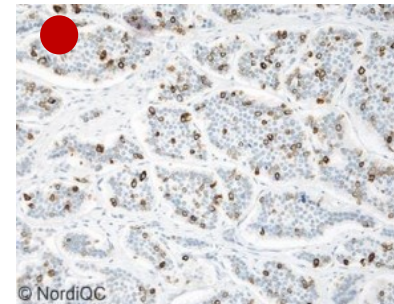
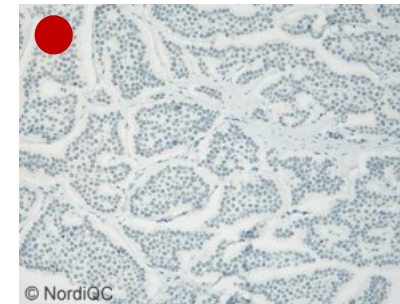
Clone CL0246



pAb 69995



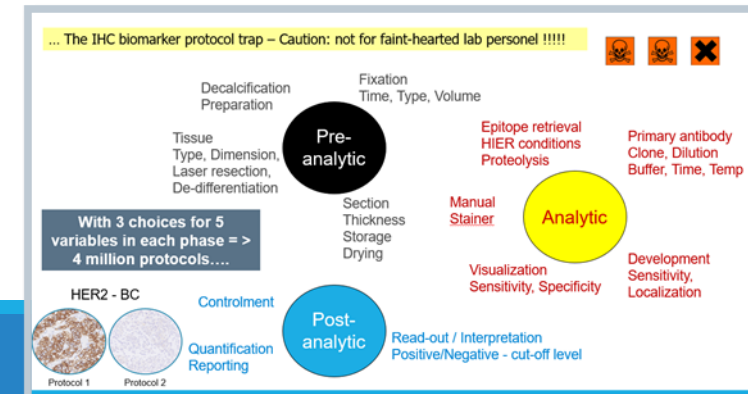
NET



Nielsen, Søren, Bzorek, Michael, Vyberg, Mogens, Røge, Rasmus.

Lessons Learned, Challenges Taken, and Actions Made for "Precision" Immunohistochemistry. Analysis and Perspectives From the NordiQC Proficiency Testing Program.

Applied Immunohistochemistry & Molecular Morphology ():10.1097/PAI.0000000000001071, October 5, 2022. | DOI: 10.1097



The quality of IHC precision testing – Make the right choices....

Impact of choice of primary antibody....

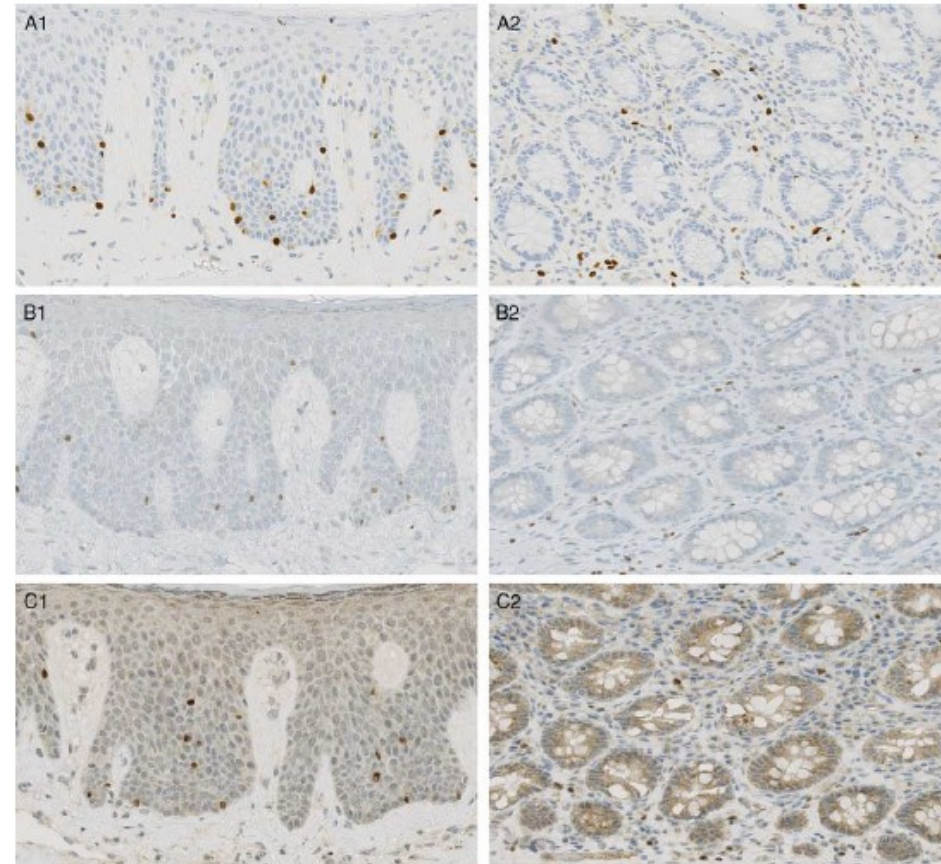
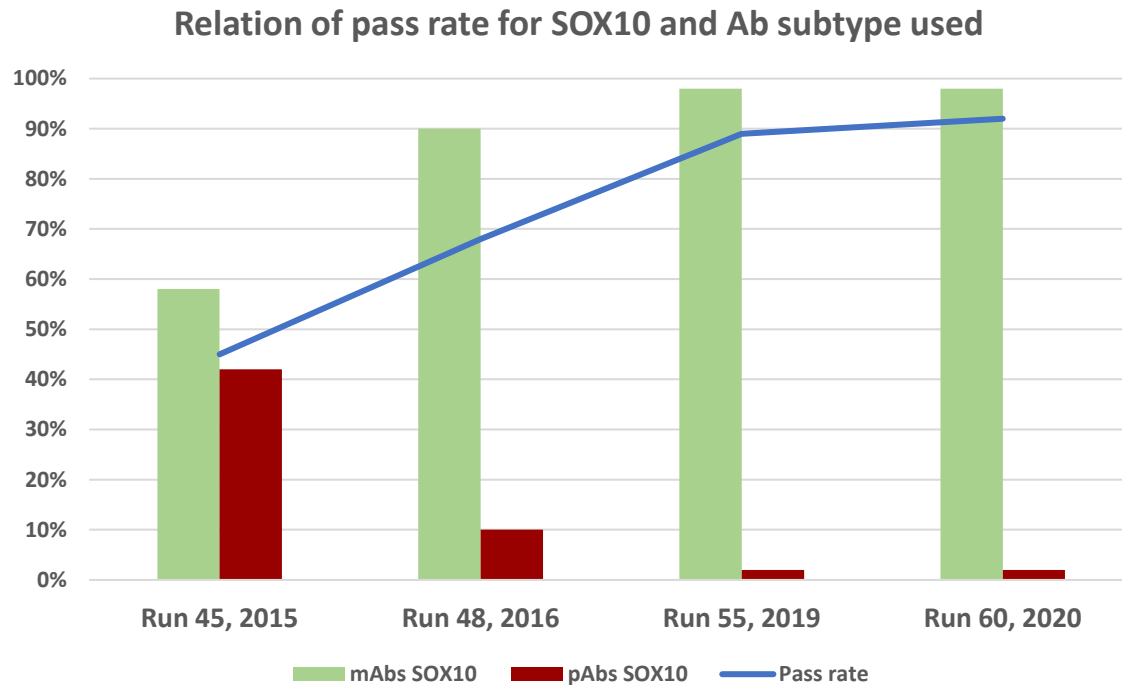
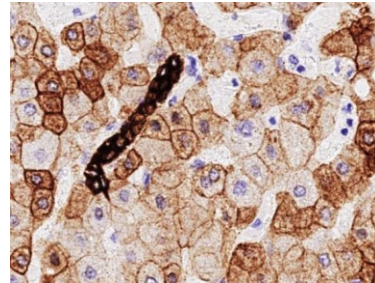
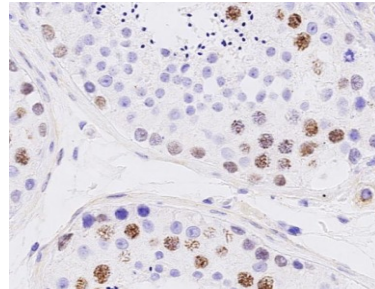
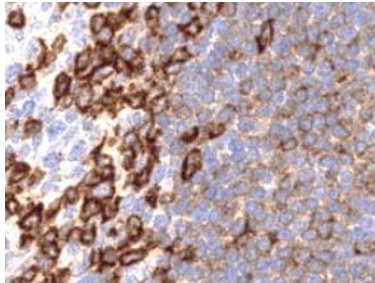


FIGURE 1. Serial sections from the SOX10 tissue microarray, NORDIQ run 45, comprising normal skin (A1 to C1, left column) and normal colon (A2 to C2, right column), IHC analyzed for SOX10 in 3 laboratories (A–C). A1, A2 (laboratory A) show an optimal result based on mAb EP268: strong nuclear staining of melanocytes in the skin and Schwann cell nuclei in the colon mucosa, whereas all other cells are unstained. A similar optimal result is obtained using mAb BC34. B1, B2 (laboratory B) show an insufficient results based on a pAb: the melanocyte and Schwann cell nuclei are weakly or equivocally stained. C1, C2 (laboratory C) show insufficient results also based on a pAb: the melanocyte and Schwann cell nuclei are moderately stained but there is a diffuse cytoplasmic cross-reaction (weak in the squamous epithelium and moderate in the columnar epithelium). All analyses based on pAbs used in this assessment gave staining patterns similar to laboratory B or C. mAb indicates mouse monoclonal antibody; NORDIQ, Nordic Immunohistochemical Quality Control; pAb, polyclonal antibody.

Positive and negative tissue controls – Potentials and Limitations

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

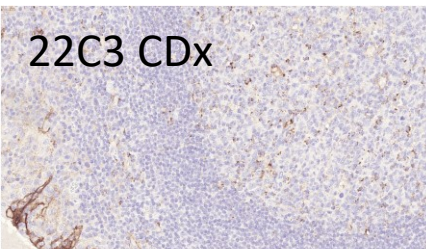
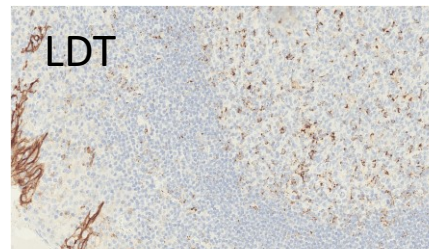
CD markers, transcription factors, tumour lineage markers...



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

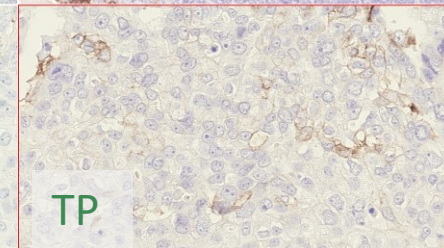
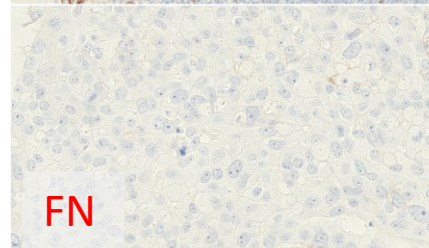
HER2, PD-L1, Claudin 18.2...

Control ok



Control ok

Tumour
PD-L1 neg
CPS <10



Tumour
PD-L1 pos
CPS ≥10

A *battle royale* for "accurate" controls for "precision" testing.....

www.histocyte.com

Cell lines

ALK and ROS1 being +/-

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

www.acepibio.com

Cell lines

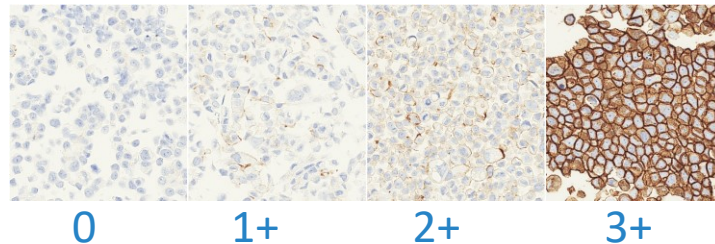
HER2, PD-L1 with dynamic range

www.arrayscience.com

Cell lines

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

MMR, melanoma markers...

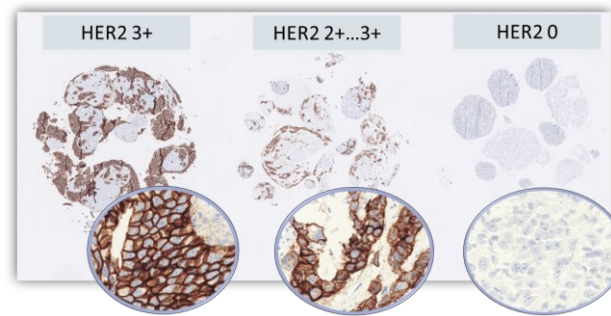


www.statlab.com

Histoids / Faux tissue

ALK +/-

HER2, PD-L1 with dynamic range

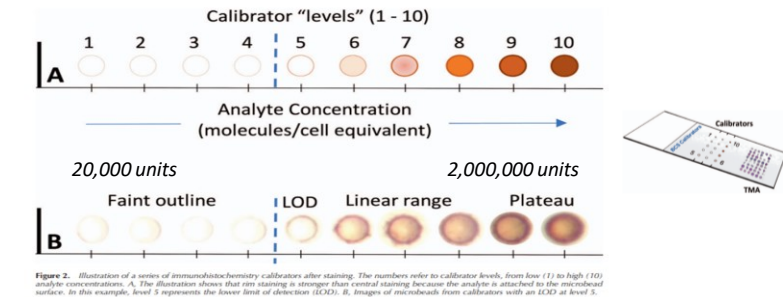


www.bostoncellstandards.com

Microbeads; Calibrators / Controls

ALK, p53, PD-L1 +/-

HER2, ER, (PD-L1) with dynamic range



Role of cell lines for IHC test development

HER2 Analyte Control^{DR}

Cell line controls for immunohistochemistry and in situ hybridization.

Research Use Only

PRODUCT AVAILABILITY

Product Code	Product Description
HCL026	X2 Cut slides
HCL027	X5 Cut slides
HCL028	X1 Cell microarray block

APPLICATION

This product is suitable for use in immunohistochemistry and in situ hybridization.

MATERIALS

Four formalin fixed paraffin embedded cell lines with a dynamic range (DR) of expression for Human Epidermal growth factor Receptor 2 (HER2).

Cell line A: Breast adenocarcinoma

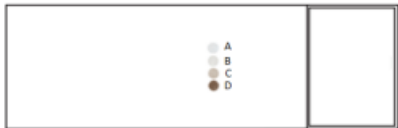
Cell line B: Breast adenocarcinoma

Cell line C: Gastric adenocarcinoma

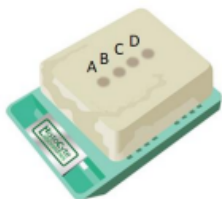
Cell line D: Breast adenocarcinoma

Cells are fixed in 10% neutral buffered formalin and paraffin wax embedded.

Sections are cut at 4µm, mounted on positively charged slides and baked overnight at 37°C.



Cell microarrays (CMA) contain cores that are 1.5-2mm in diameter and 3-3.5mm in length. It is possible to obtain over 300 sections depending on thickness.



Expression Profile

Cell Line	IHC for HER2	FISH for HER2 gene amplification
A	0	Non-amplified
B	1+	Non-amplified
C	2+	Equivocal
D	3+	Amplified

Storage and Handling

Store at 2-8°C. Do not freeze (for expiration date please see the product label)

WARNINGS AND PRECAUTIONS

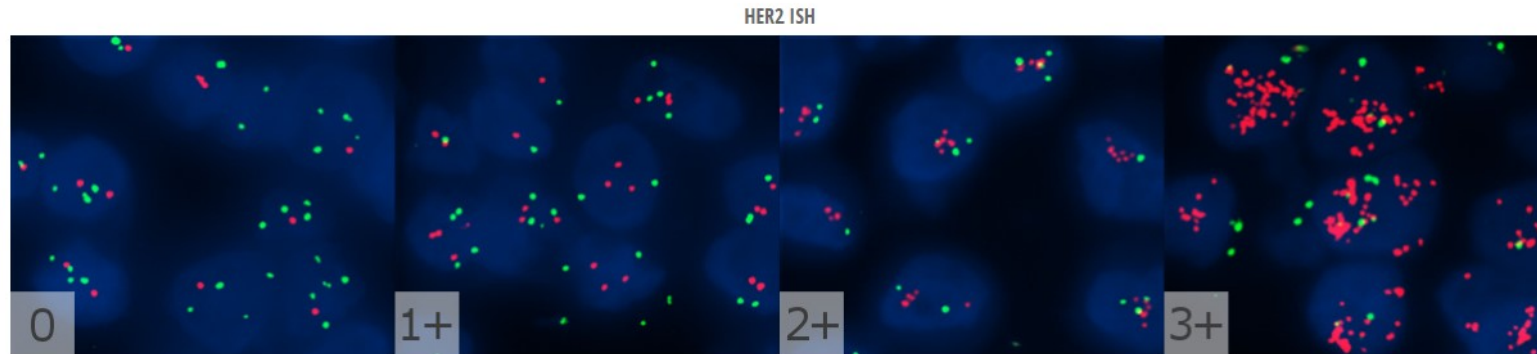
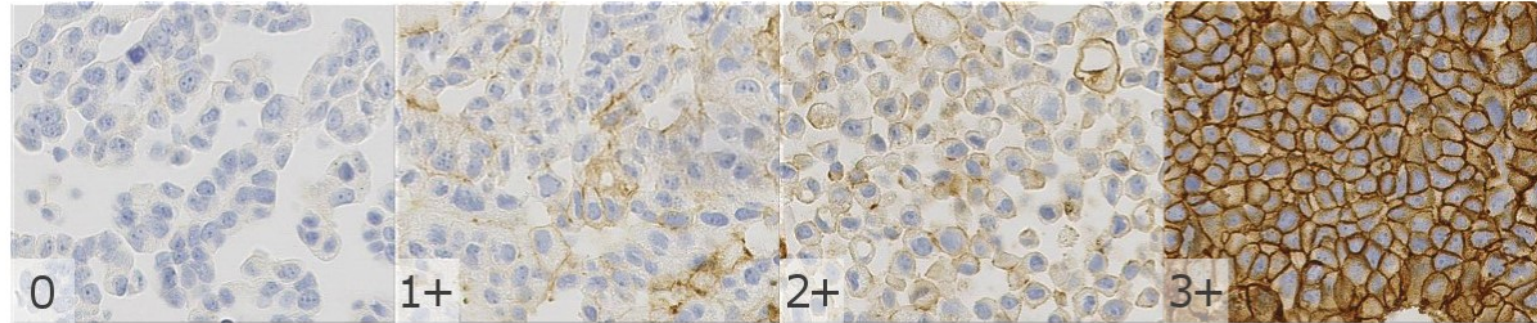
1. The product is intended for research use only.
2. It is the responsibility of the end user to determine suitability with their reagents and procedures within their laboratory.
3. Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those specified in the package insert.

TROUBLE SHOOTING

For further help please feel free to contact HistoCytel Laboratories Ltd at info@histocytel.com or call +44 (0)191 603 1007.



For updates and additional product information please visit: www.HistoCytel.com



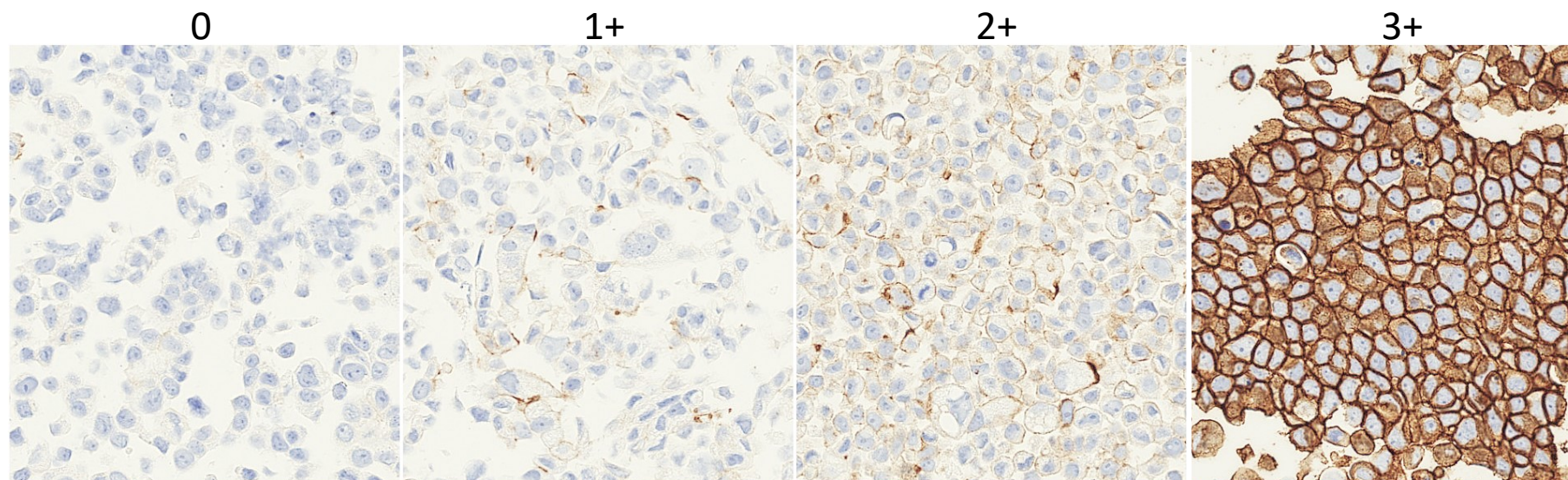
Still need evidence/proof (VALIDATION) how to correlate any change in staining pattern in cell lines for accuracy in tissues of breast carcinoma.

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?

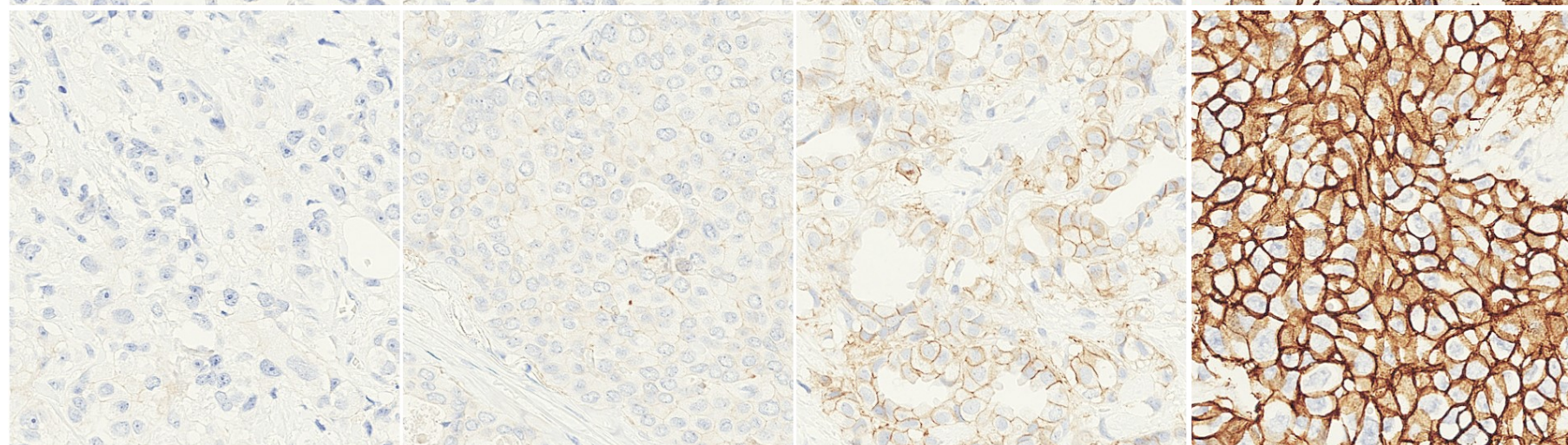
In NordiQC run
B39 15% of the
participants
used cell lines as
onslide control

Correlation of IHC for HER2 – accurate PATHWAY – cell lines and tissues

Cell lines
(HCL028 Histocyte)



Breast carcinomas



NordiQC run B34

0

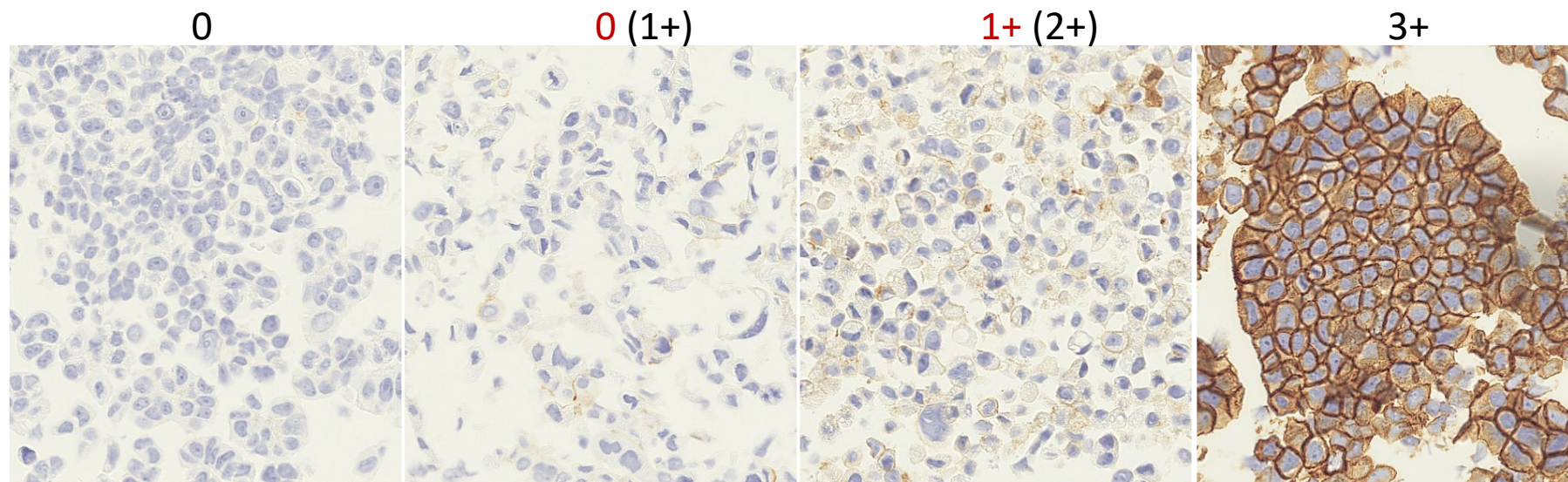
1+

2+ AMP

3+

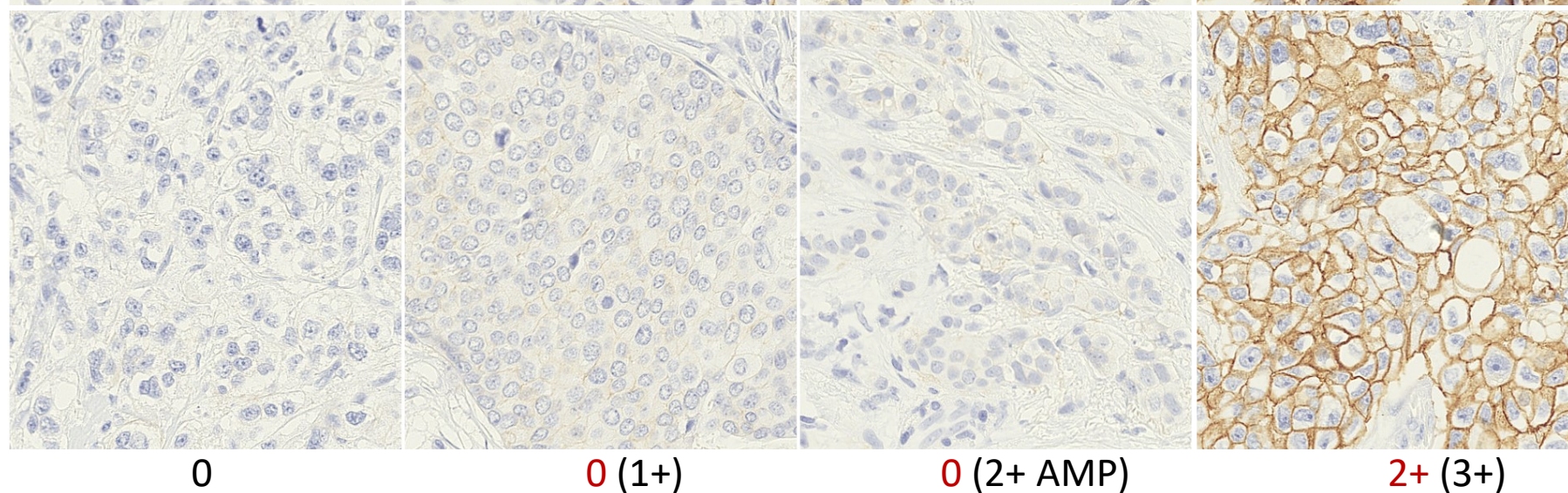
Correlation of IHC for HER2 – (inaccurate) PATHWAY – cell lines and tissues

Cell lines
(HCL028 Histocyte)



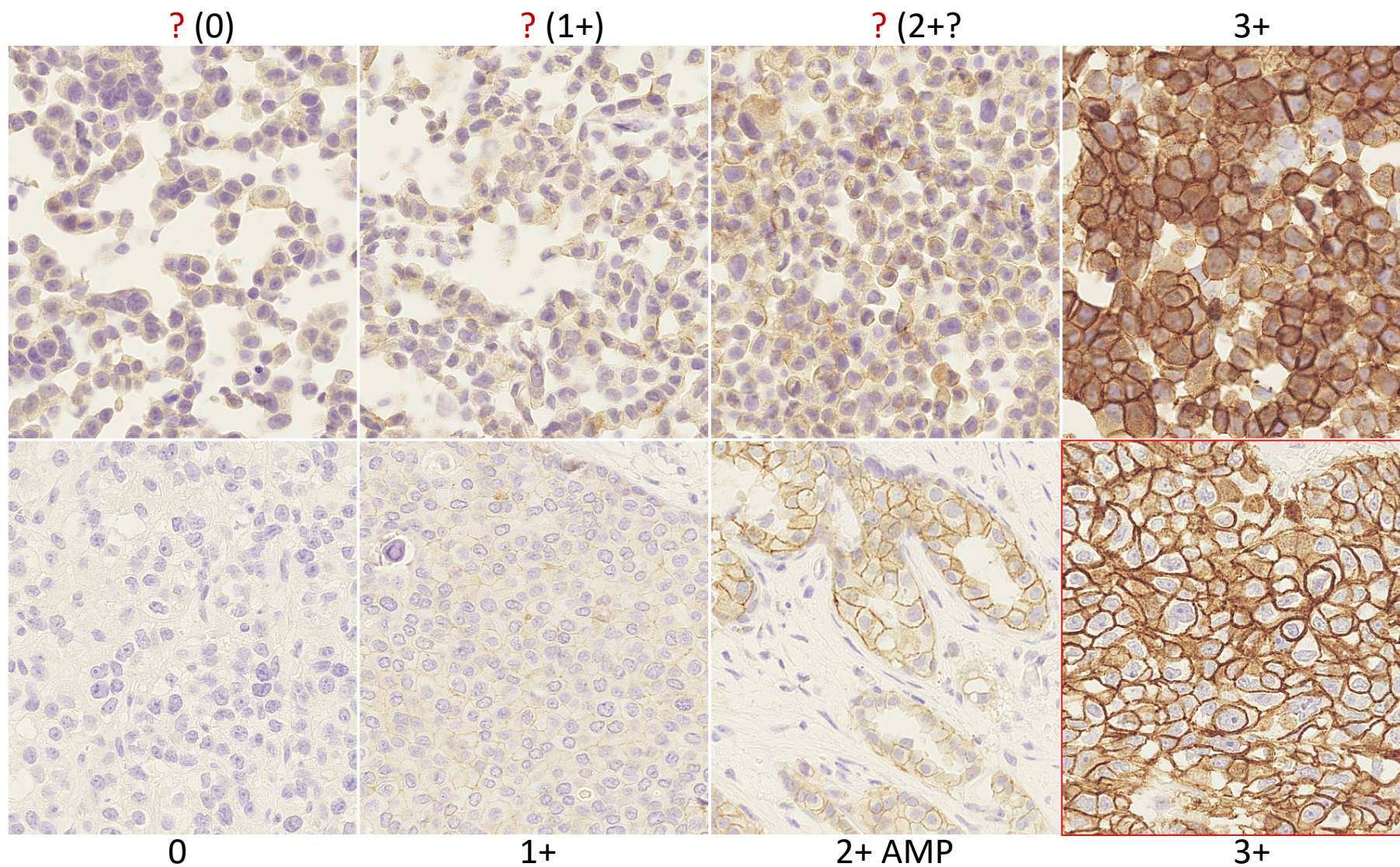
Breast carcinomas

NordiQC run B34



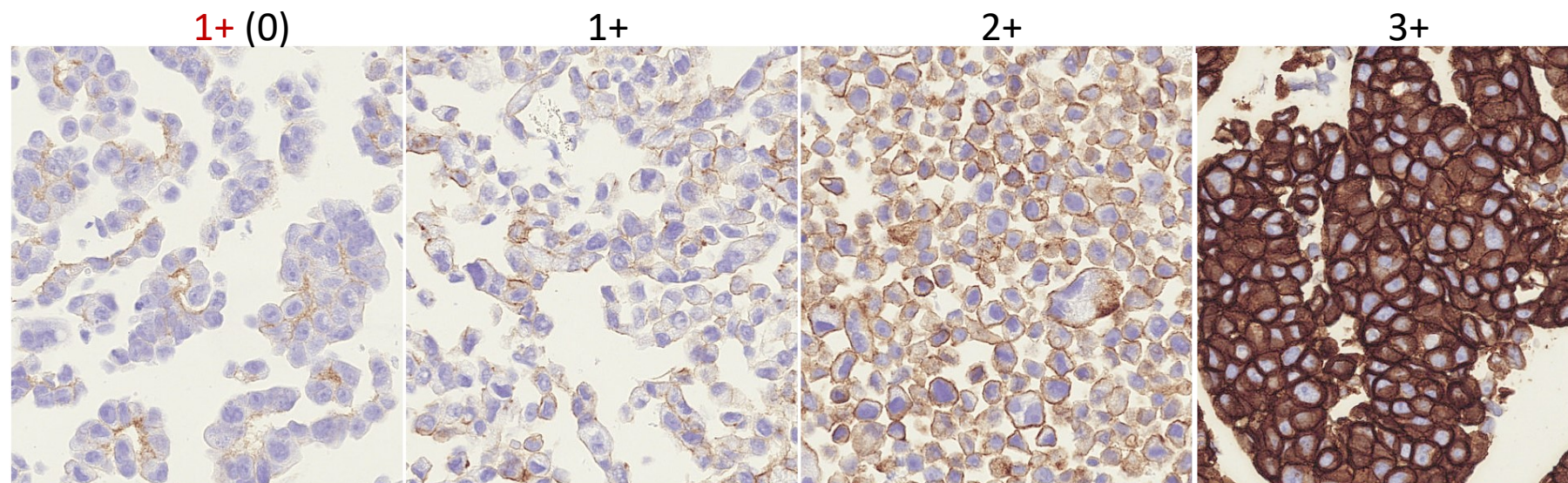
Correlation of IHC for HER2 – SP3 – cell lines and tissues

Cell lines
(HCL028 Histocyte)



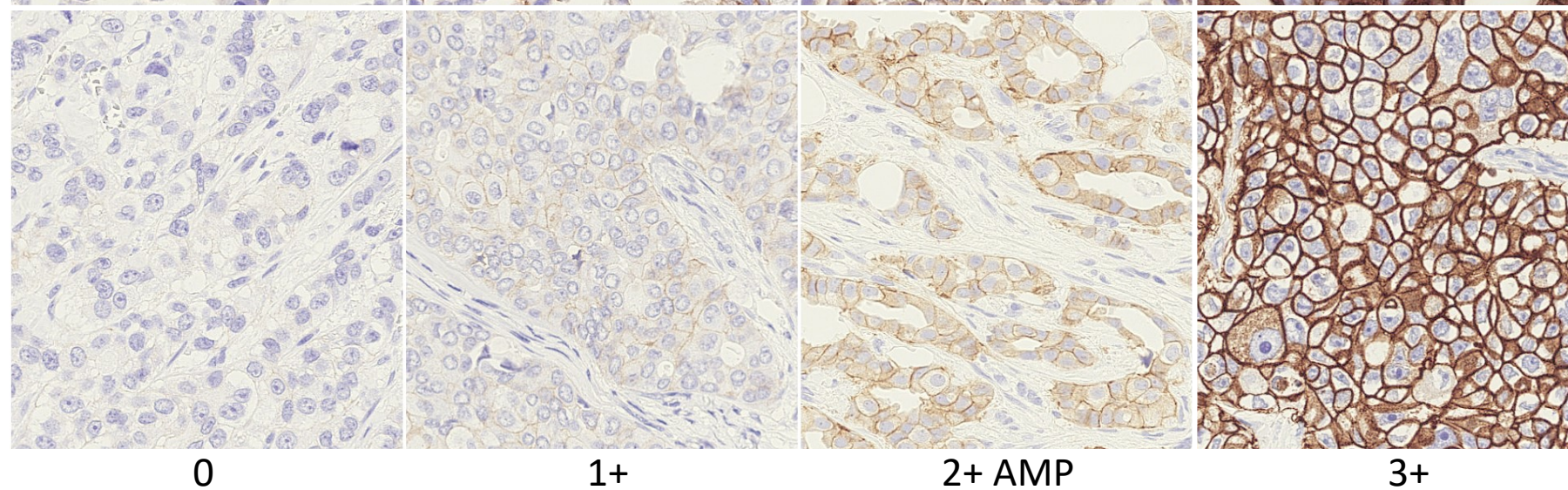
Correlation of IHC for HER2 – HercepTest 2' Gen – cell lines and tissues

Cell lines
(HCL028 Histocyte)



Breast carcinomas

NordiQC run B34

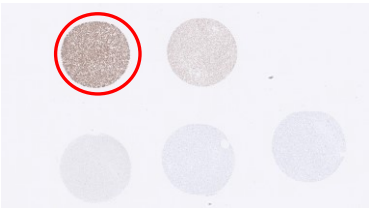
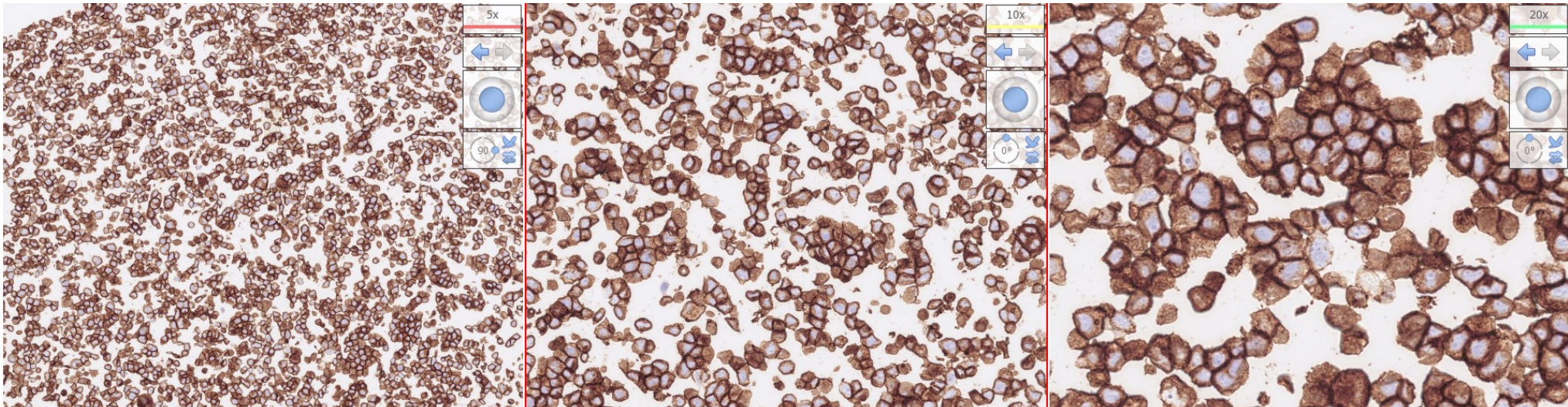


Preliminary observations
A newly designed cell line TMA

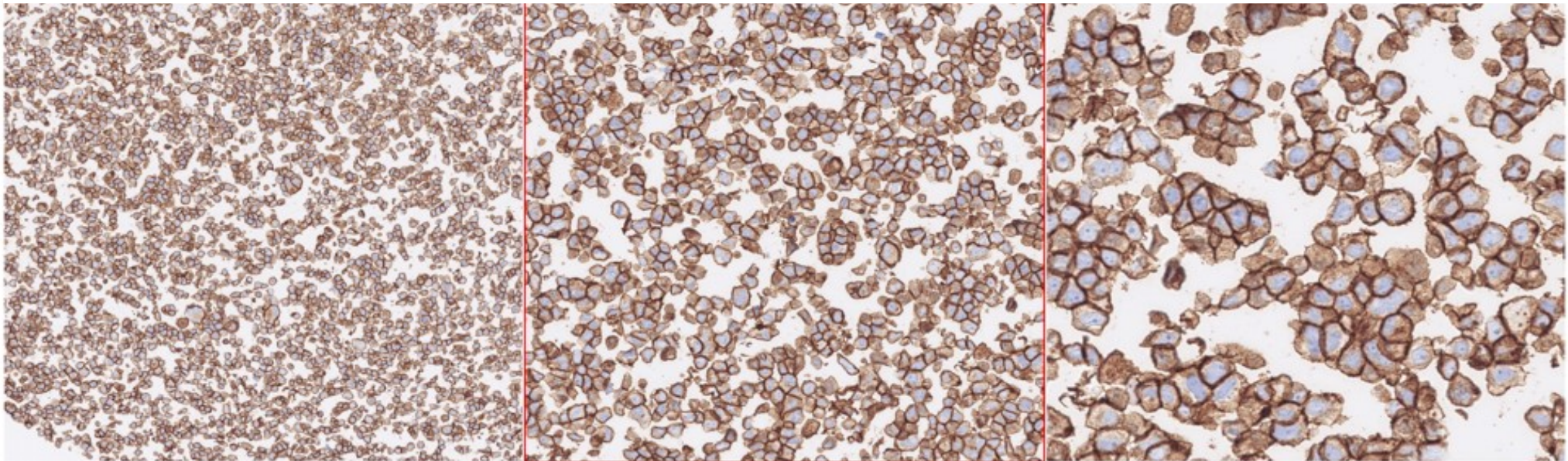
HER2 Ultralow, Low, "Classical Pos

Acepix HER2 Array 3700-0210

Acepix V4 HER2 cell lines - HercepTest 2' Gen Agilent / PATHWAY; 3+

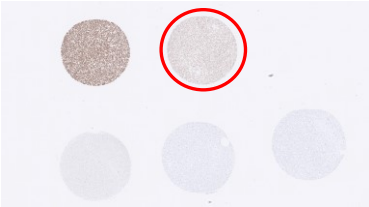
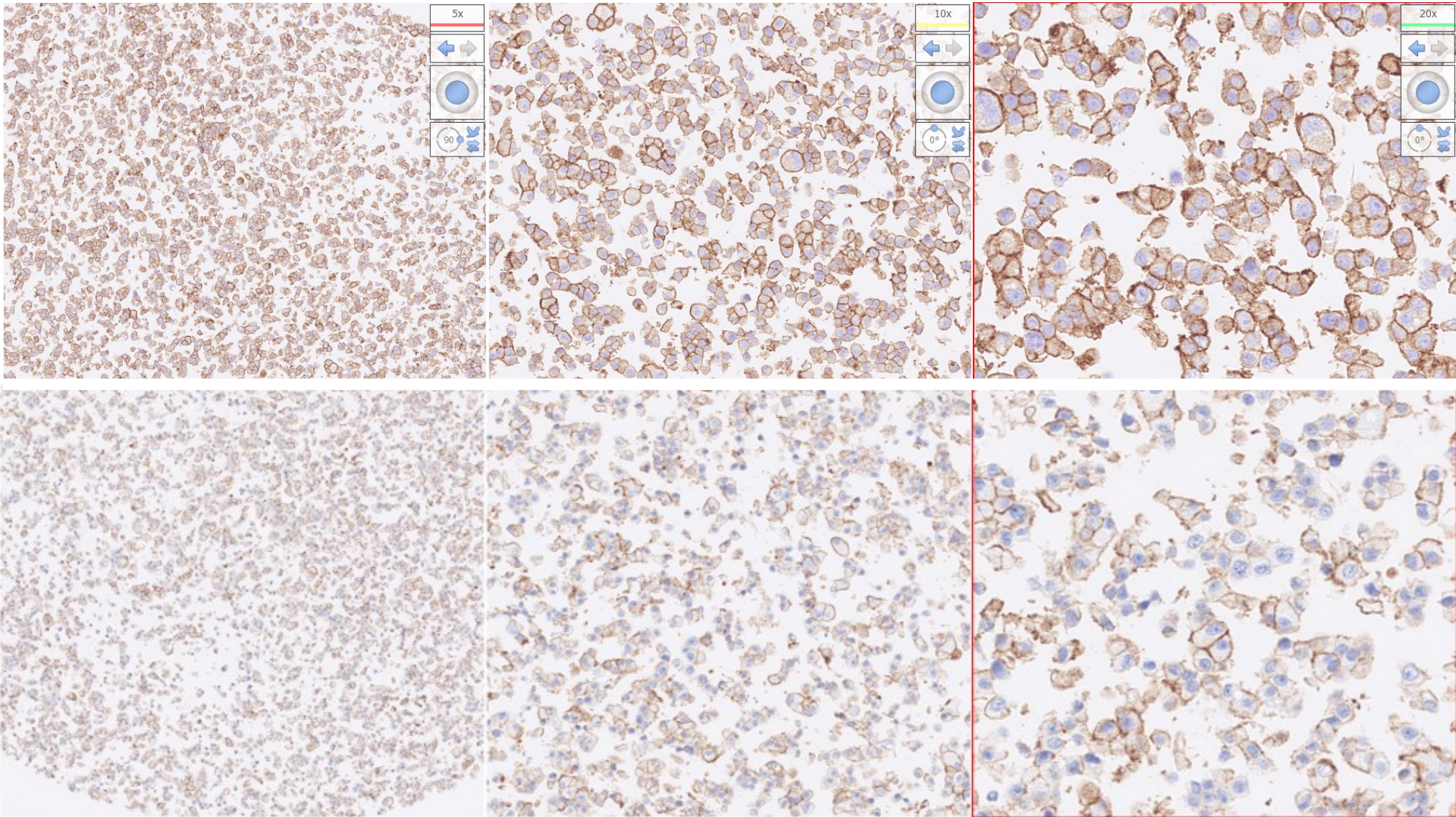


HercepTest



PATHWAY

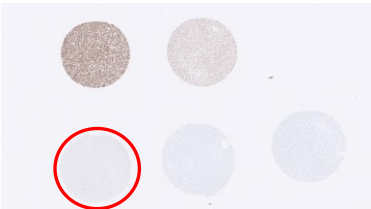
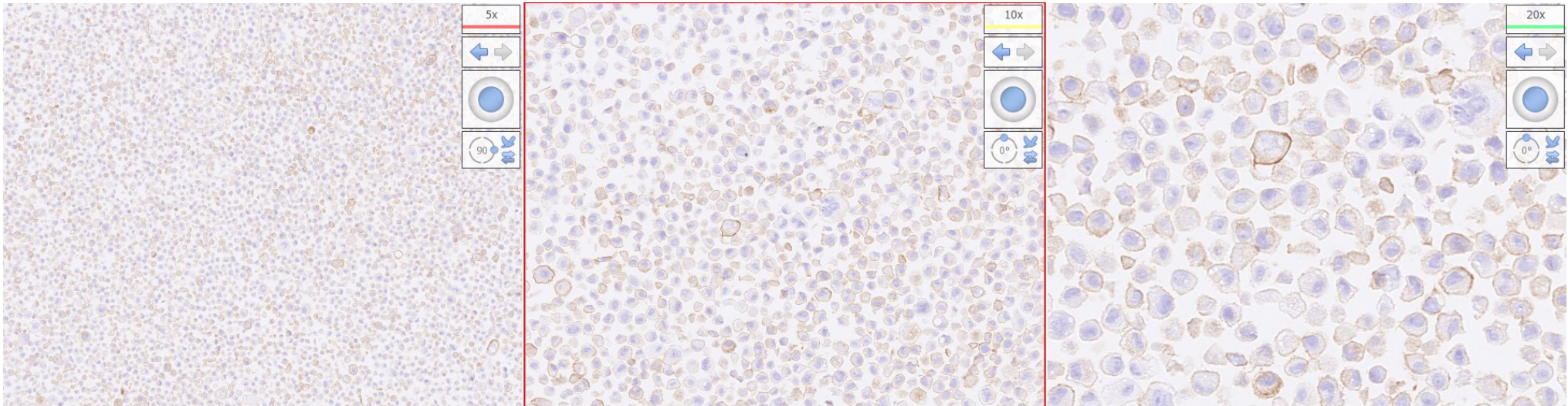
Acepix V4 HER2 cell lines - HercepTest 2' Gen Agilent / PATHWAY; 2+



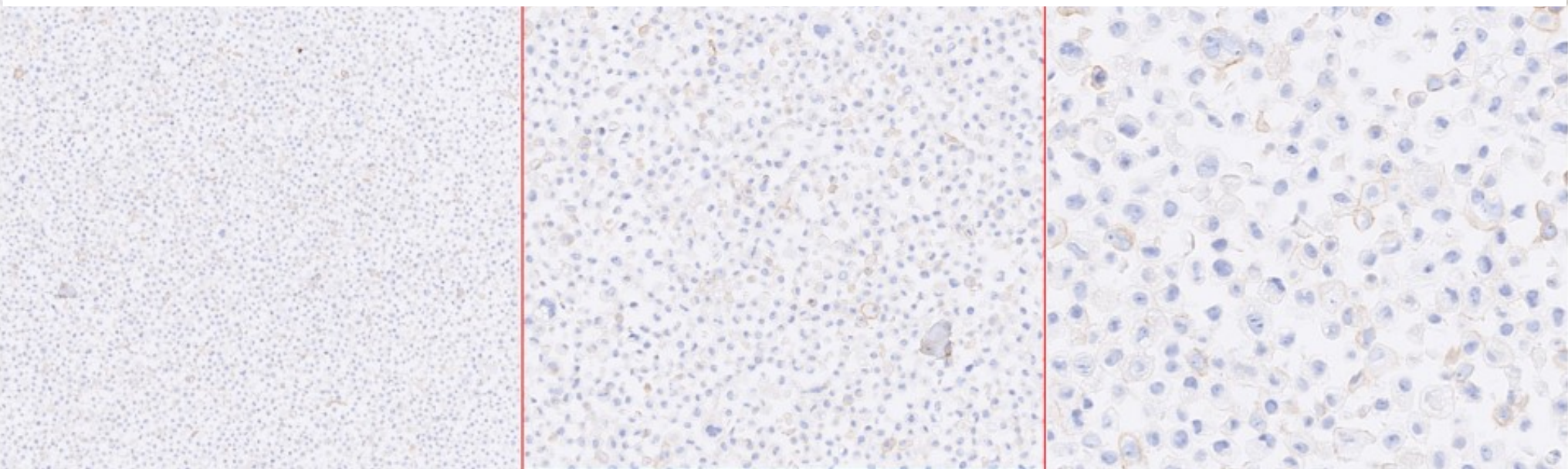
HercepTest

PATHWAY

Acepix V4 HER2 cell lines - HercepTest 2' Gen Agilent; 1+

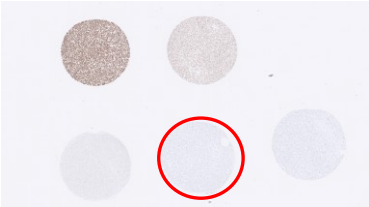
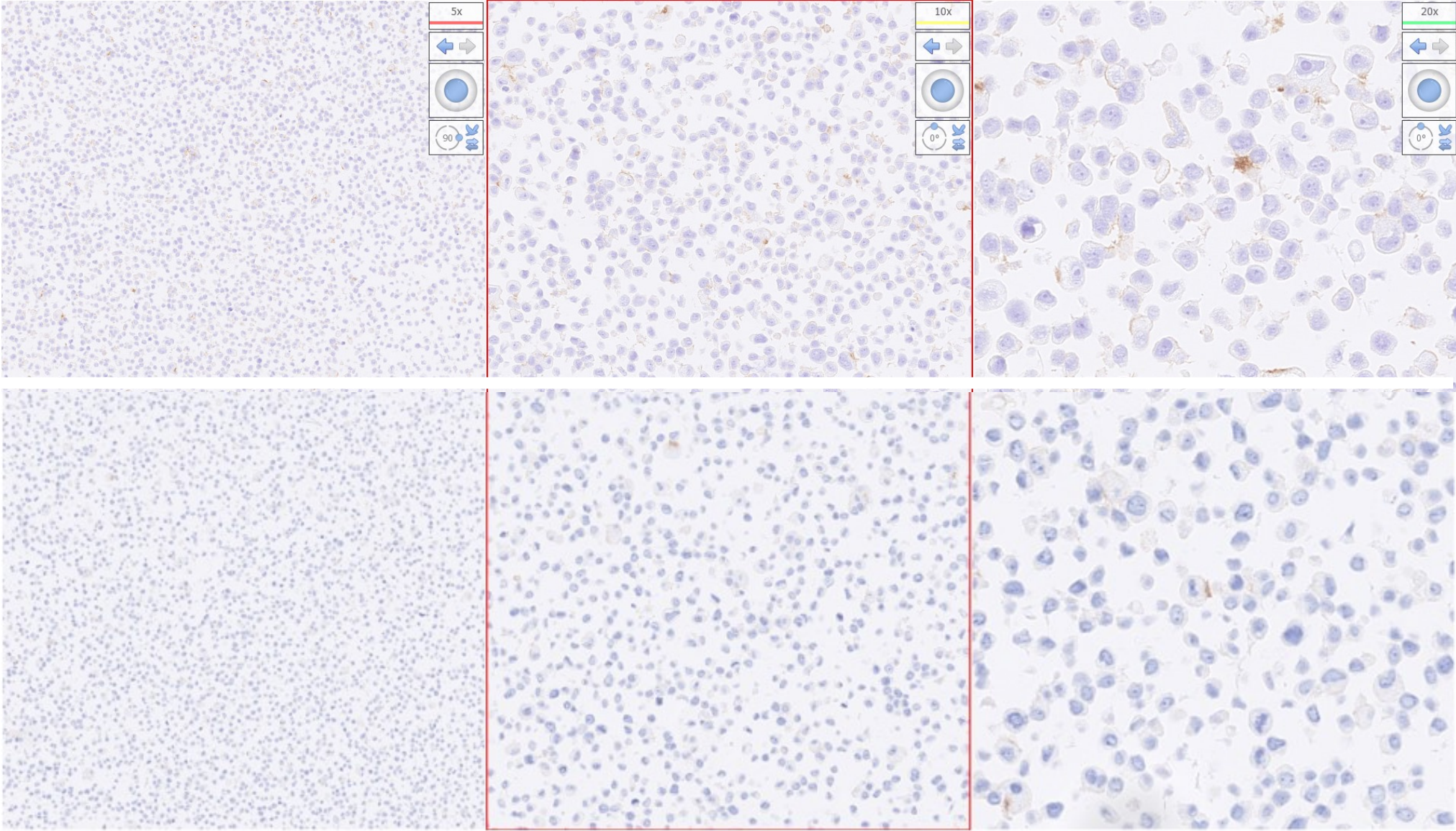


HercepTest



PATHWAY

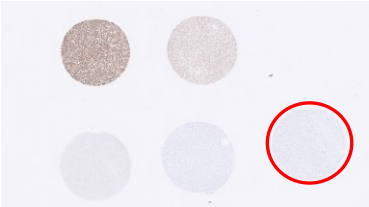
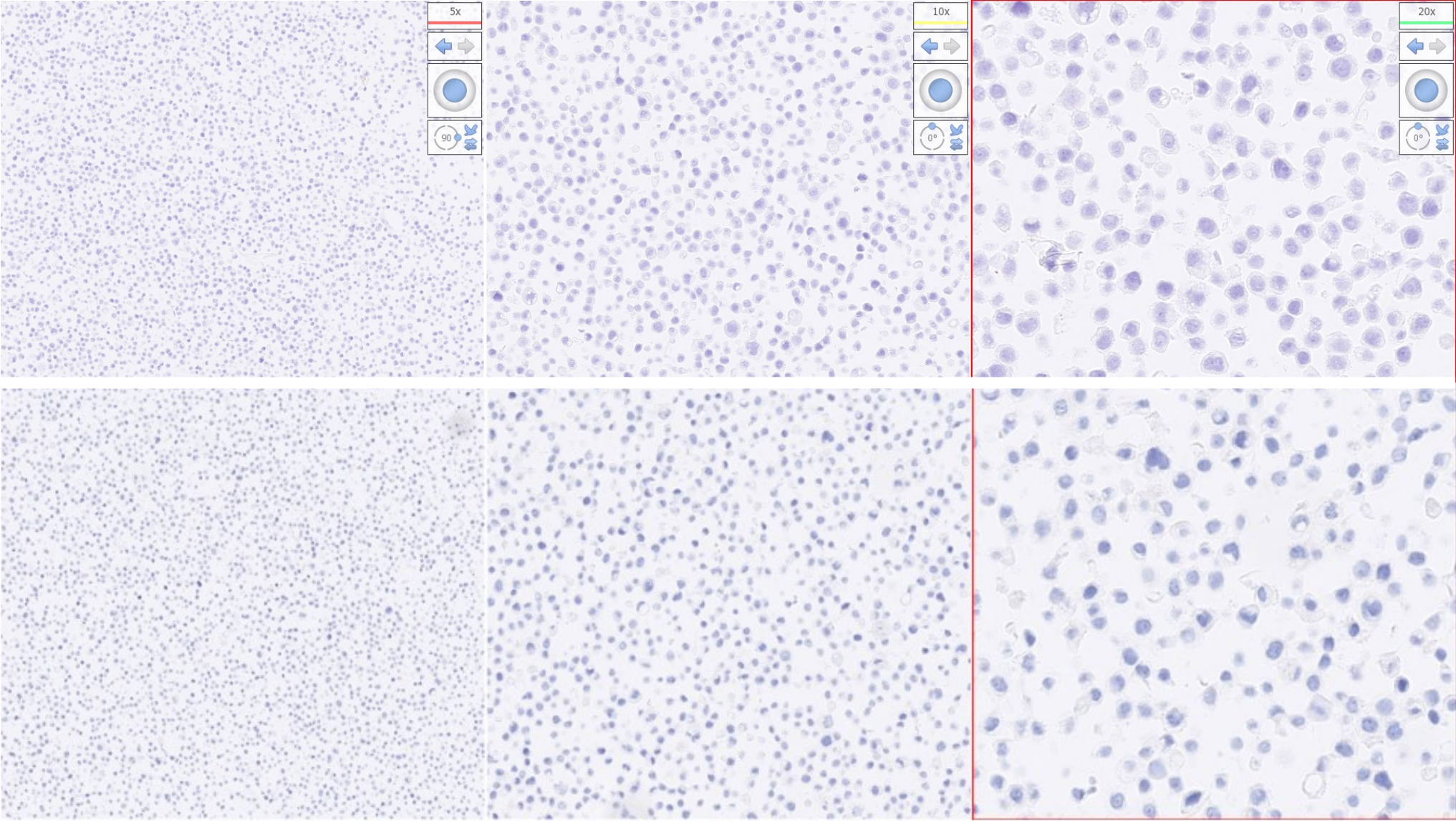
Acepix V4 HER2 cell lines - HercepTest 2' Gen Agilent / PATHWAY; >0 <1+



HercepTest

PATHWAY

Acepix V4 HER2 cell lines - HercepTest 2' Gen Agilent; 0

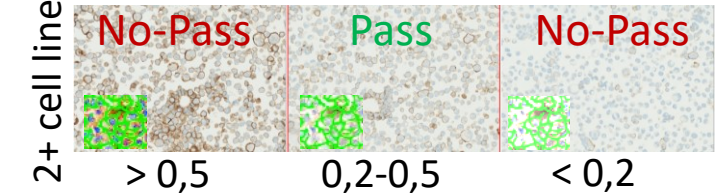
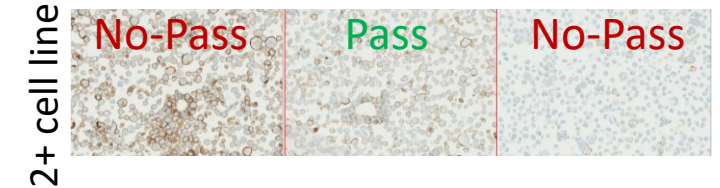
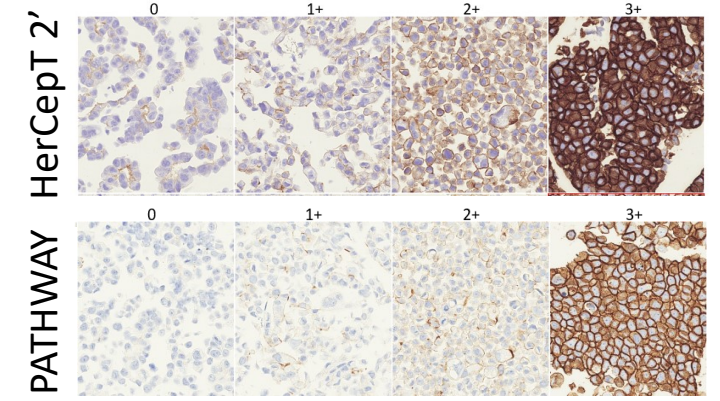


HercepTest

PATHWAY

The needs for cell lines as Quality tool for Accuracy/Precision

- Need to map staining characteristics for most commonly used IHC assays
 - The different assays will provide different patterns
- Need to identify change in patterns being critical with risk of false negative / false positive results
 - Each assay most likely will have different patterns / thresholds
- Need to integrate software as digital image analysis (DIA) or artificial intelligence (AI) to secure reproducibility
 - Identification of DIA/AI QC-score for successful versus unsuccessful test
- The DIA/AI QC-scores must be validated for each IHC assay both with focus on expected level and critical levels
 - Large scale testing on e.g. breast carcinomas with the dynamic and critical range of the target analyte
 - Both to identify e.g. "classical" HER2 overexpression and the novel HER2 low category



Analytical standards – IHC versus clinical chemistry; Calibrators

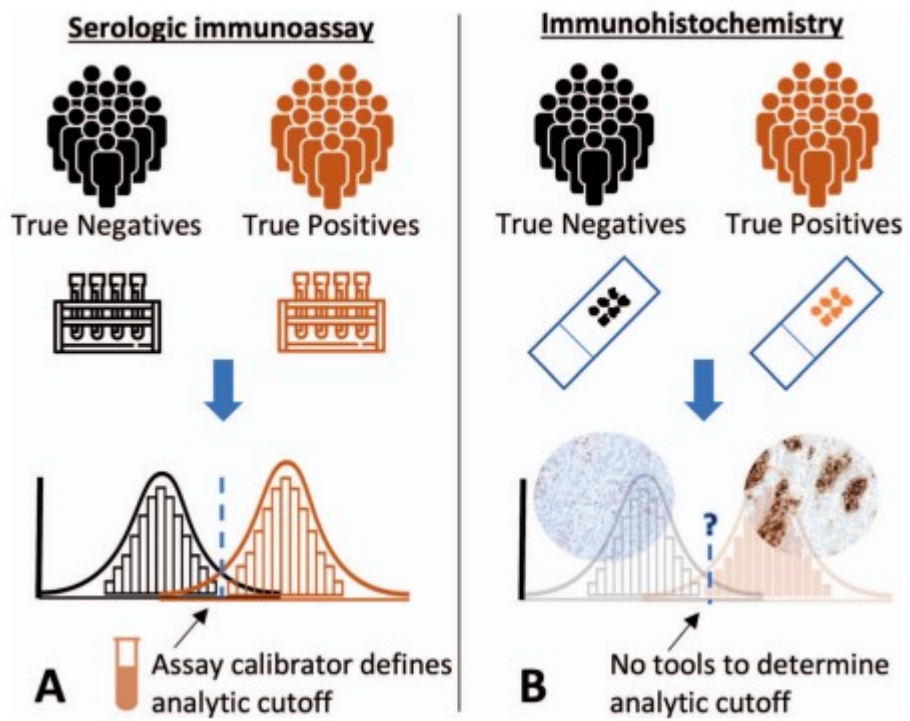
CA125; 35u/ml

CA-19; 37-40u/ml

....

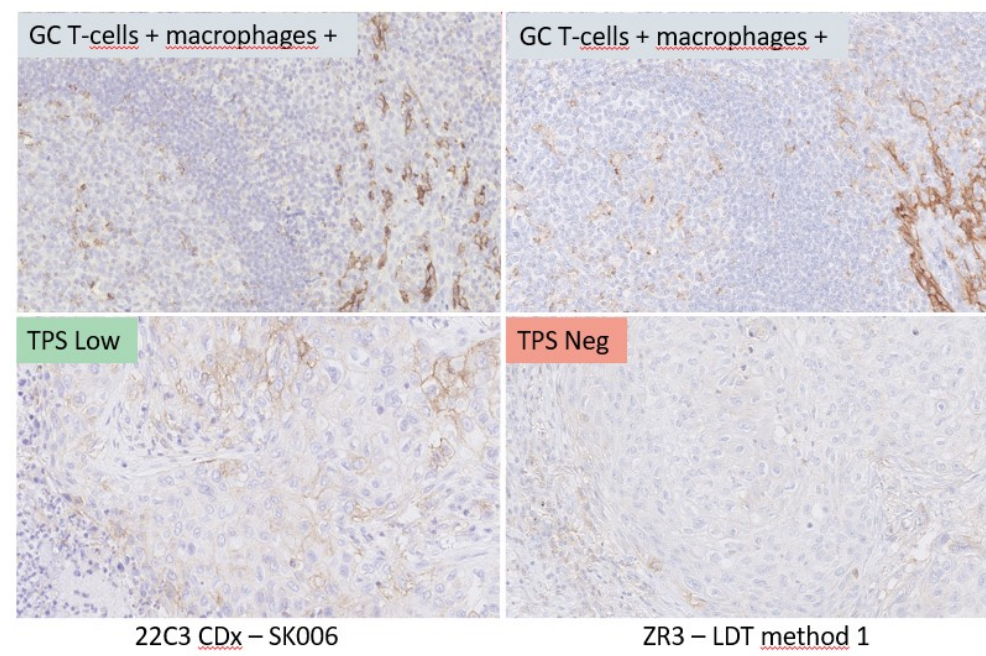


Reference standard materials



ICAPCs

No reliable tissue controls identified for semi-quantitative biomarkers as especially HER2 and PD-L1...



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;
Emma E. Todorova, MD, PhD
(Arch Pathol Lab Med. doi: 10.5858/arpa.2022-0031-RA)

Analytical standards – IHC versus clinical chemistry; Calibrators

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

ER, HER2, PD-L1 and p53

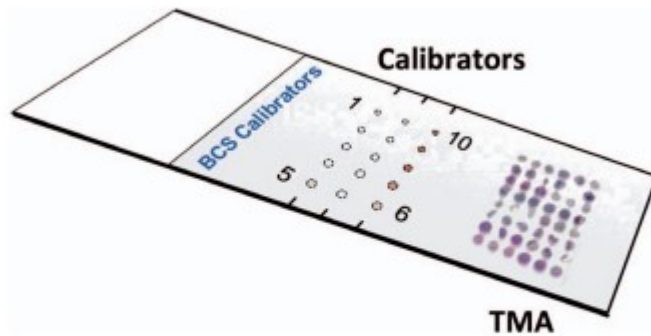
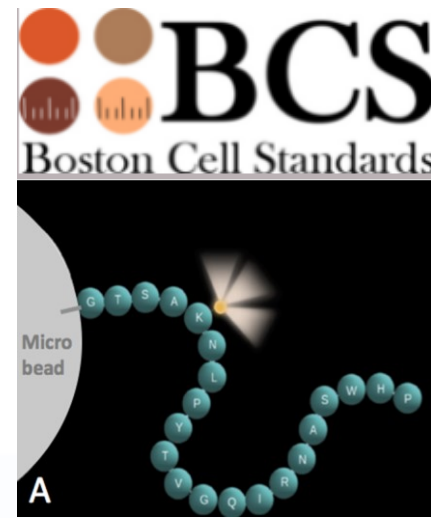


Figure 5. Illustration of the survey tool for correlating clinical accuracy (from the tissue microarray data) with analytic sensitivity (from the calibrator data). The calibrators are at up to 10 different concentrations, for example levels 1–10. The middle row depicts negative controls. Abbreviations: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

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

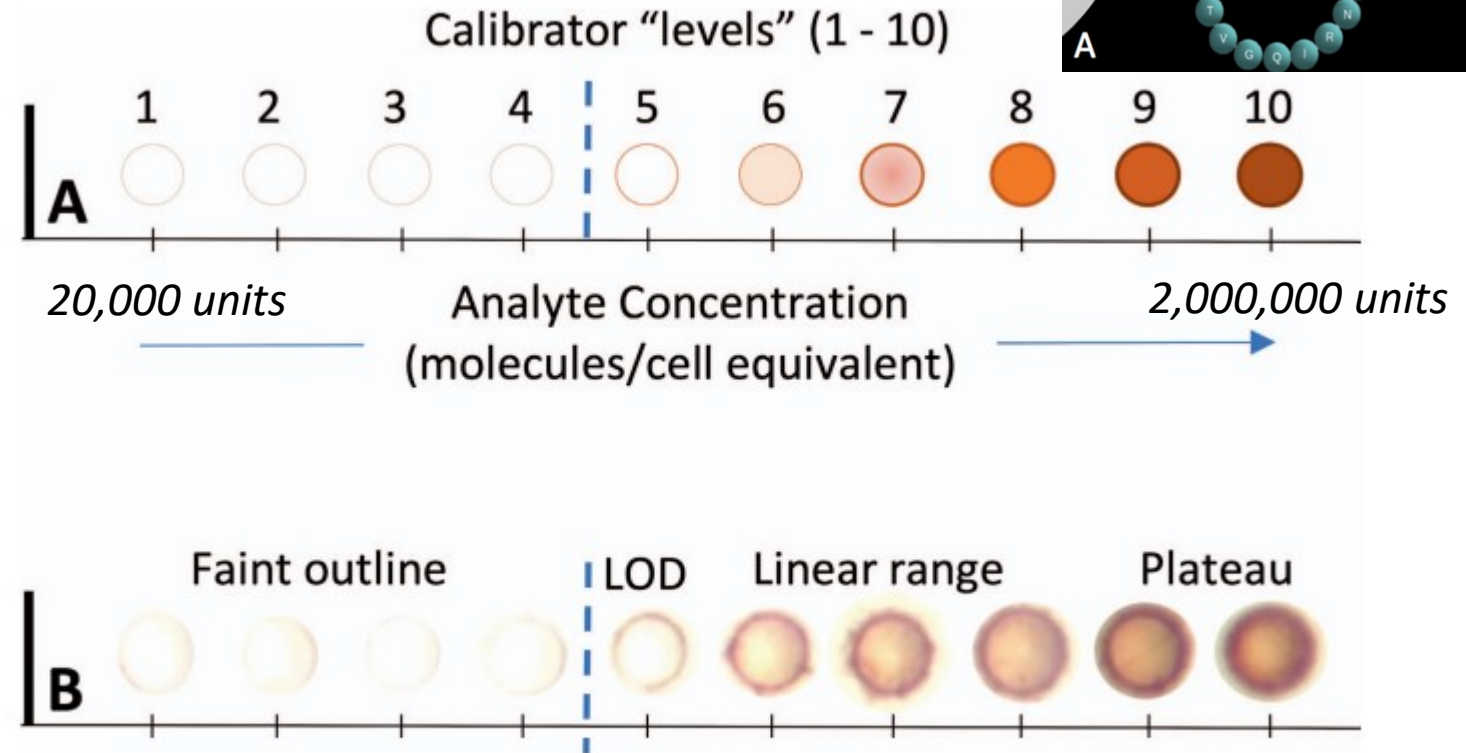


Figure 2. Illustration of a series of immunohistochemistry calibrators after staining. The numbers refer to calibrator levels, from low (1) to high (10) analyte concentrations. A, The illustration shows that rim staining is stronger than central staining because the analyte is attached to the microbead 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.

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

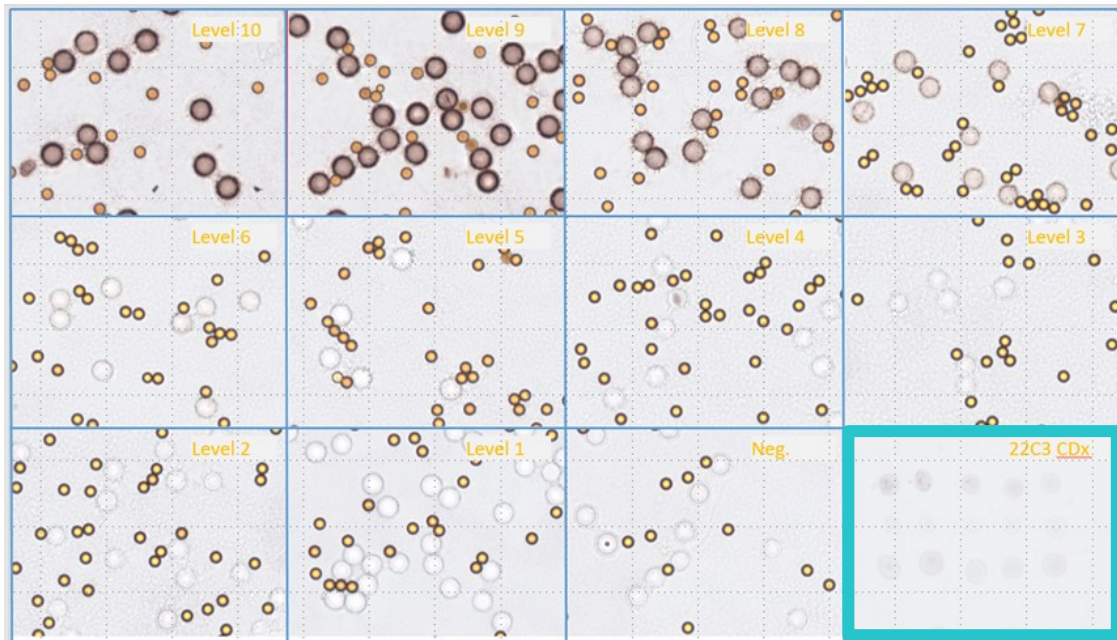
ARTICLE OPEN

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 CDx 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*

Mod Pathol. 2022;35(3):326–332.

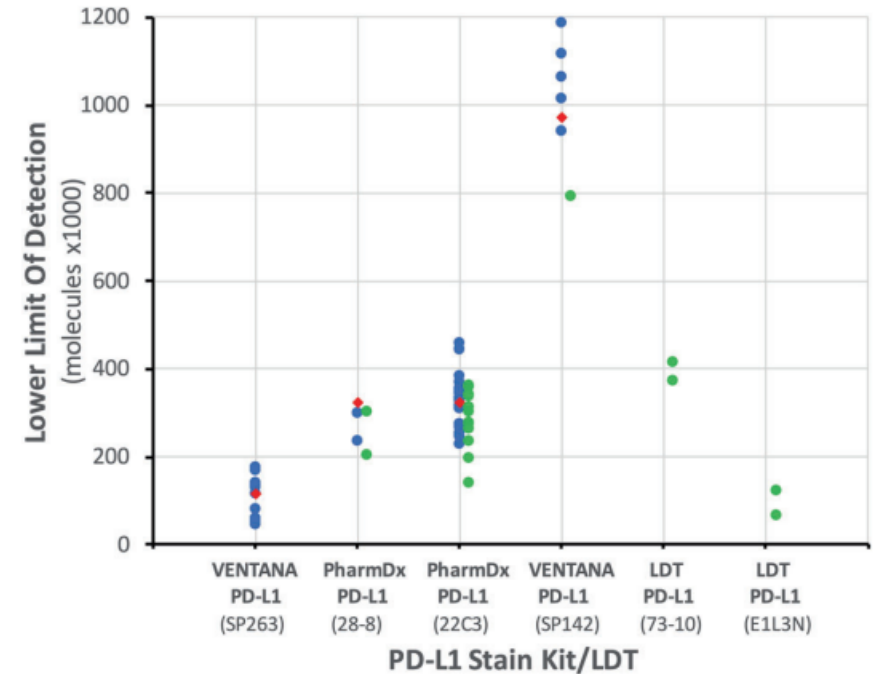
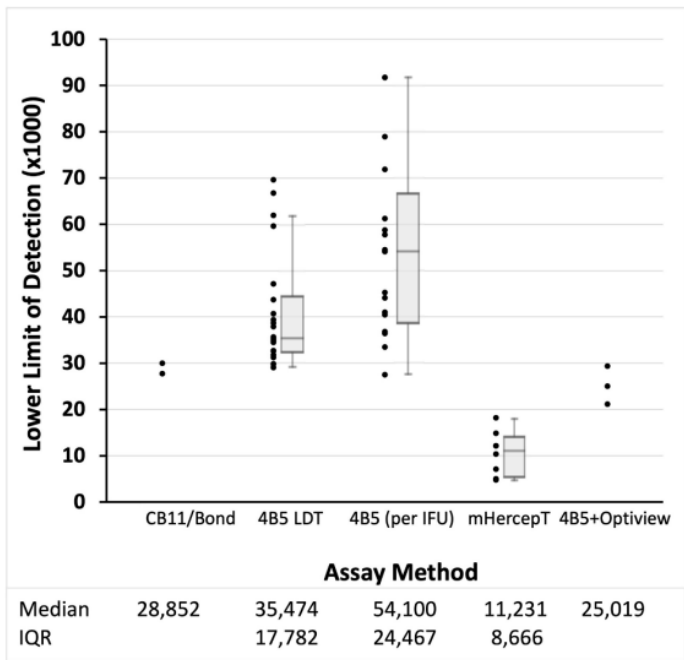
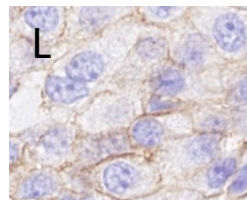
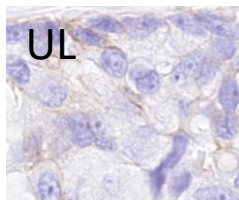


Fig. 2 Lower limit of detection (LOD) of various PD-L1 assays (x axis). Lower numbers (on the y axis) equate to greater sensitivity. Each dot represents a separate IHC laboratory test. Blue dots depict FDA-cleared assays in clinical laboratories, green dots for laboratory-developed tests (LDTs), and 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.

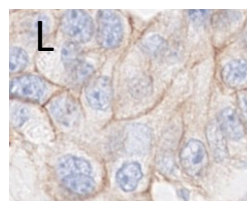
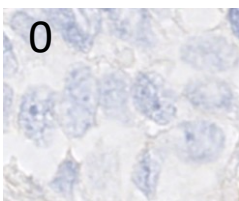
1' large feasibility study published on calibrators as IHC QC tool



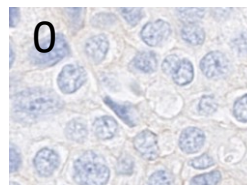
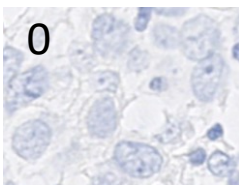
LOD
11.000



LOD
54.000



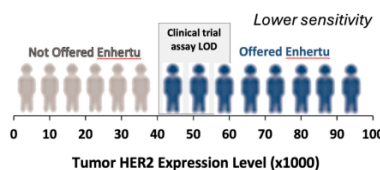
LOD
90.000



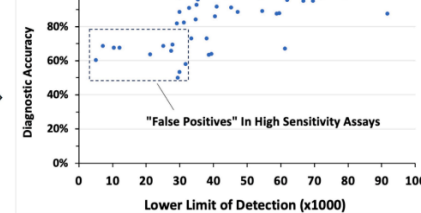
BC 1

BC 2

A Clinical Trial Assay Sensitivity



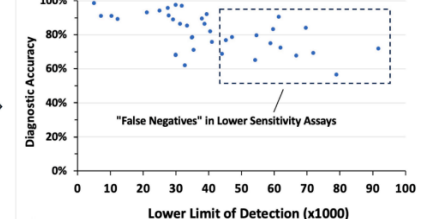
B Commercial Lab Assay Sensitivity



C



D



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

www.thelancet.com Vol 120 October, 2025

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

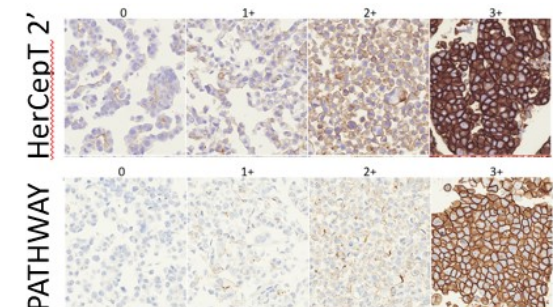
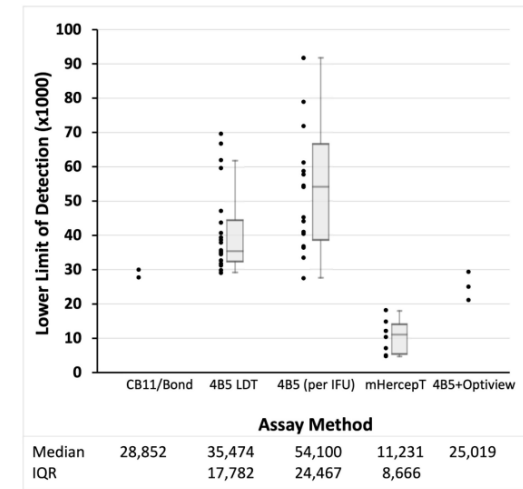
David J. Dabbs,^{a,b} Emina Torlakovic,^{a,c} Søren Nielsen,^{a,d} Suzanne C. Parry,^{a,e} Jing Yu,^b Catherine Stoos,^f Beth Clark,^b Henrik Høeg,^g Jeppe Thagaard,^g Seshi R. Sompuram,^h Stephen P. Naber,ⁱ Yukako Yagi,^j James Sayre,^k Kodala Vani,^h Méli ssande Cossutta,^l Françoise Soussaline,^l 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,*}



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

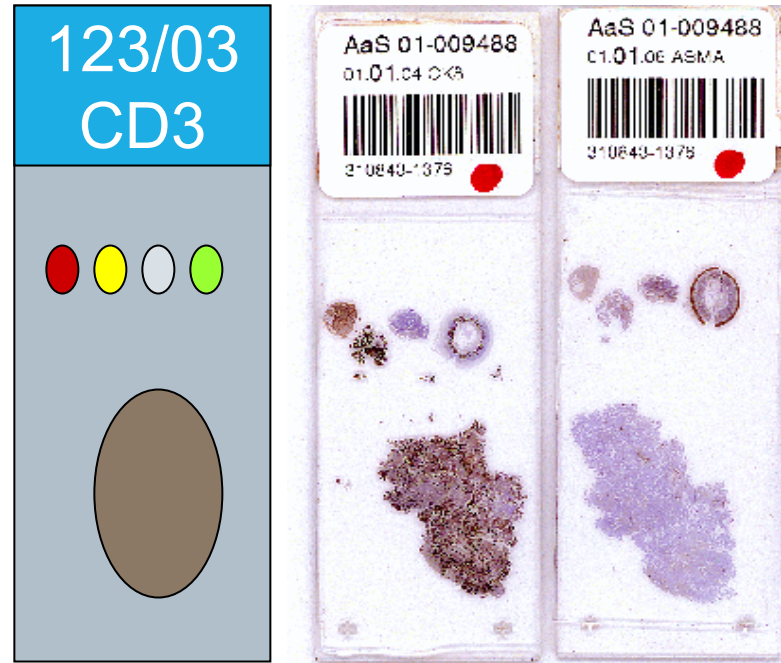
Different performances related to different IHC assays
Different thresholds for adequate vs inadequate result
Software DIA/AI QC-tools to be developed and verified



Application of TMA for QC of diagnostic IHC

Daily IHC control for the majority of routine markers:

Appendix
Liver
Pancreas
Tonsil



Each slide stained and evaluated has essential information of the obtained sensitivity and specificity

In contrast only using 1 external tissue run control, no information is available for the single slide evaluated

Application of TMA for QC of diagnostic IHC

	TMA On-slide control	TMA Run / batch control	Remarks
Missing reagent FN in patient test	Yes	No – only control slide	Potential internal pos. control only indicator of protocol performed
Wrong antibody FP in patient test	Yes	No – only control slide	
Inappropriate protocol performance - Drying out etc FN / FP in patient test	Yes	No – only control slide	Potential internal pos. control only indicator of protocol performed
	Errors seen for all IHC automated and semi-automated IHC platforms		

On-slide controls....

REVIEW ARTICLE

(Appl Immunohistochem Mol Morphol 2015;23:1–18)

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

Emina E. Torlakovic, MD, PhD,*† Soren 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*§

RESEARCH ARTICLE

(Appl Immunohistochem Mol Morphol 2017;25:308–312)

An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls

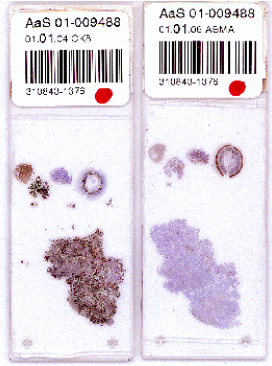
Carol C. Cheung, MD, PhD, JD,*† Clive R. Taylor, MD, DPhil,‡ and Emina E. Torlakovic, MD, PhD†

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, PhD¹²; Meredith M. Regan, ScD¹³; 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

“even for automated stainers, where it cannot be guaranteed that every slide in fact receives identical treatment”.



Use of on-slide controls in NordiQC

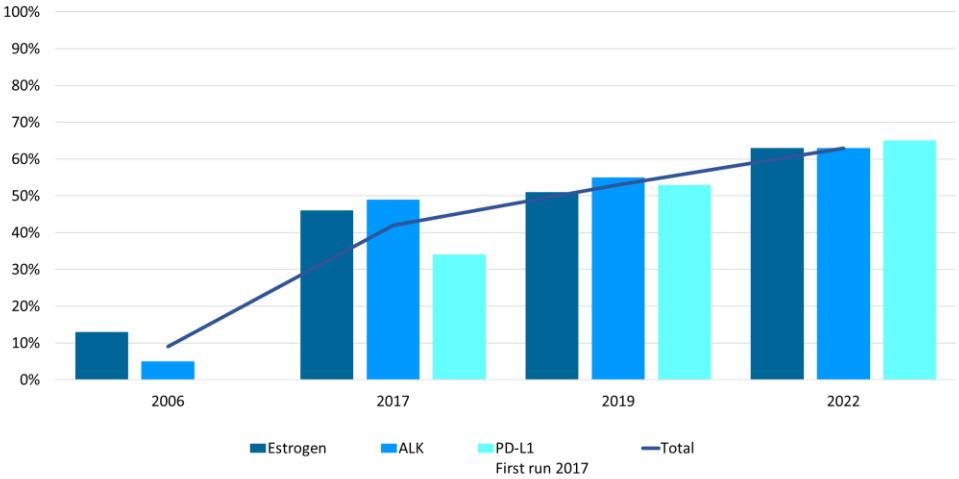
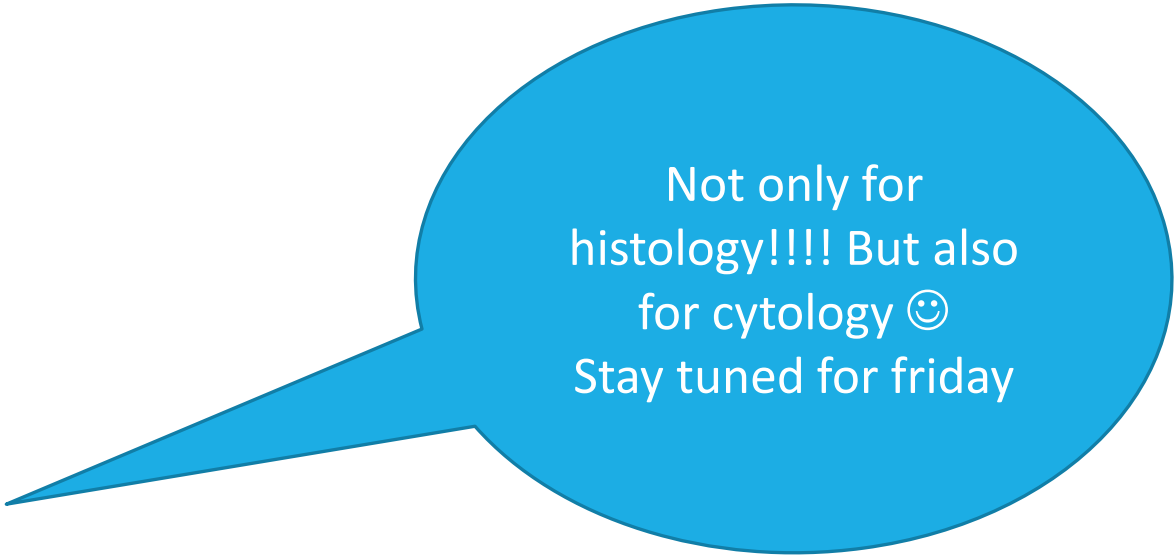


Fig. 5 Evolution of use of on-slide controls in NordiQC

Evolution in the Use of On-Slide Controls for Diagnostic Immunohistochemistry in the Era of Precision Testing
Heidi Lykke Kristoffersen, Rasmus Røge, Søren Nielsen. NordiQC, Aalborg Universityhospital, Denmark.
USCAP 2023

On-slide controls....



Not only for
histology!!!! But also
for cytology 😊
Stay tuned for friday

RESEARCH ARTICLES | JULY 19 2024

Integrated On-Slide Positive Controls for Immunocytochemistry on Cytology Slides

Subject Area:  [Pathology and Cell Biology](#)

[Irena Srebotnik Kirbiš](#) ; [Ruben Rodrigues Roque](#); [Margareta Strojjan Fležar](#)

Acta Cytologica 1–7.

<https://doi.org/10.1159/000540413>  [Article history](#)

PubMed:39033750

Application of TMA for QC of diagnostic IHC

RESEARCH ARTICLE

(*Appl Immunohistochem Mol Morphol* 2017;25:308–312)

An Audit of Failed Immunohistochemical Slides in a Clinical Laboratory: The Role of On-Slide Controls

Carol C. Cheung, MD, PhD, JD,*† Clive R. Taylor, MD, DPhil,‡ and Emina E. Torlakovic, MD, PhD†

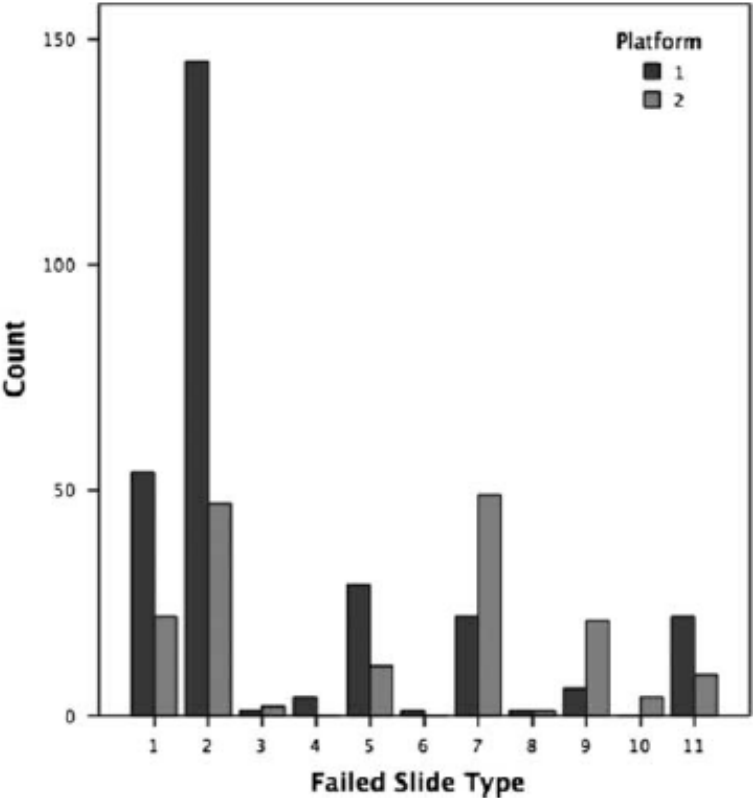


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

TABLE 1. Categories of Failed IHC Slides

Failed IHC Slide Category	Description	Comments
1	On-slide control too weak, patient tissue negative	Correct primary Ab was applied, but test sensitivity is possibly too low
2	On-slide control negative, patient tissue negative	Total slide failure; the result of the test does not suggest possible cause of the failure
3	On-slide control too weak, patient tissue weakly positive but no internal control	May indicate decreased technical sensitivity
4	On-slide control negative, patient tissue weakly positive but no internal control	There is uncertainty whether the correct primary Ab was applied or if there was significantly decreased sensitivity
5	No on-slide control, patient tissue negative	Uncertain results; cannot distinguish if the staining was optimal, suboptimal, or total failure
6	No on-slide control, patient tissue positive	No internal control present; lesion positive; failed only if there is uncertainty over whether the proper primary Ab was applied
7	Failed signal-to-noise ratio	Usually too high background; potential false positive, involving both patient sample and on-slide external control
8	Counter staining problem	If severe, may render result uninterpretable
9	Wrong protocol	Wrong protocol selected when > 1 protocol for the given primary Ab exists in the system
10	Uneven staining	Large or critical areas of the patient tissue or controls were missed by uneven staining
11	Wrong control	Either wrong tissue control or areas relevant to the test were missing (detached during staining or paraffin block with control tissue cut through)

IHC indicates immunohistochemistry.

2% error rate;

Type I 0,8%*

Type II 9,0%**

(452/22.234 slides)

* 2 or 3 step IHC assay (e.g. Env Flex/FLEX+)

** 5 step IHC assay (OptiView + AMP)

Application of TMA for QC of diagnostic IHC

A: On-slide controls

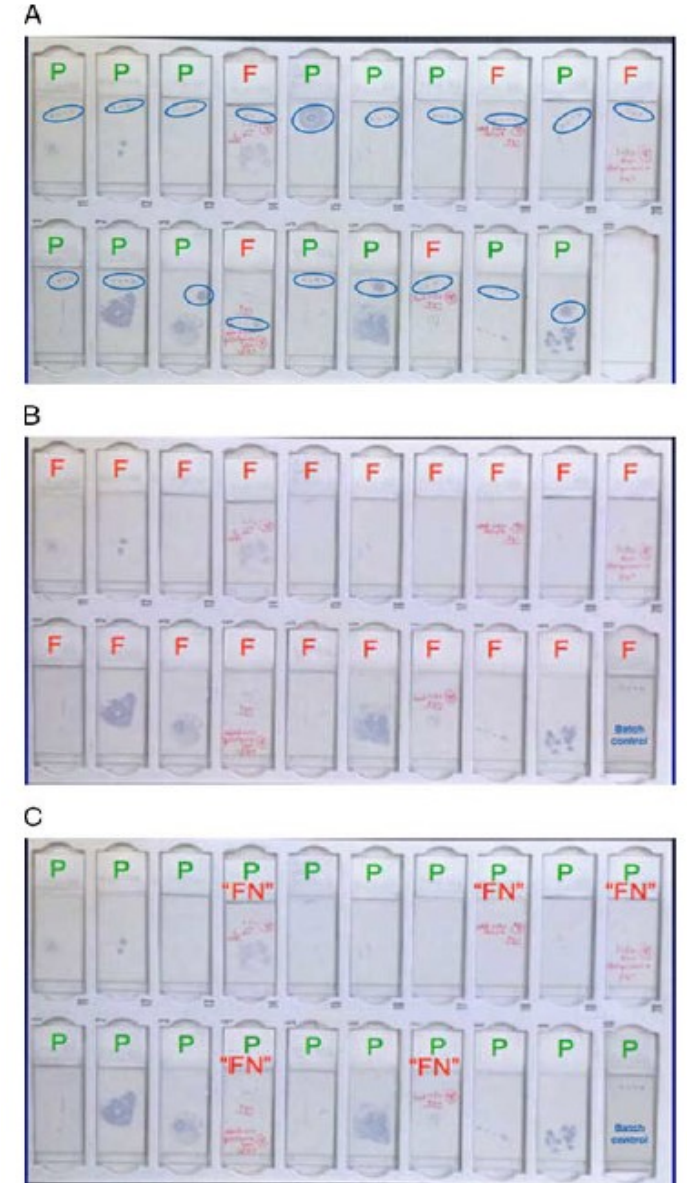
IHC slides stained for ALK (Class II),
same run, same instrument, same protocol
14/19 passed
5/19 failed (5 x 150 USD)

B: Batch-control - Theoretically:

Batch control **failed** by same conditions as above
0/19 passed
19/19 failed (no consistent internal control...) (20 x 150 USD)

C: Batch-control - Theoretically:

Batch control **passed** by same conditions as above
19/19 passed
0/19 failed (the 5 failed slides not identified....) (Cost...???)



Conclusions

Focus on external tissue controls is central to standardize, optimize and verify/validate IHC:

- Tissue controls are used to calibrate, verify and validate IHC assays
- Internal tissue controls are of limited value, but central for IHC assays with loss of target analyte as MMR
- Need to generate consensus guidelines on ICAPCs for all IHC tests – which tissues, which staining pattern etc. Interaction of industry, EQA and pathology organisations and societies being required
- Need to identify best practice controls – tissues, beads, cell lines.. – for type 2 IHC
- On-slide controls are preferable to 1 batch control

External tissue control tool box

E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification	Range of relevant expression levels With expression No expression	Range of relevant expression levels High expression Low expression No expression	Reproducibility Method of transfer proof
High expression Low expression No expression	With expression No expression		10/20 of each Type I/II IHC	+ relevant cut-off	



Questions and/or comments



Thank You for the attention and.....