NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 4-6 2023

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 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 !!!!!

Decalcification
Preparation

Fixation
Time, Type, Volume

Tissue
Type, Dimension,
Laser resection,
De-differentiation

Pre-
analytic

Section
Thickness
Storage
Drying

Epitope retrieval
HIER conditions
Proteolysis

Primary antibody
Clone, Dilution
Buffer, Time, Temp

Manual
Stainer

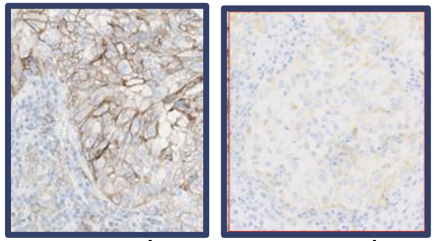
Analytic

Visualization
Sensitivity, Specificity

Development
Sensitivity,
Localization

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

PD-L1 - NSCLC



Protocol 1 Protocol 2

Controlment

Quantification
Reporting

Post-
analytic

Read-out / Interpretation
Positive/Negative - cut-off level

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

Diagnostic Cytopathology, Vol 39, No 4 2011

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

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.

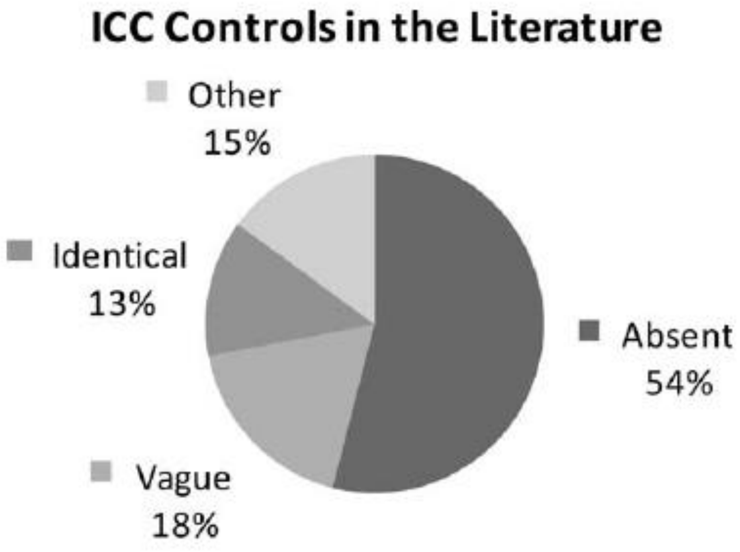


Fig. 1. Description of immunocytochemistry controls in articles reviewed.

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

IHC controls to guide reliability of data...

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

But....

Can PAX8 expression be seen in breast carcinoma??

Central for subtyping of unknown primary 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…

HE MRQ-50 BC12

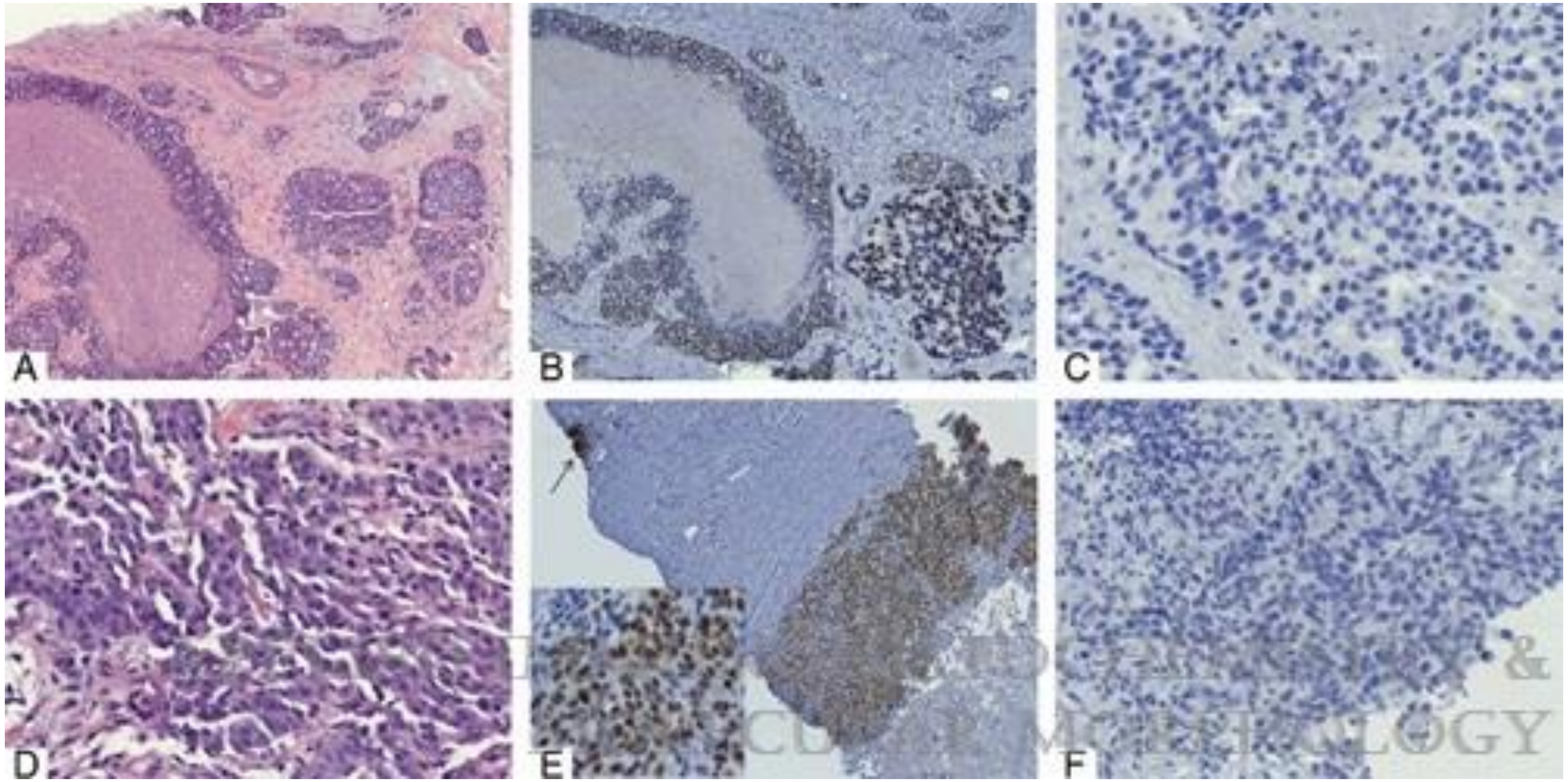


FIGURE 1

Aberrant Immunostaining of Breast Carcinoma by MRQ-50 PAX8 Antibody

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

Applied Immunohistochemistry & Molecular Morphology28(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…

NordiQC Assessments of PAX8 Immunoassays
Rasmus Røge, MD,† Ole Nielsen, HT,‡ Michael Bzorek, HT,§ Søren Nielsen, HT,* and Mogens Vyberg, MD*†*

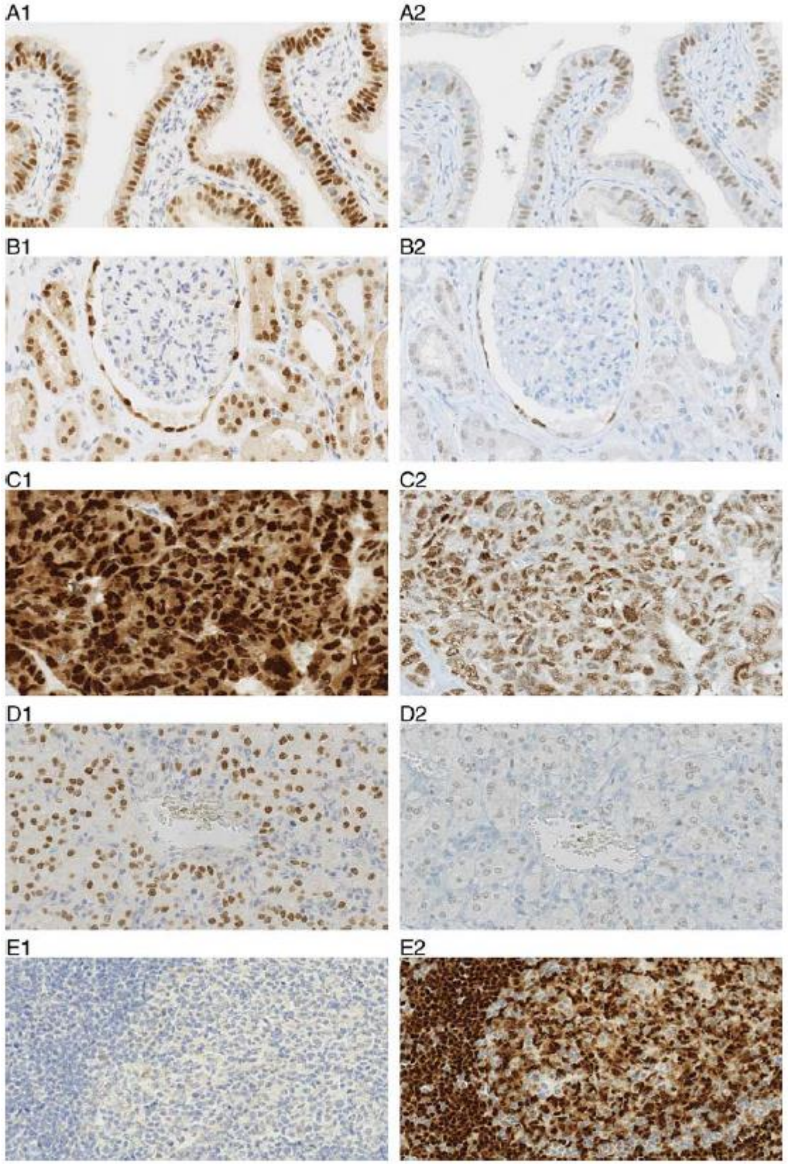
Positive tissue control 1
Fallopian tuba

Positive tissue control 2
Kidney

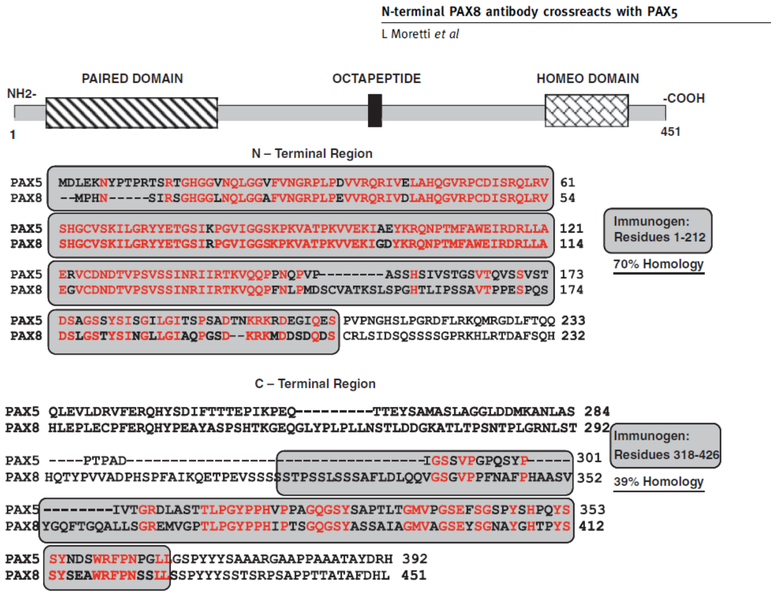
Tumour type 1
Ovarian carc.

Tumour type 2
Renal cell carc.

Negative tissue control 1
Tonsil



Level of analytical sensitivity



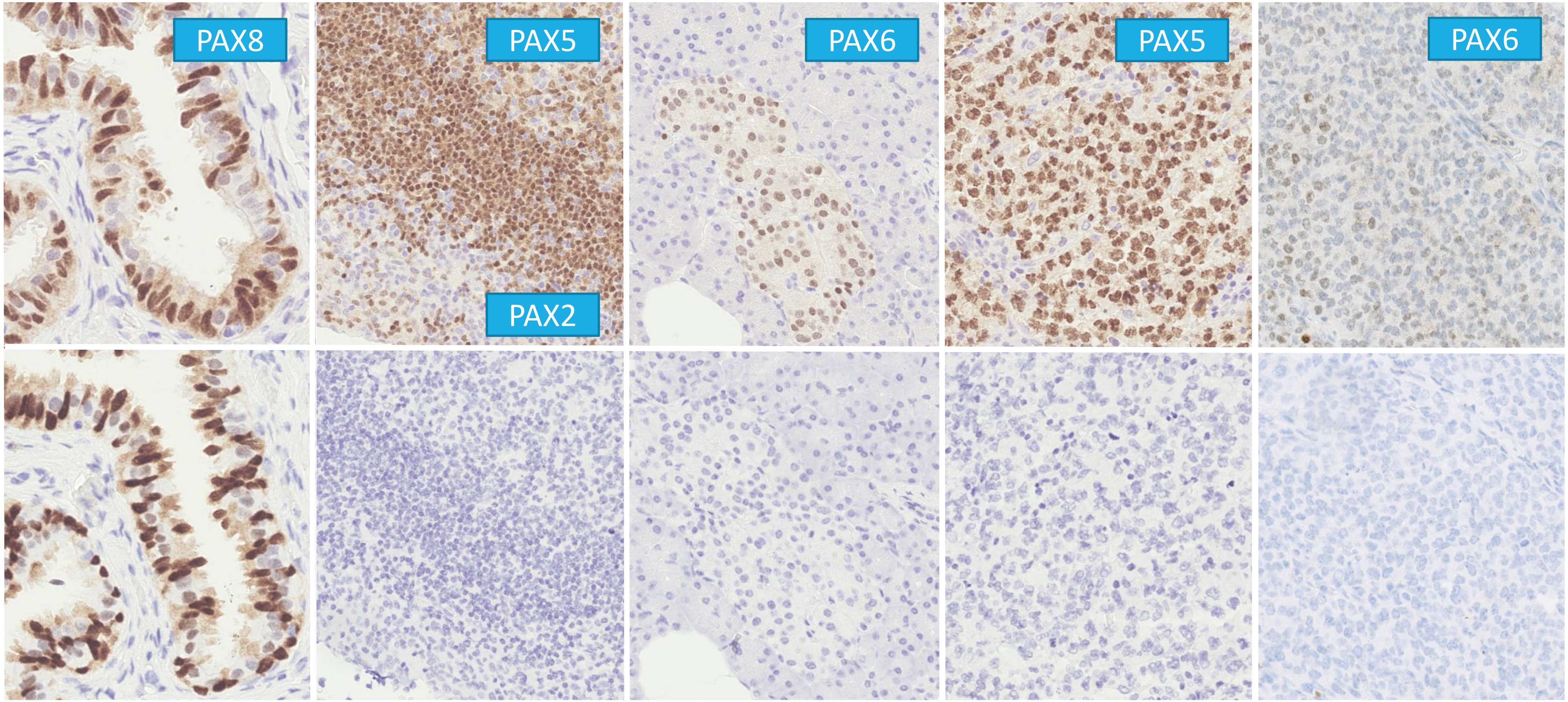
Level of analytical specificity

BC12 / SP348 MRQ-50 / pAb

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 of 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. Ruhul Quddus, MD, MPhil (Path)

41% MRQ-50
0% BC12

No data on controls

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

No data on controls

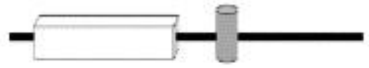
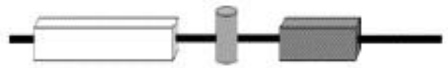
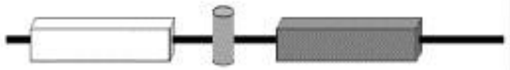
Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

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

< 5%

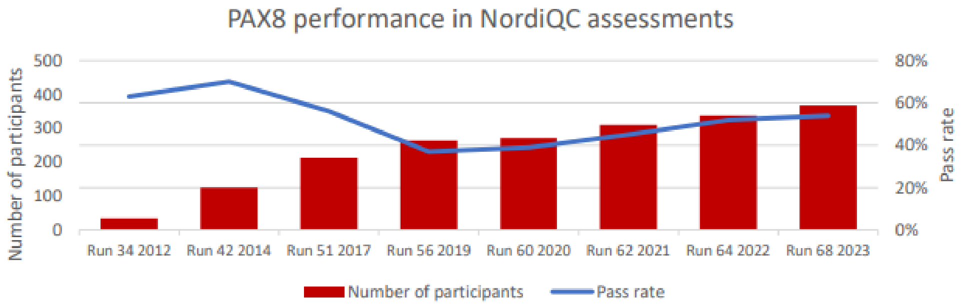
No data on controls

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

PAX family Group	Protein structure/domains (Paired, octapeptide, homeodomain)	Protein family member	Embryonic Expression Domain	Expression/Mutation in human disease
I		PAX1	Skeleton, thymus 3rd/4th pharyngeal pouch	Klippel-Feil Syndrome, Jarcho-Levin Syndrome
		PAX9	Skeleton, Teeth, Thymus	Jarcho-Levin Syndrome, Oligodentia
II		PAX2	Kidney, CNS	Hyperproliferative dysplastic kidney, Renal hyperplasia, Bladder and renal cancer,Coloboma Syndrome
		PAX5	B-Cells, CNS	Lymphomas
		PAX8	Kidney, Thyroid, CNS	Congenital hypothyroidism, Thyroid carcinomas/adenomas
III		PAX3	Neural Crest, CNS somites/muscle	Waardenburg Syndrome Types I/III, Melanoma, Rhabdomyosarcoma
		PAX7	Neural Crest, CNS somites/muscle	Rhabdomyosarcoma
IV		PAX4	Pancreas, gut	Diabetes
		PAX6	Pancreas, gut, CNS and eye	Aniridia,GI tumors Cataracts/Peter's Anomaly

NordiQC data – PAX8

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



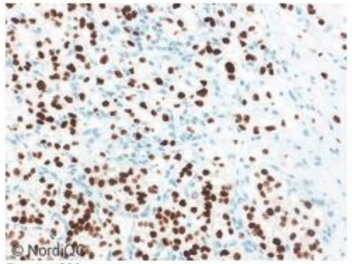


Fig. 4a x200
Optimal PAX8 staining of the RCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig. 4b.

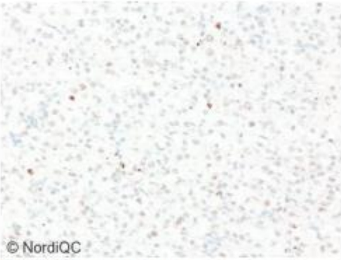


Fig. 4b x200
Insufficient PAX8 staining of the RCC using the same protocol as in Figs. 1b-3b.
Only a faint nuclear staining is seen in the vast majority of neoplastic cells. Compare to Fig. 4a.

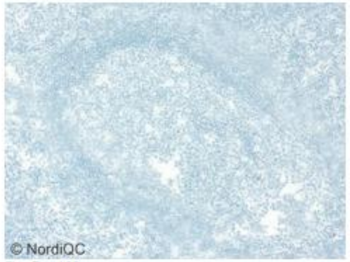


Fig. 5a x100
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

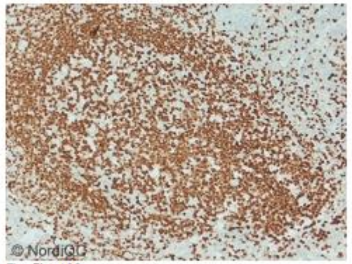


Fig. 5b x100
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.

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 4	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 4	Cell Marque Epitomics	-	5	8	1	36%	-
rmAb clone **SP348***	146	Abcam Gennova Spring Bioscience	102	31	9	4	91%	70%
rmAb clone **ZR-1***	2 2	Zeta Corporation BioSite	1	-	2	1	-	-
rmAb clone **BP6157***	2	Biolynx	-	1	1	-	-	-
rmAb clone **QR016***	7	Quartett	3	3	1	-	86%	43%
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 Diagomics	-	-	3	-	-	-
Conc total	223		106	54	43	20	72%	48%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **MRQ-50, 760-4618 (VRPS)[3]**	6	Ventana/Roche	-	-	-	6	0%	0%
mAb clone **MRQ-50, 760-4618 (LMPS)[4]**	49	Ventana/Roche	-	3	34	12	6%	0%
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%	0%
mAb clone, **BC12* API438**	6	Biocare Medical	-	2	4	-	33%	0%
mAb clone **IHC008 PII77R06**	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%	0%
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%	0%
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%	0%
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%	8%
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.

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[‡§]*

References central for the area of IHC controls

The 4-paper evolutions series

Recommendations and road-map for IHC QA provided by

International Society For Immuno-Histochemistry and Molecular Morphology (ISIMM)

International Quality Network for Pathology (IQN-PATH)

Published AIMM 2017 (Jan-April)

Main elements to develop & validate IHC assays

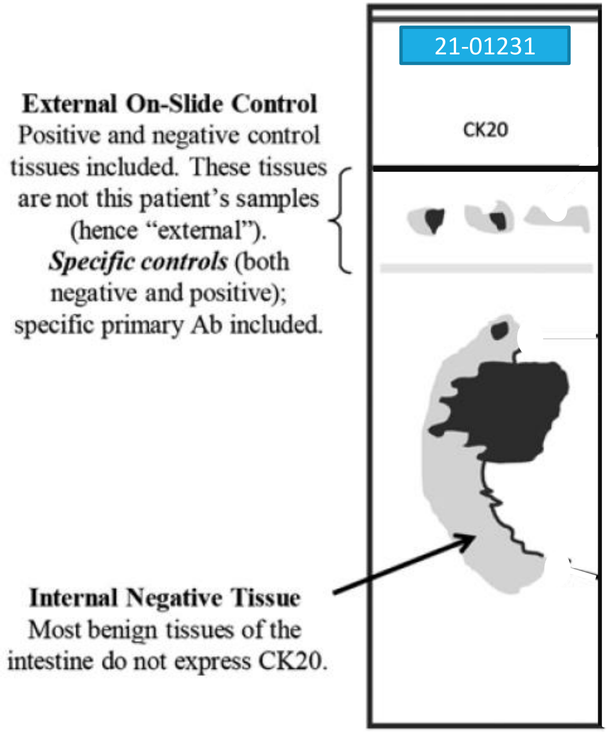
1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of analytical sensitivity/specificity
4. Identification of IHC performance controls providing information that the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

The journey from an antibody to a diagnostic IHC assay with a specific purpose
Based on external tissue control.

Tissue controls

- Reagent and **tissue** controls are necessary for the validation of immunohistochemical staining results.

- Tissue controls are the most valueable tool to monitor the 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

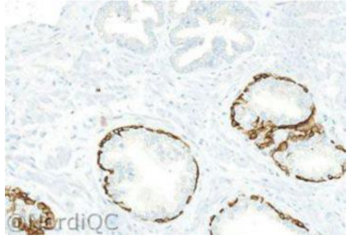


How to use internal tissue controls

Appl Immunohistochem Mol Morphol • Volume 22, Number 4, April 2014 *Standardization of Negative Controls*

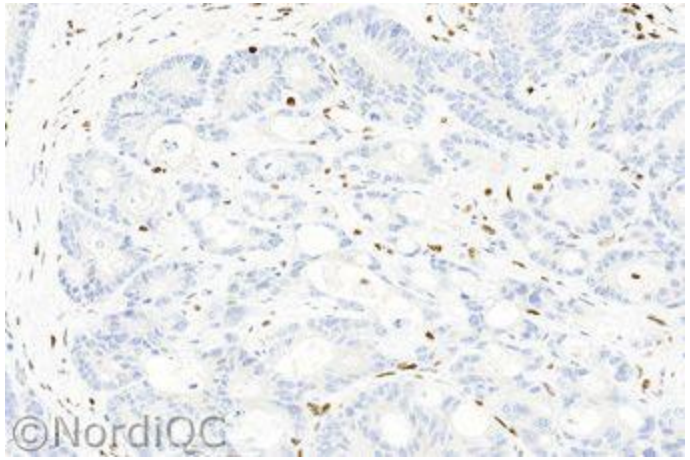
TABLE 2. Examples of IHC Assays Where Preferential Use of Internal Positive Controls Recommended

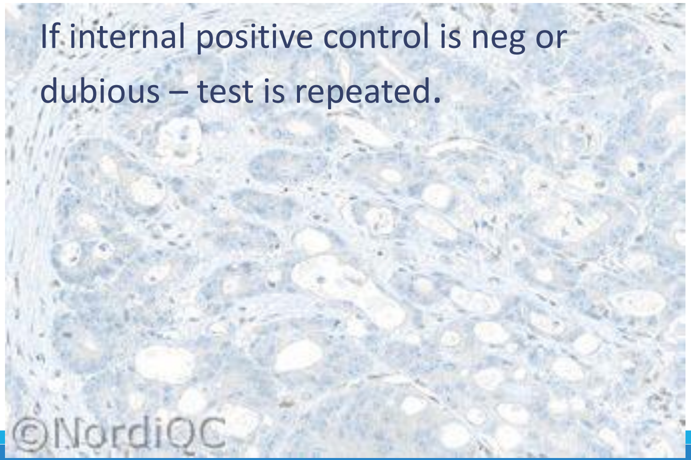
IHC Assay	Use	Comments
Cytokeratin 5	Demonstration of basal cells in glandular structures of prostate to differentiate between benign (positive) and malignant (negative) glands	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control Tested sample 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



Target analyte	Application	Internal control to confirm ”true” loss
BAP1, MTAP	Mesothelioma	Stromal cells
p53	Gynelogical carc.	Stromal cells
PTEN	Lung and gynecological carc.	Stromal and benign cells
MMR (MLH1, MSH2, MSH6,PMS2)	Lynch syndrome	Stromal cells / lymphocytes
SMAD4	Pancreas and GI carc.	Stromal and benign cells

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





Limitations of internal tissue controls

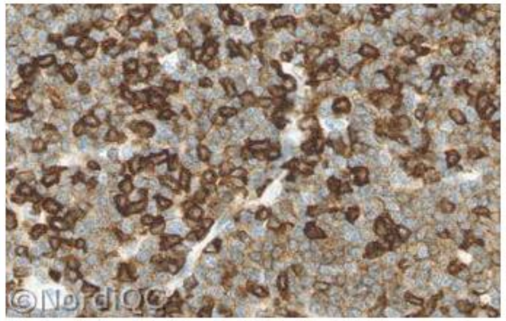


Fig. 4a. Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

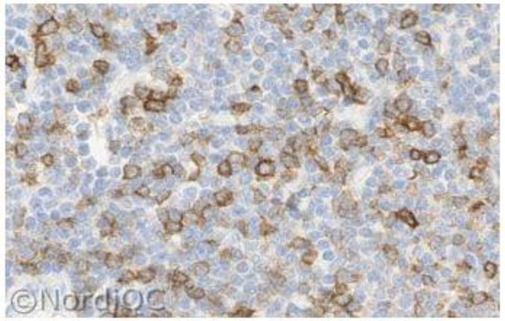


Fig. 4b. Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a. The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

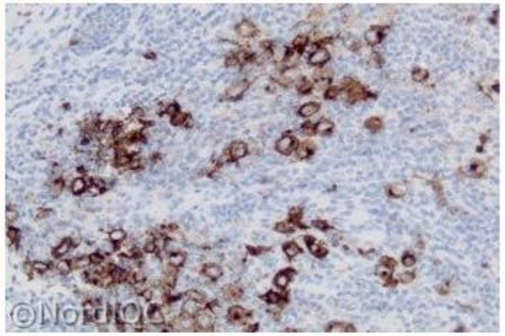


Fig. 2a. Optimal CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

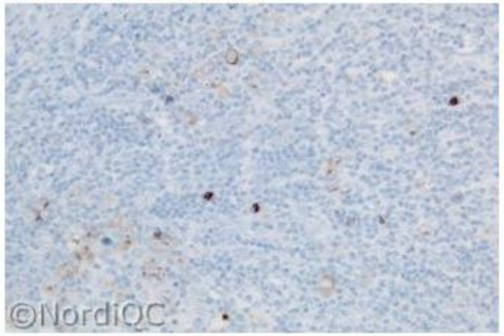


Fig. 2b. CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a.

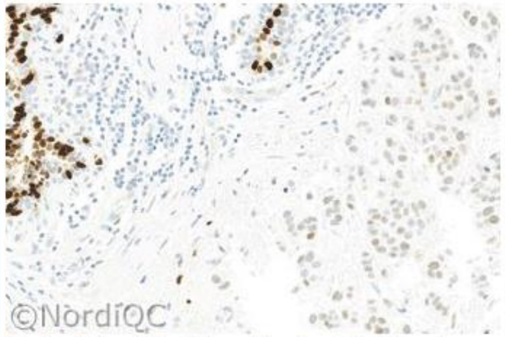


Fig. 3a. Optimal ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

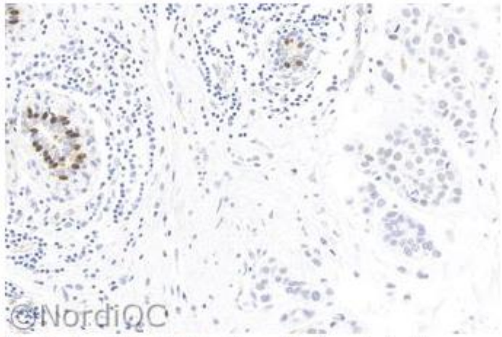


Fig. 3b. Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 – 80 % cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. Only dispersed neoplastic cells show an equivocal staining

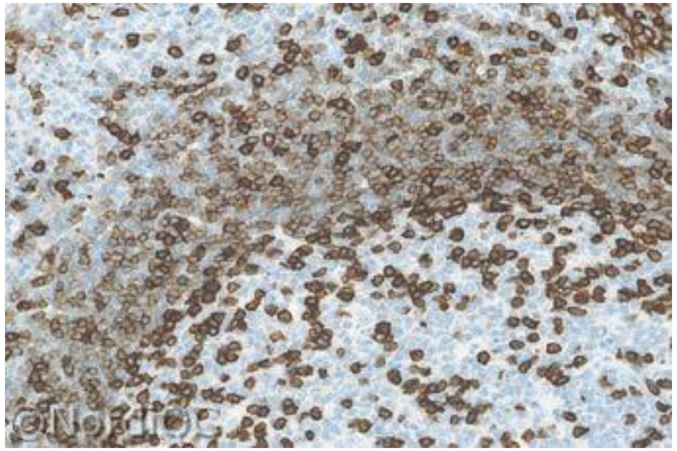
Internal positive tissue controls;

In general not applicable as positive controls due to levels of expression may not be relevant for level of test calibration

e.g. CD5, CD15, CD34, CD45, CD56, S100, ER, PD-L1 etc

CD5;
Tonsil
Mantle zone

Critical control



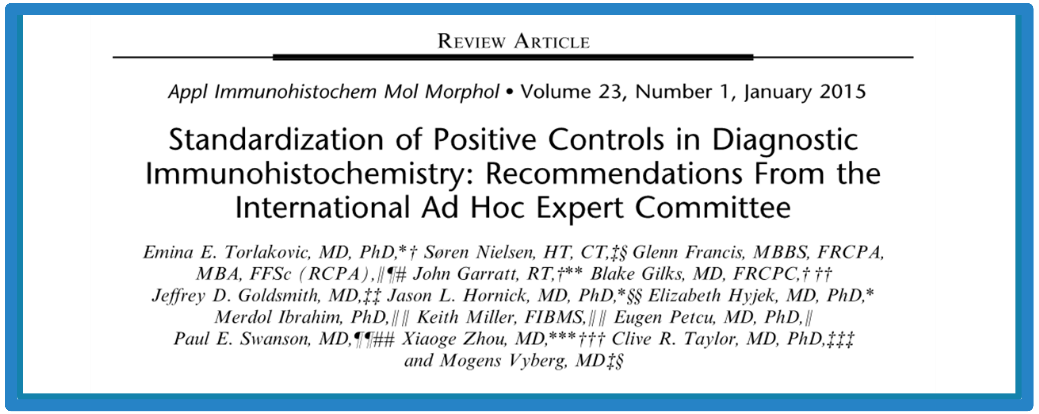
Critical tissue controls = ICAPCs

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



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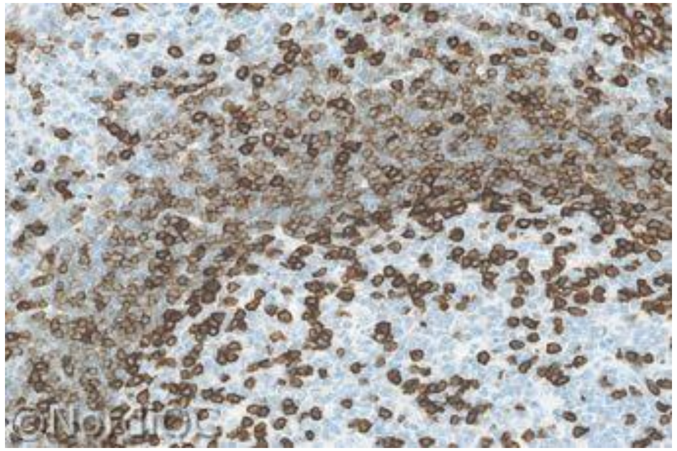
CD5;
Tonsil
Mantle zone

Critical
control



Main elements to develop & validate IHC assays

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

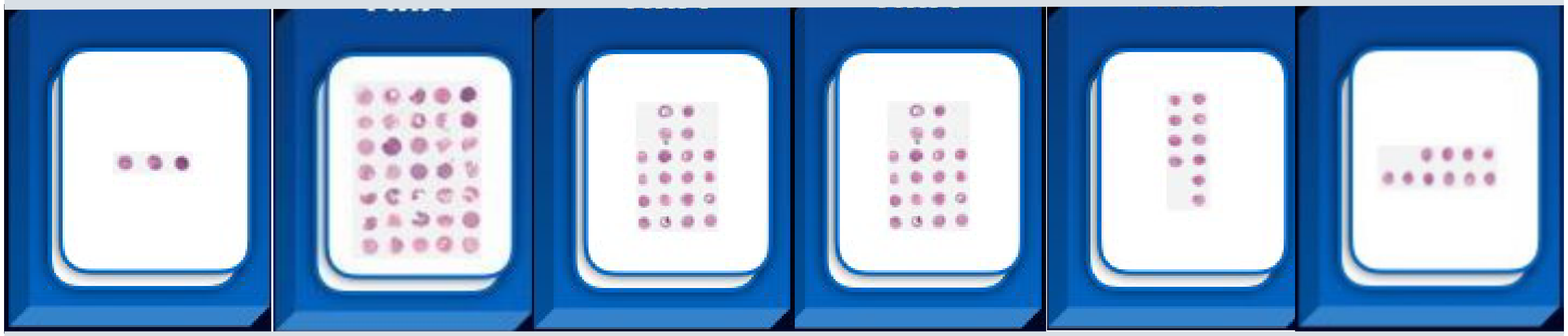
1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of analytical sensitivity / specificity
4. Identification of IHC performance controls providing information that 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 Method of transfer proof
High expression Low expression No expression	With expression No expression		With expression No expression 20/40 of each Type I/II IHC	High expression Low expression No expression + relevant cut-off	

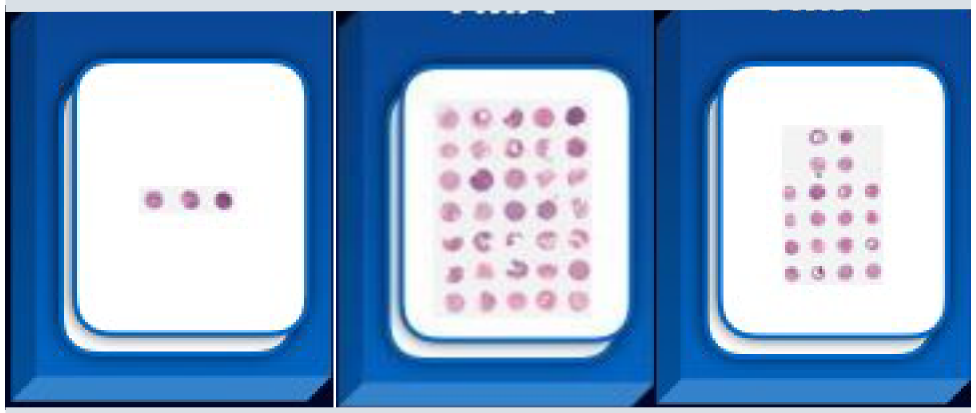
*Immunohistochemical critical assay performance controls

External tissue control tool box

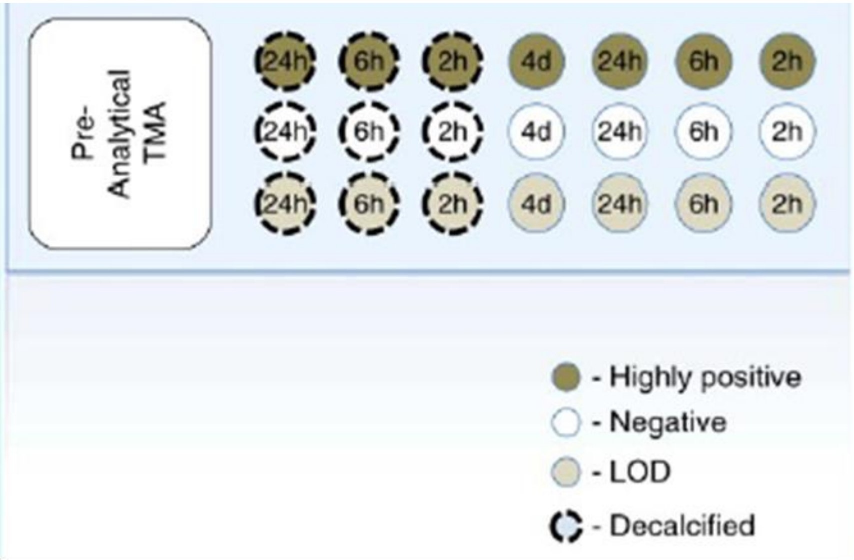
E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

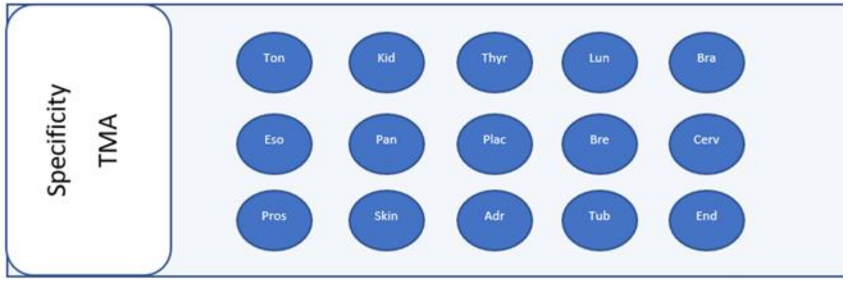
Calibration TMA's

iCAPC* TMA	Specificity TMA	Pre-analyt. TMA



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	





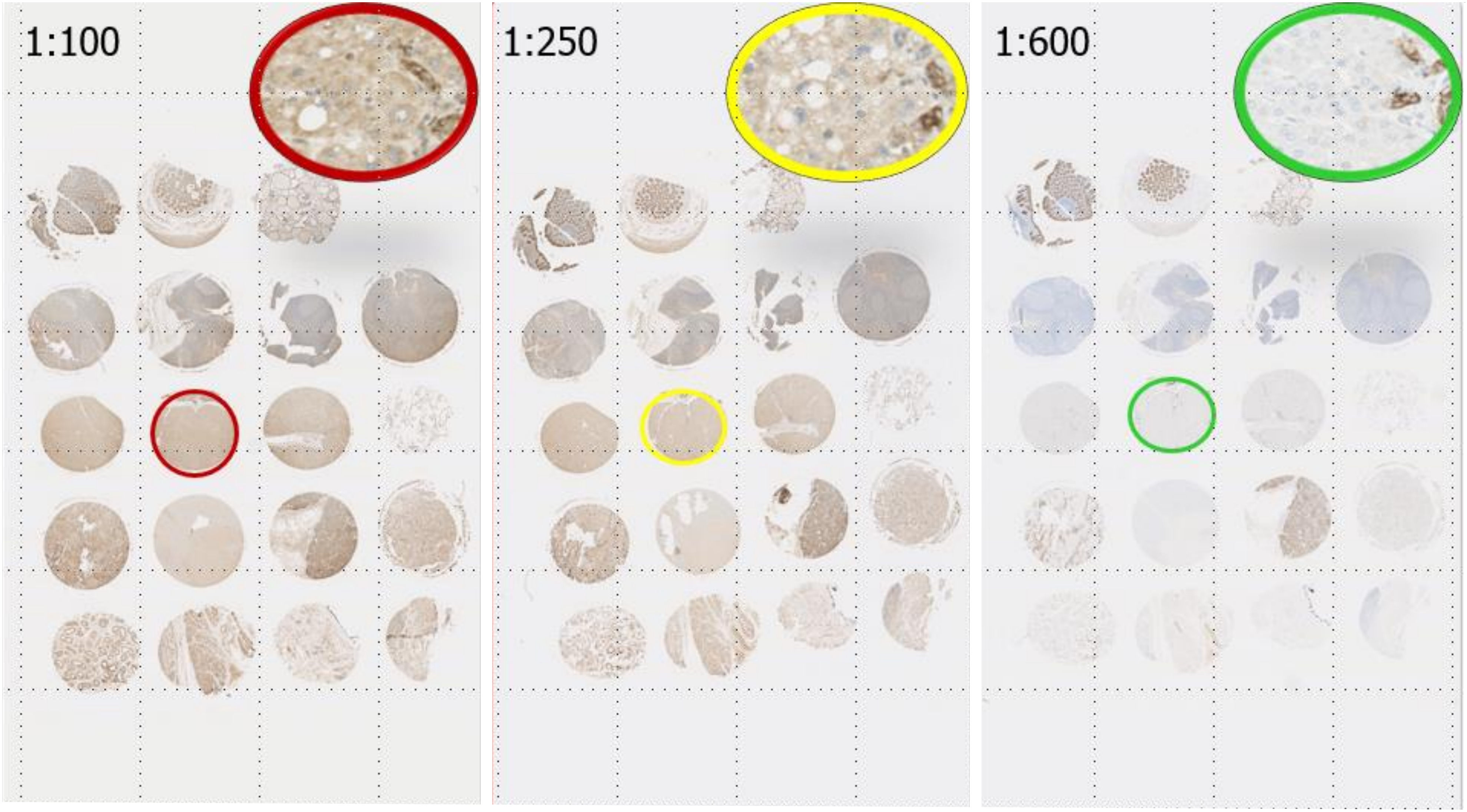
*Immunohistochemical critical assay performance controls

”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

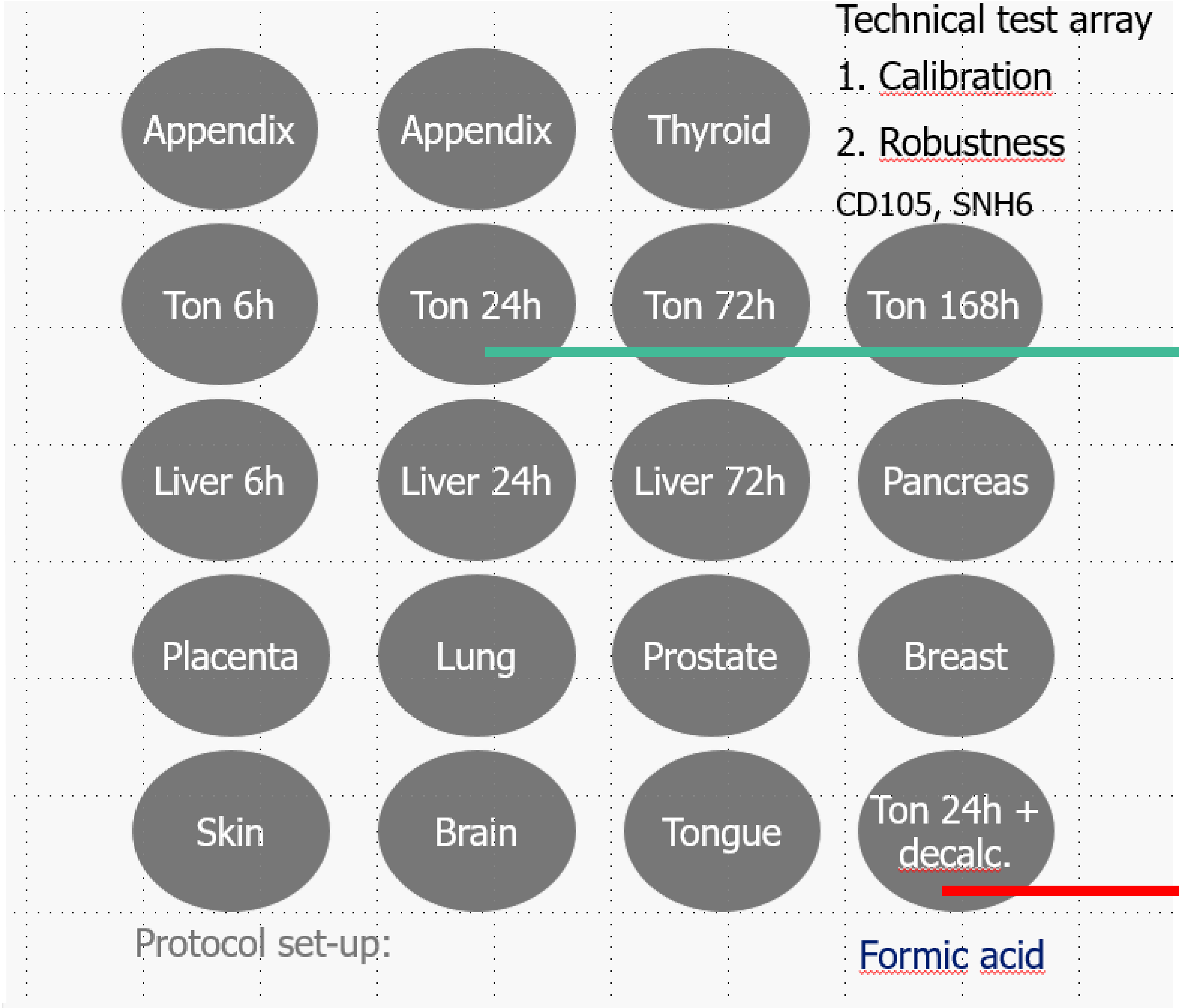


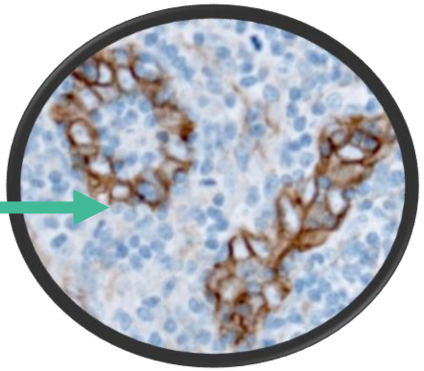
EPCAM calibration

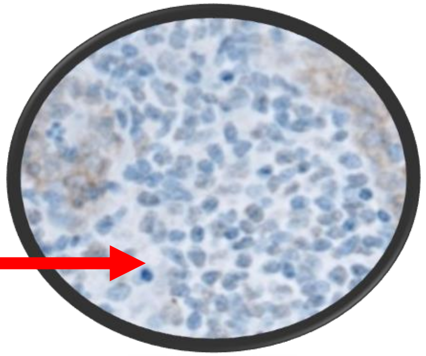
Tissue cores are used to identify best practice protocol providing highest signal-to-noise ratio for qualitative IHC markers

Source:
NordiQC and Aalborg University Hospital

”Poor mans” specificity and pre-analytical TMAs





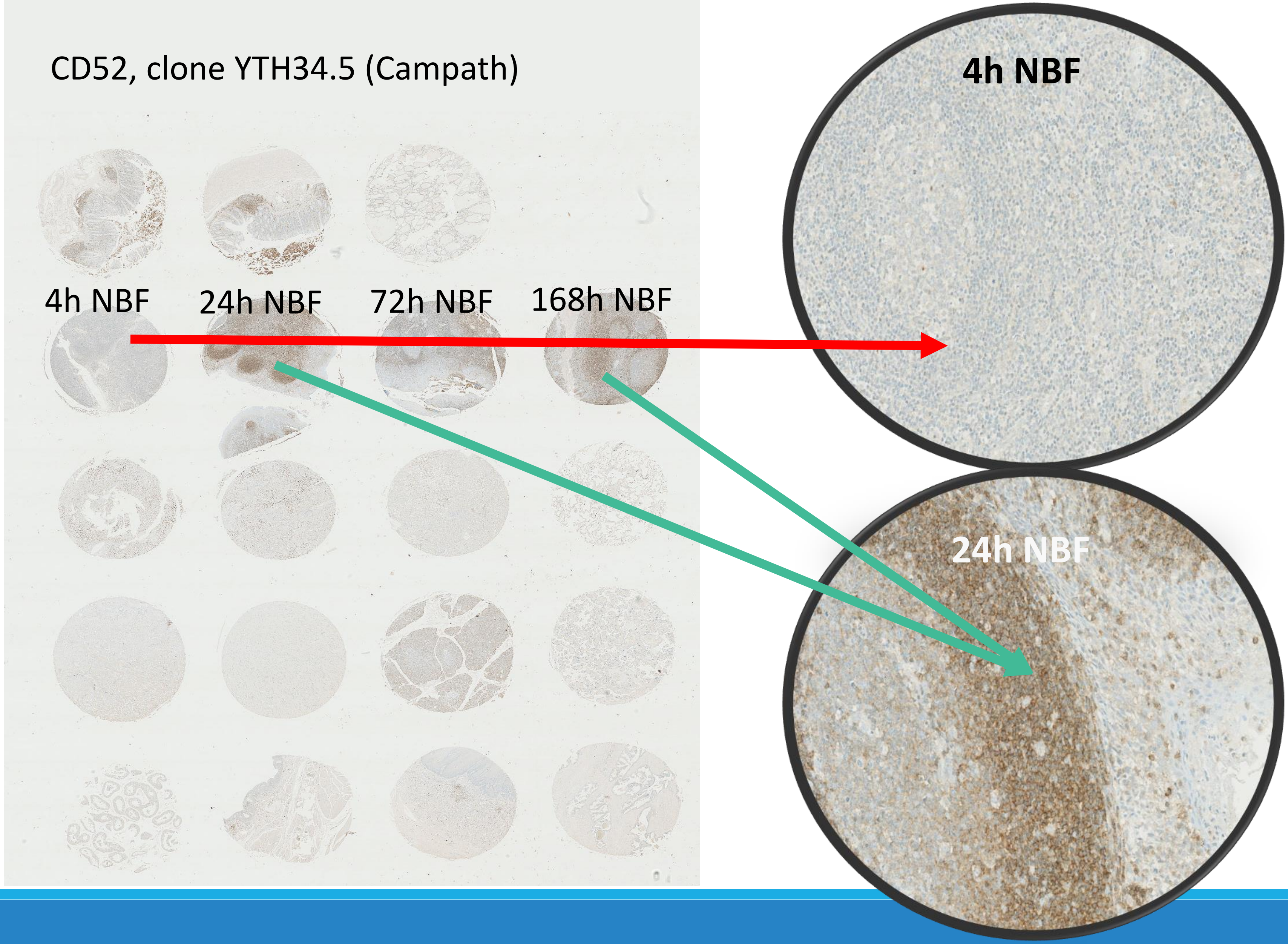


CD105 calibration

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

Source:
NordiQC (Ole Nielsen) and Aalborg University Hospital

”Poor mans” specificity and pre-analytical TMAs

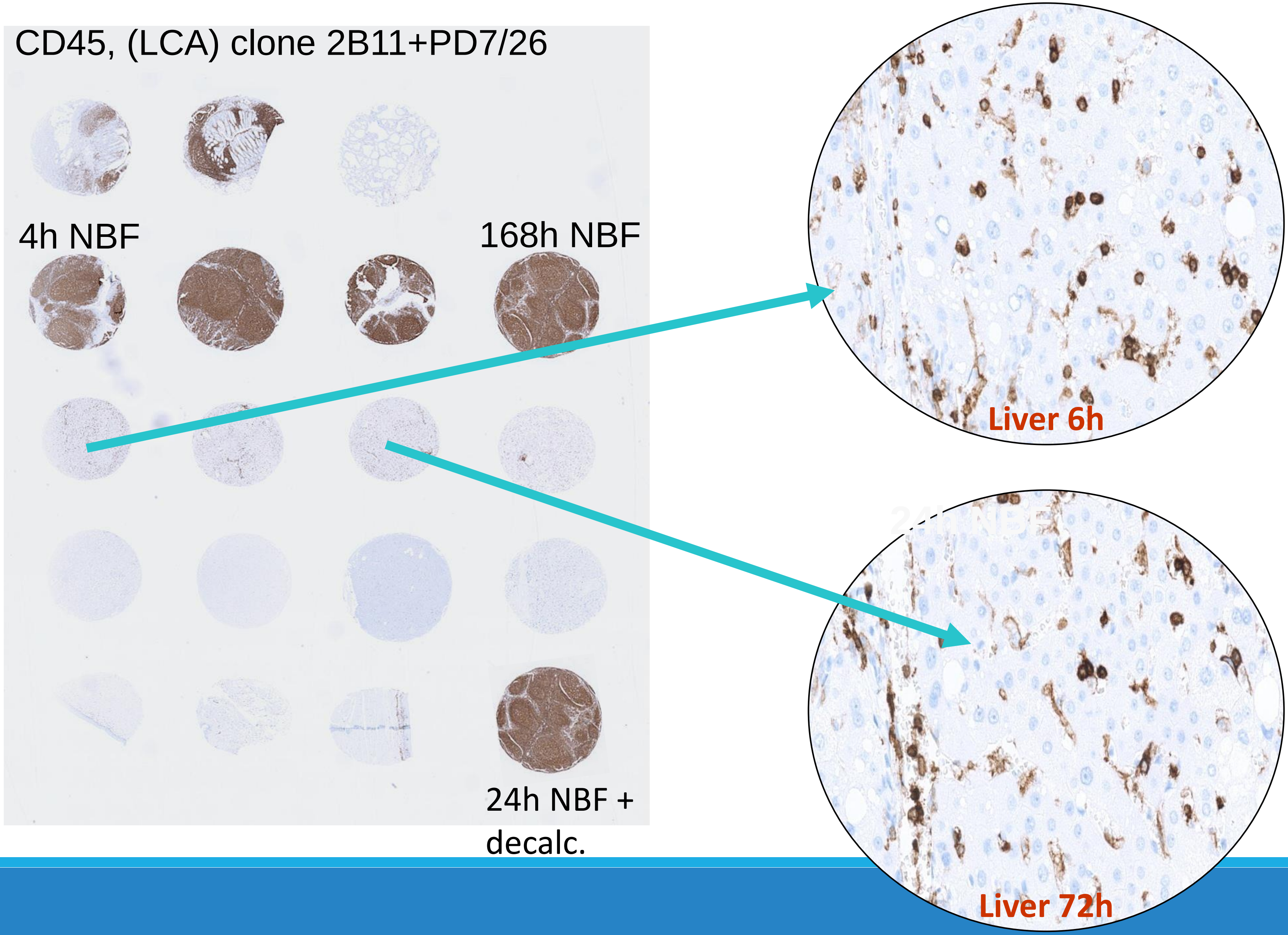


CD52 calibration

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

Source:
NordiQC and Aalborg University Hospital

”Poor mans” specificity and pre-analytical TMAs



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

Test Performance Characteristics - TPCs

Test performance characteristics;

Which staining pattern characterizes an optimally calibrated IHC assay for a specific purpose?

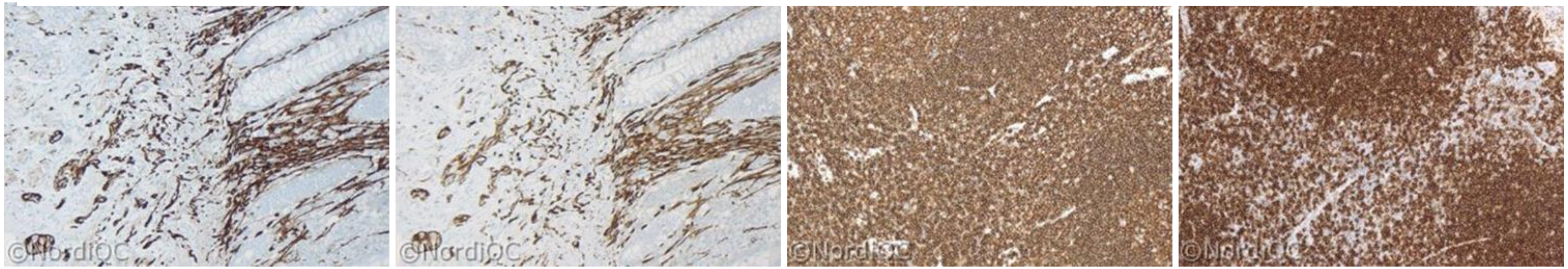
- Analytical sensitivity
- Analytical specificity
- Precision / reproducibility of IHC assay

Which tissues / cellular structures show the clinical relevant range of the target analyte with focus on required low level of demonstration – **CRITICAL CONTROLS - ICAPCs**?

Fit For Purpose; the selection….

CD56 CD45

Appendix



Tonsil

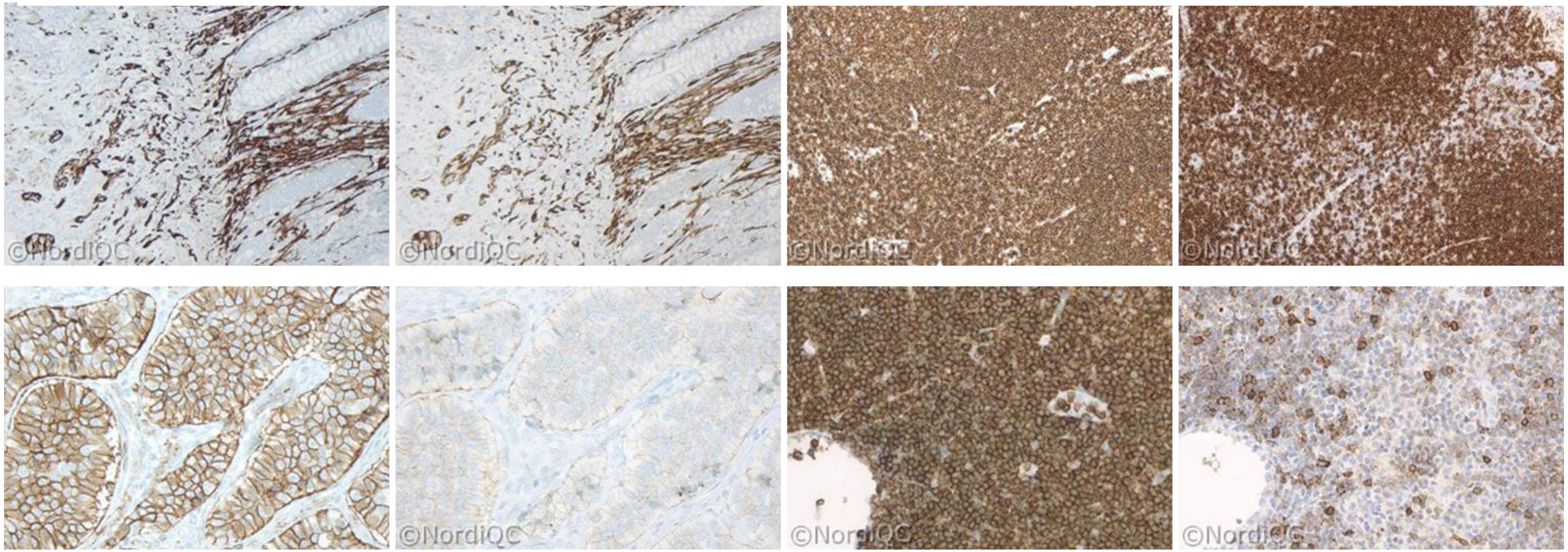
Test A Test B Test A Test B

Fit For Purpose; the selection….

CD56 | CD45

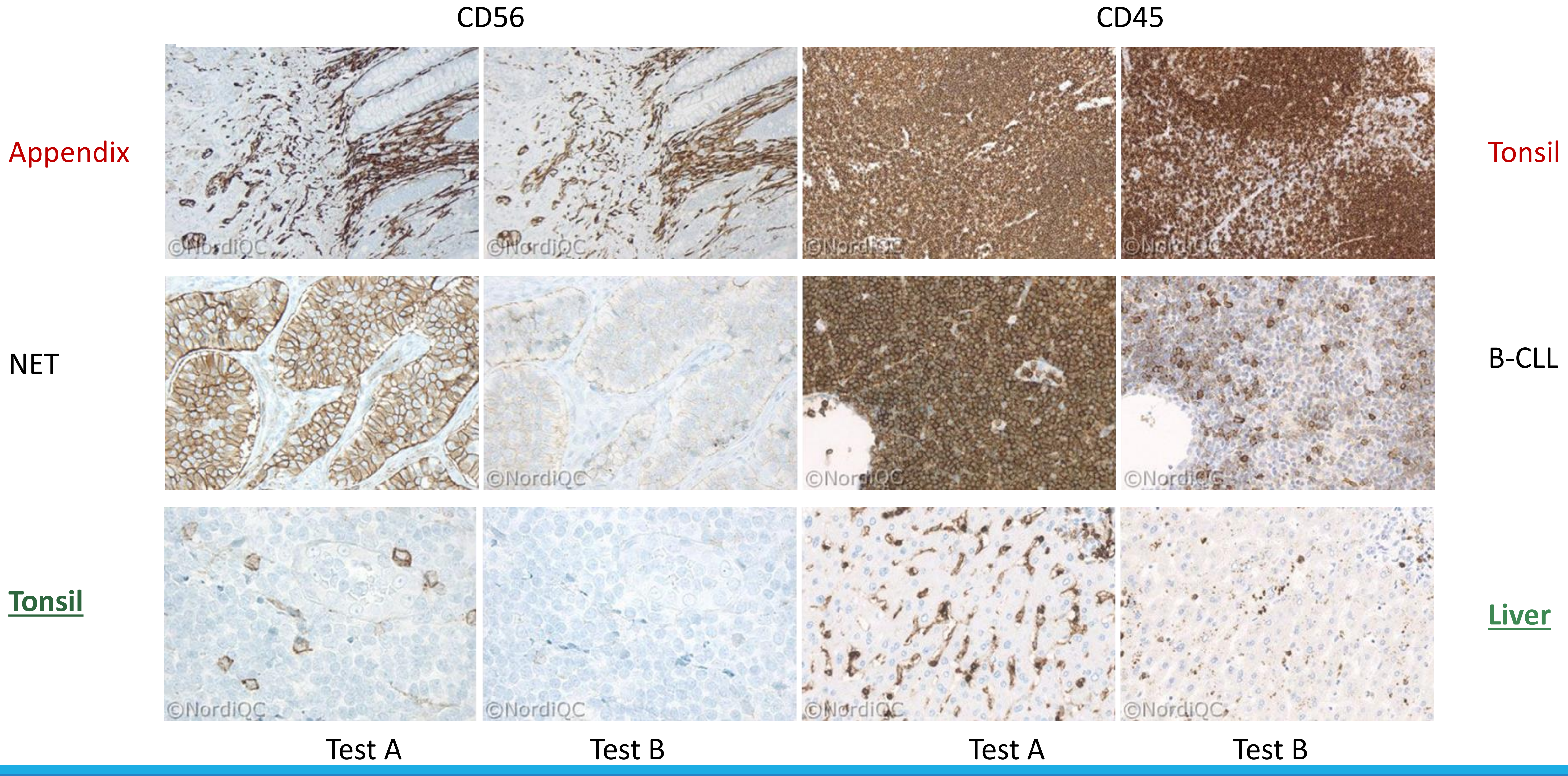
Appendix | Tonsil

NET | B-CLL

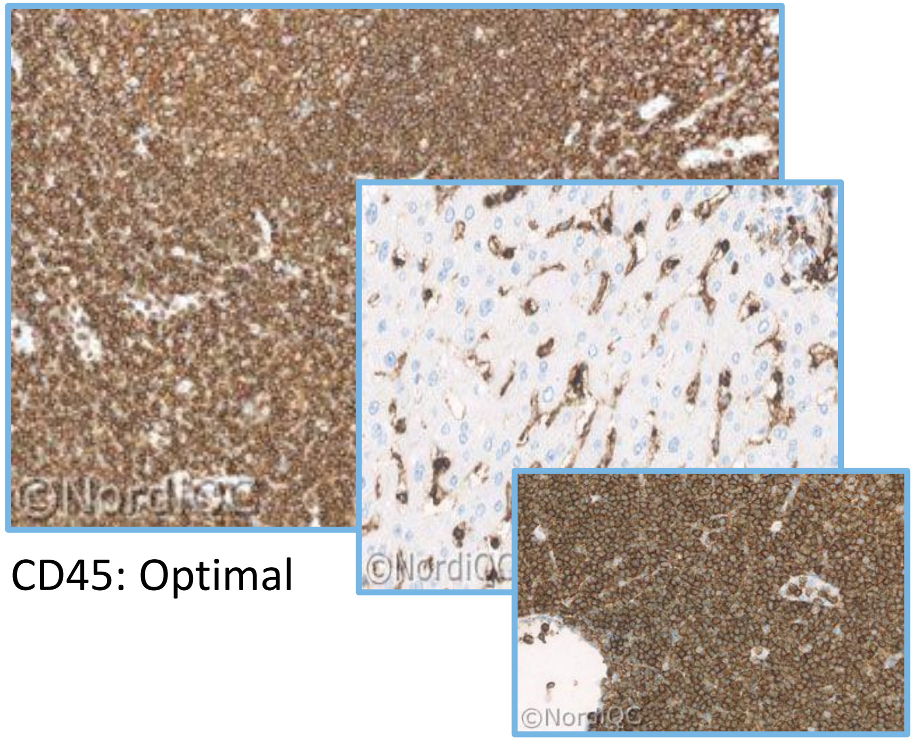


Test A | Test B | Test A | Test B

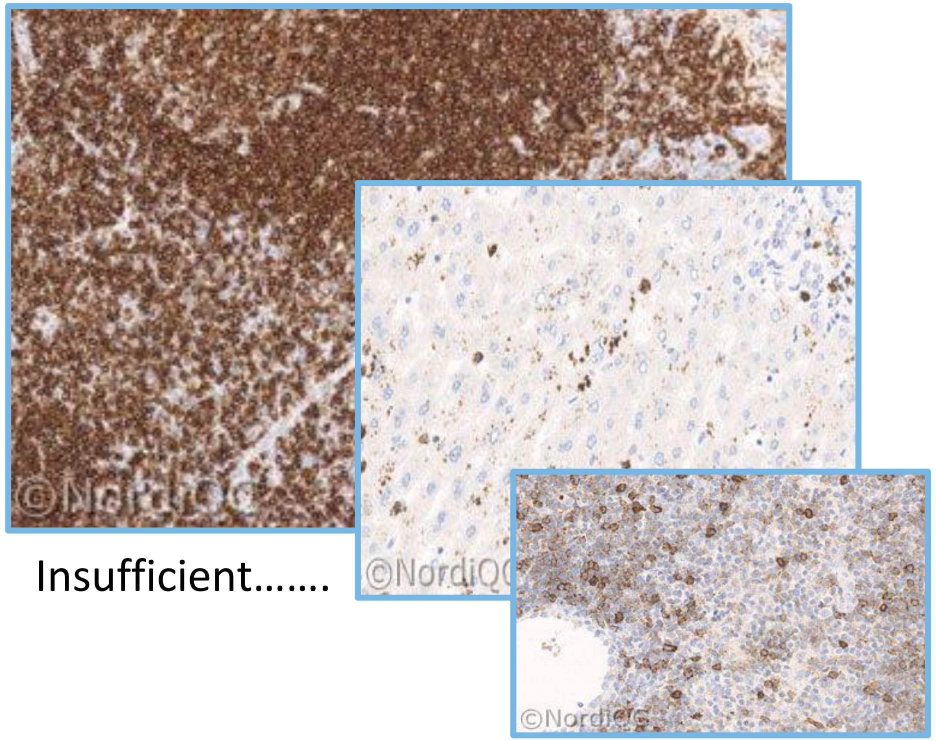
Fit For Purpose; the selection....



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 sensitivity in an optimally calibrated IHC assay

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

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

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‡§*

ASCO special articles

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

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

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

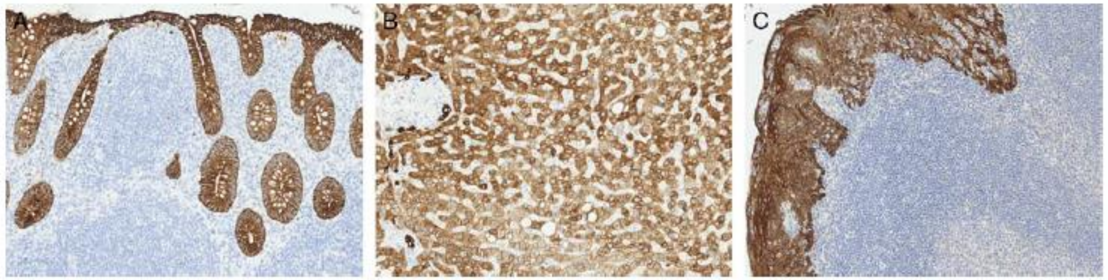


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining 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.

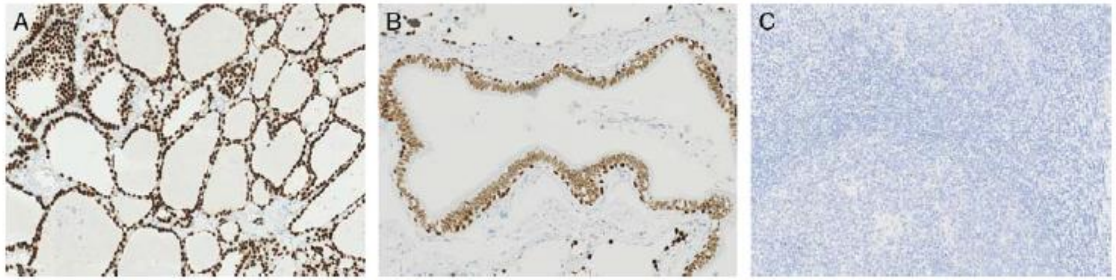


FIGURE 7. TTF-1 iCAPC. A, Thyroid: virtually all epithelial cells must show a strong nuclear staining reaction. B, Lung: virtually all pneumocytes and basal cells of terminal bronchi must show a moderate to strong nuclear staining reaction. Columnar epithelial cells of terminal bronchi must show an at 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.

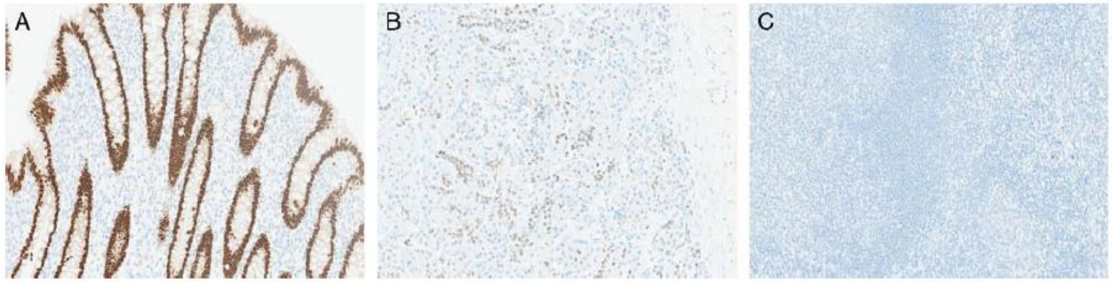


FIGURE 8. CDX-2 iCAPC. A, Appendix: virtually all epithelial cells must show a strong nuclear staining reaction. A weak cytoplasmic staining reaction in addition to strong nuclear staining is often present. B, Pancreas: the majority of epithelial cells of intercalated ducts must show a weak to moderate 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

Generel expected patterns

High expression
(Right antibody)

Low expression
(Appropriate sensitivity)

No expression
(Appropriate specificity)

Which tissue
Which cells
Which extension
Which intensity

NordiQC IHC tissue control atlas – open from 05.2022

NordiQC 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

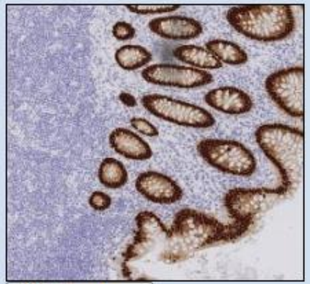
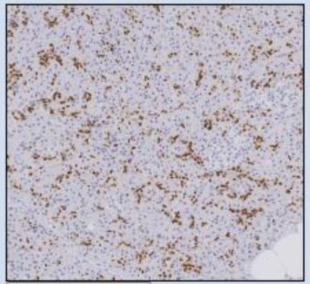
Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas – open from 05.2022

NordiQC | 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. *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.*	The vast majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction.	No staining reaction should be seen. *Note, dispersed lymphocytes can show a faint nuclear staining reaction.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Back

Available for NordiQC participants

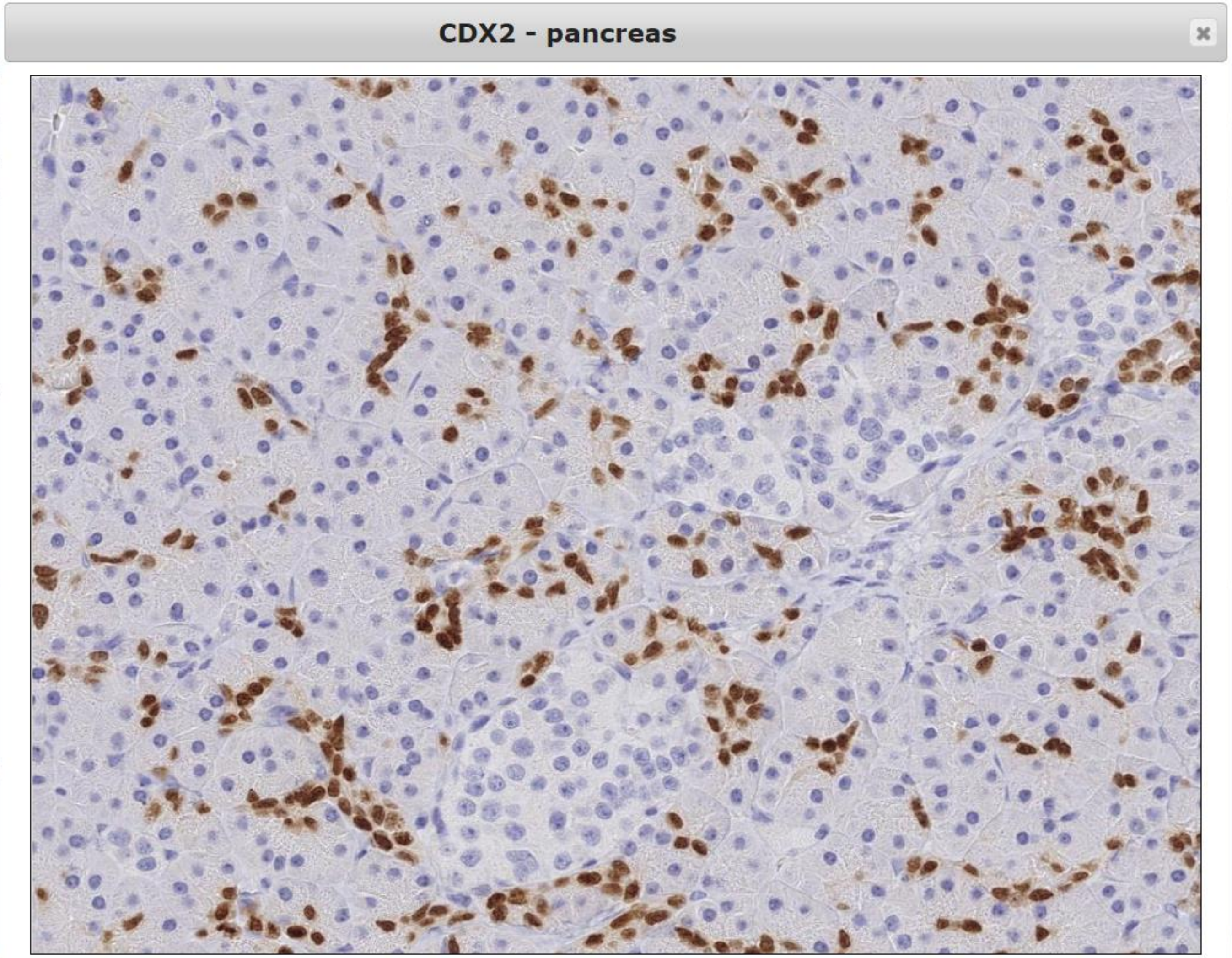
Tissues

Purpose

Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas – open from 05.2022



The vast majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction.

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
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External tissue control tool box

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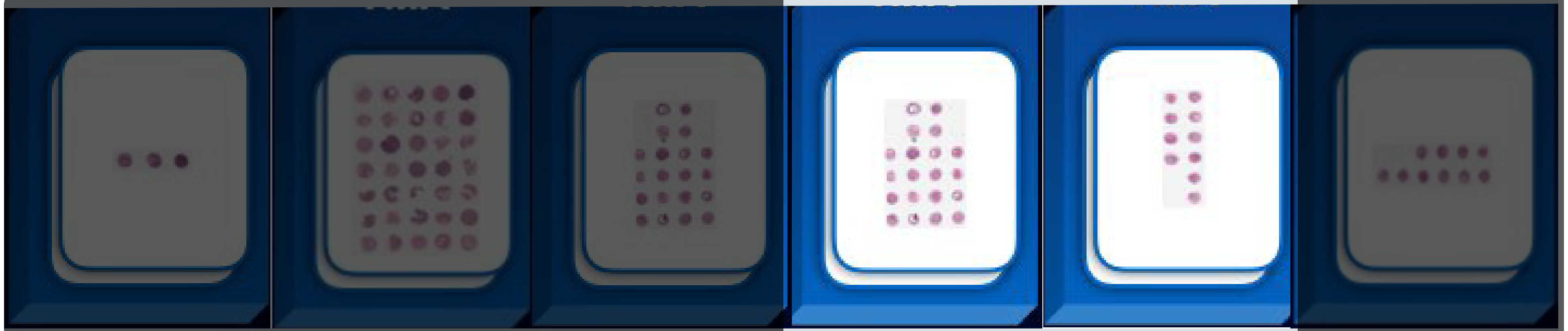

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			With expression	High expression Low expression	Method of transfer proof
High expression Low expression No expression	With expression		No expression	No expression	
	No expression		20/40 of each Type I/II IHC	+ relevant cut-off	

*Immunohistochemical critical assay performance controls

Sample sets for technical / analytical validation of IHC

- Technical / Analytical validation
  - Laboratory developed tests (concentrates and RTU formats being applied modified to official protocol)
  - Non-predictive markers (- ER, PR, HER-2..)
    - CLSI*: 20 cases per entity relevant (pos, neg)
    - CAP**: 10 positive, 10 negative

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 quantitatively.

- Ad-Hoc: 10 strongly pos, 10 interm. to low, 5 neg.

Number perhaps less important compared to use of tissue with full range of expression patterns reflecting the diagnostic use and purpose of test

* Clinical and Laboratories Standards Institute ** College of American Pathologists

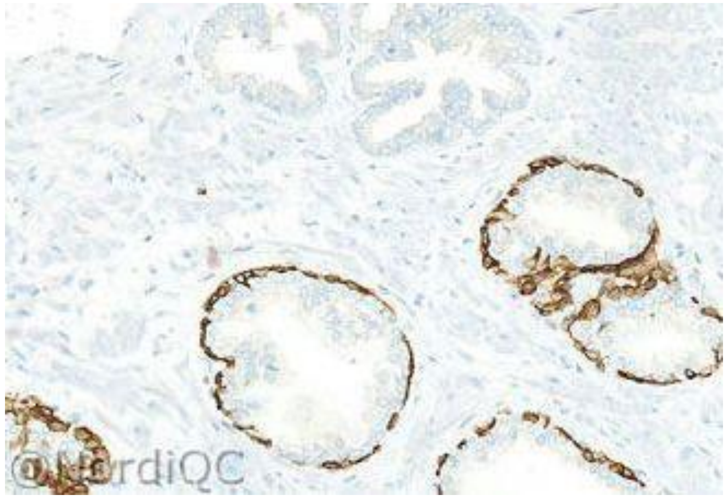
Identification of purpose of the test

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

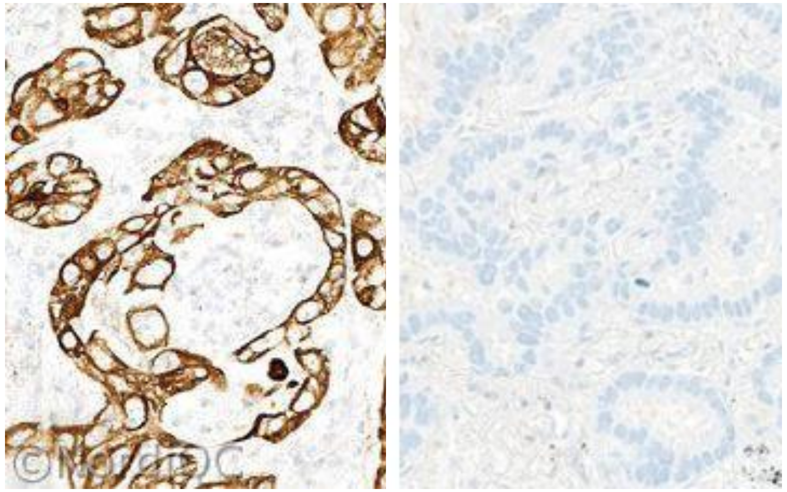
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

IHC for CK5

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



Prostate sample



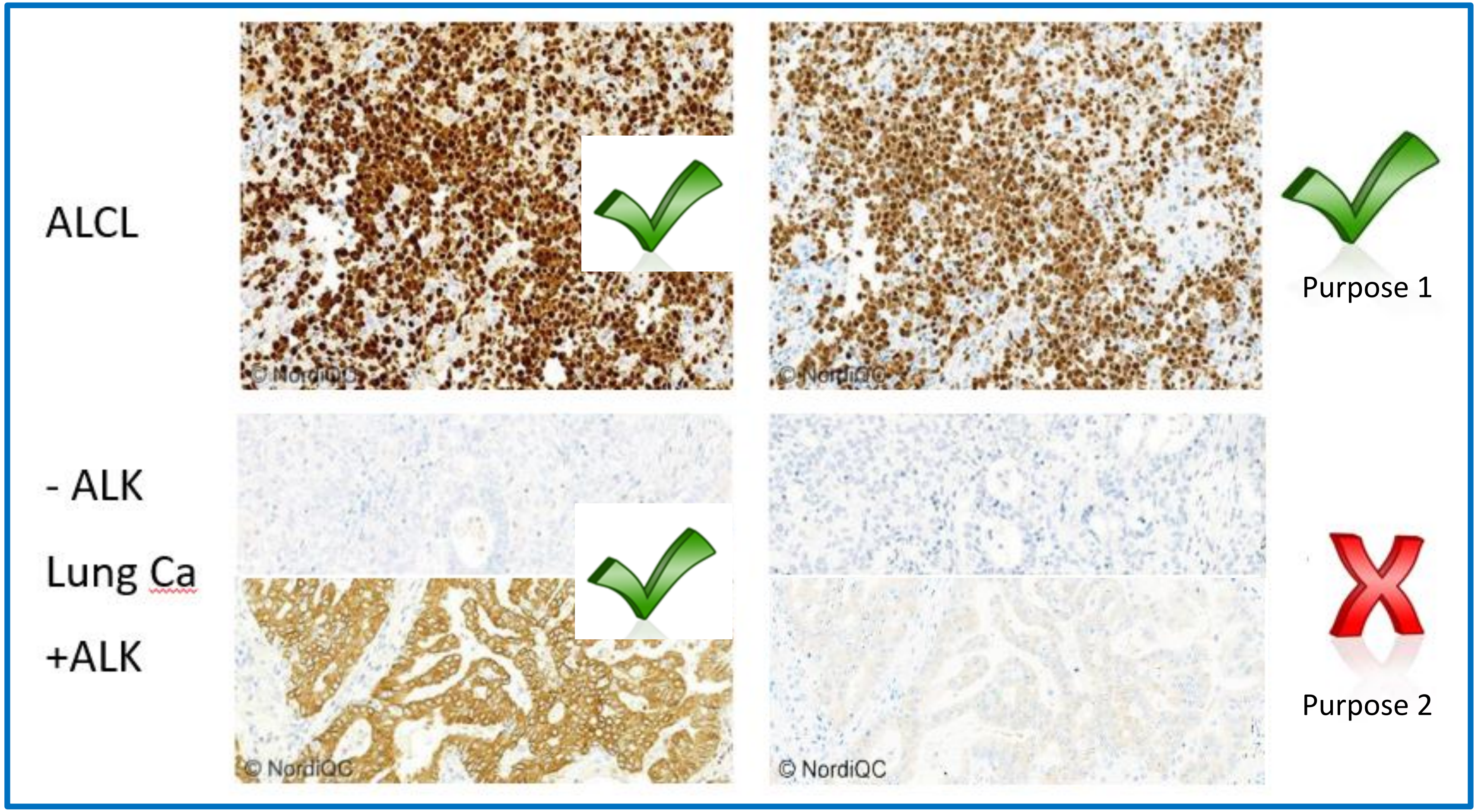
Lung sample

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



Typically **high** antigen expression level

Typically **low** antigen expression level

IHC method 1

IHC method 2

IHC for ALK

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

E Torlakovic et al. AIMM 2017;25:4-11
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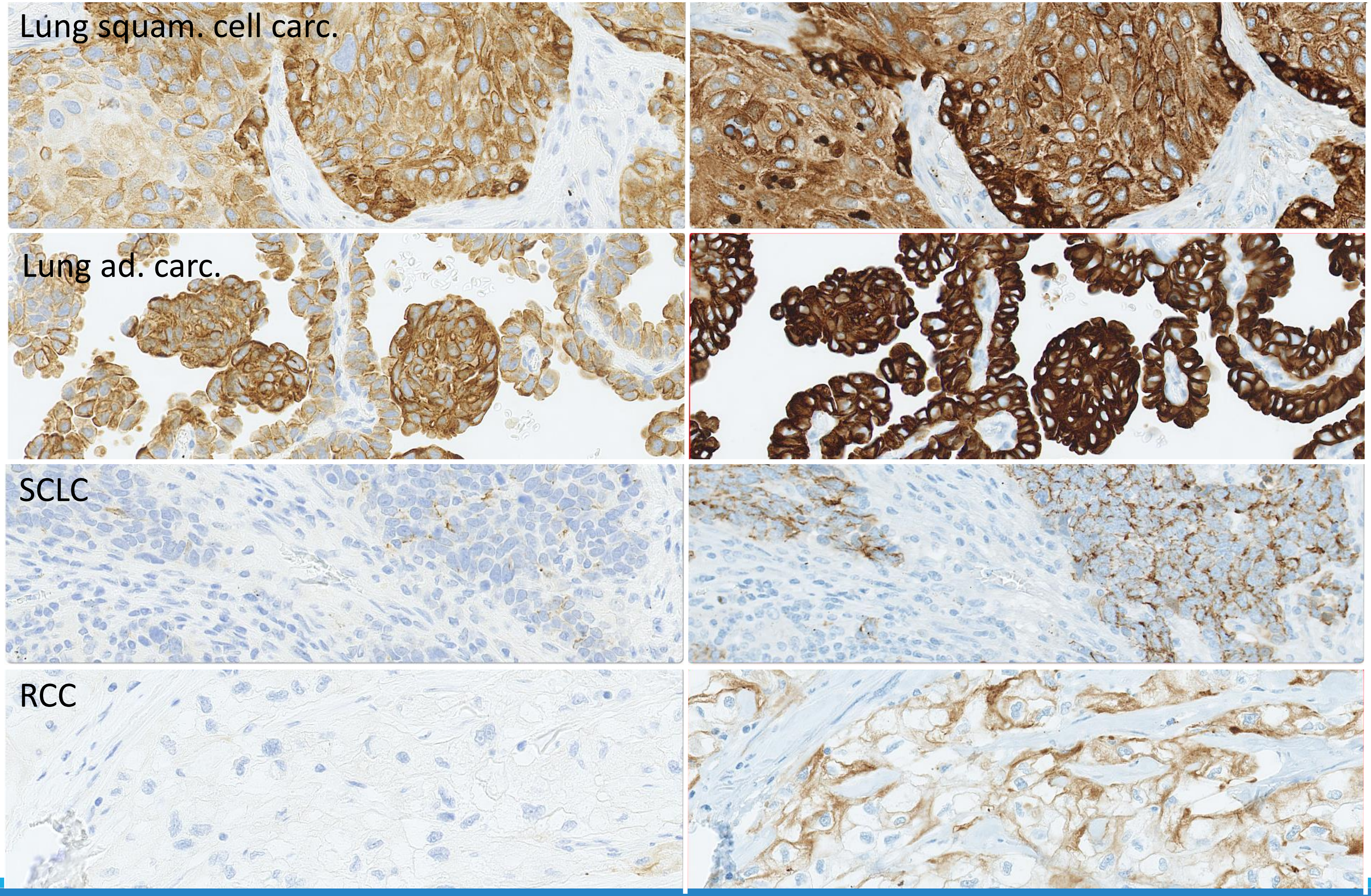
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

	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 squamus 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 squamus cell carc vs adenocarcinoma	

* CUP= Cancer Unknown Primary

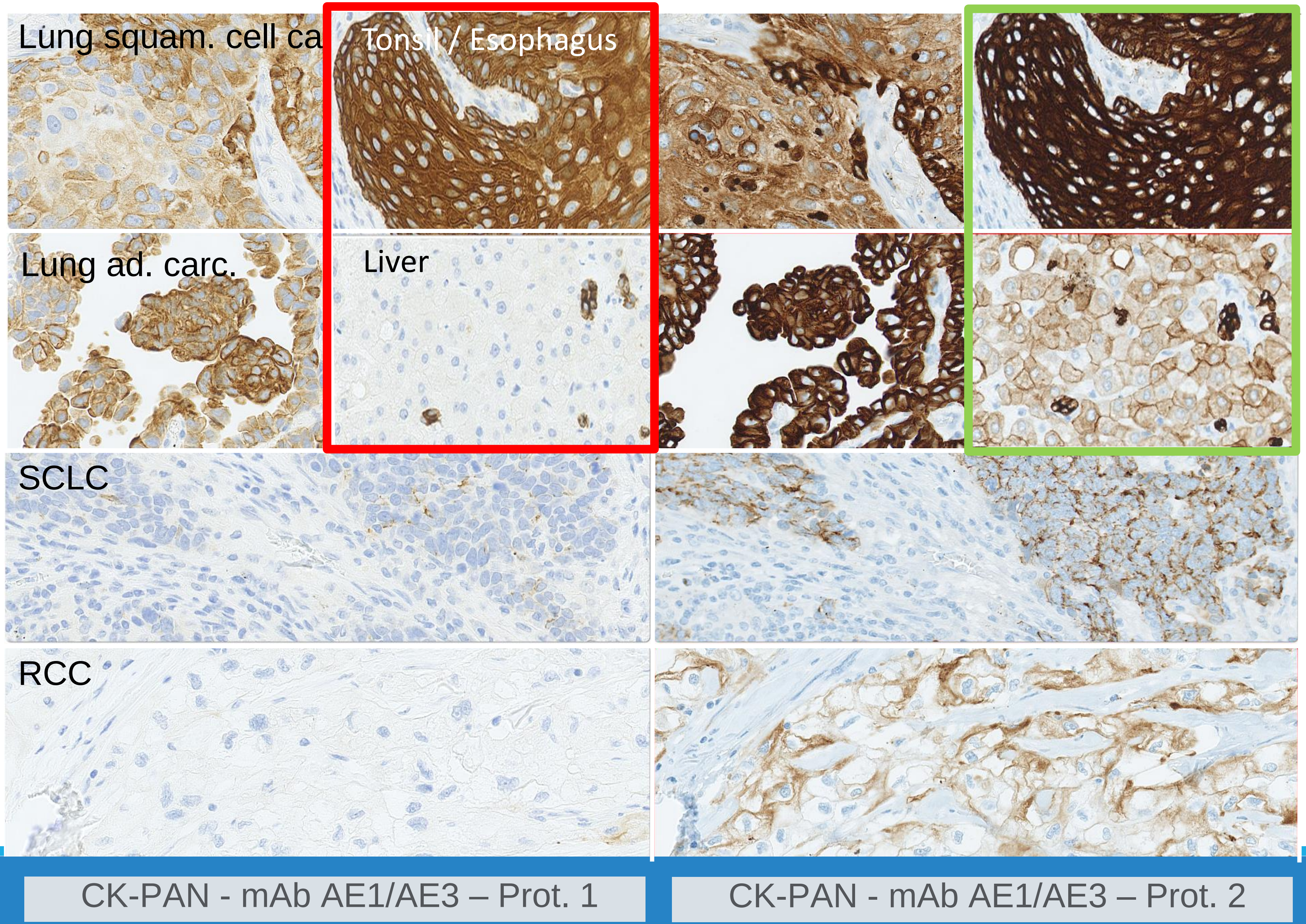
Use of samples for technical / analytical validation of IHC



CK-PAN - mAb AE1/AE3 – Prot. 1

CK-PAN - mAb AE1/AE3 – Prot. 2

Use of samples for technical / analytical validation of IHC



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

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

	Purpose I	Purpose II	Influenc. factors
CK-Pan	CUP - carcinoma lineage	Sentinel node – carcinoma metastatis	Clone, titer, retrival
CK 19	Sentinel node – carcinoma metastatis	Thyroid adenoma vs carcinoma	Titer, retrieval
EPCAM	CUP - carcinoma lineage	Lung carcinoma vs mesothelioma	Titer, retrieval
TTF1	CUP - lung adenocarcinoma	Lung adenocarcinoma vs squam.	Clone, titer
…….			

High analytical sensitivity can compromise clinical utility…..

Protocol developed, optimized and validated for purpose I will most likely compromise use for purpose II due to reduced analytical selectivity and specificity

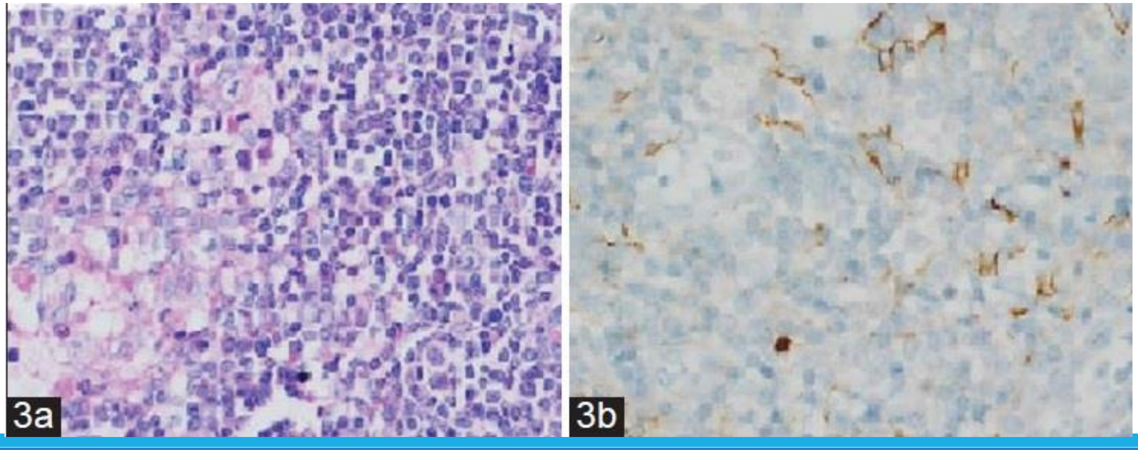
Protocol developed, optimized and validated for purpose II will most likely compromise use for purpose I due to a reduced level of analytical sensitivity

Identification of purpose of the test

Sensitivity , specificity – what to choose…?

	Purpose I	Purpose II	Influenc. factors
CK-Pan	CUP - carcinoma lineage	Sentinel node – carcinoma metastatis	Clone, titer, retrival
CK 19	Sentinel node – carcinoma metastatis	Thyroid adenoma vs carcinoma	Titer, retrieval

Jacob PM, Nair RA, Nair SP, Jayasudha A V.
Cytokeratin-positive interstitial reticulum cells in the lymph node:
A potential pitfall. Indian J Pathol Microbiol 2016;59:128-9



CK-Pan e.g. Clone AE1/AE3 with HIER

Can and will provide interpretational challenges in SN due to labelling of specialized macrophages with CK8/18

CK19 more selective
(CK19 mRNA applied for OSNA technique)

Conclusions for technical / analytical validation of IHC

1. IHC assay is calibrated (LD assay) / verified (RTU – plug-and-play) on TMA with 16-30 different normal tissues. If access to ICAPCs these must be included and submitted to pre-analytical conditions applied in the laboratory.

2. IHC assay is validated on TMAs with e.g. 30-45 commonly seen neoplasias and on TMAs with the target of interest – 10/10 or 20/20 neoplasias expected to be pos./neg. (accuracy) covering the dynamic range of expression and cut-off's (index) – note not all markers are reliable if only TMA's are used (e.g. heterogene expression)

3. Results compared to literature, reference clone etc and conclusion made.

4. *Number of specimens tested being dependant on local requirements (accreditation bodies), access to specific lesions and similar conditions*

Challenges for technical / analytical validation of IHC

1. Limited access to relevant tissues – rare incidences
   - ALK (lung), ROS1, Myogenin..

2. New markers not described in details – no data on test performance characteristics
   - SATB2, Claudin-4, PRAME, TRPS1….

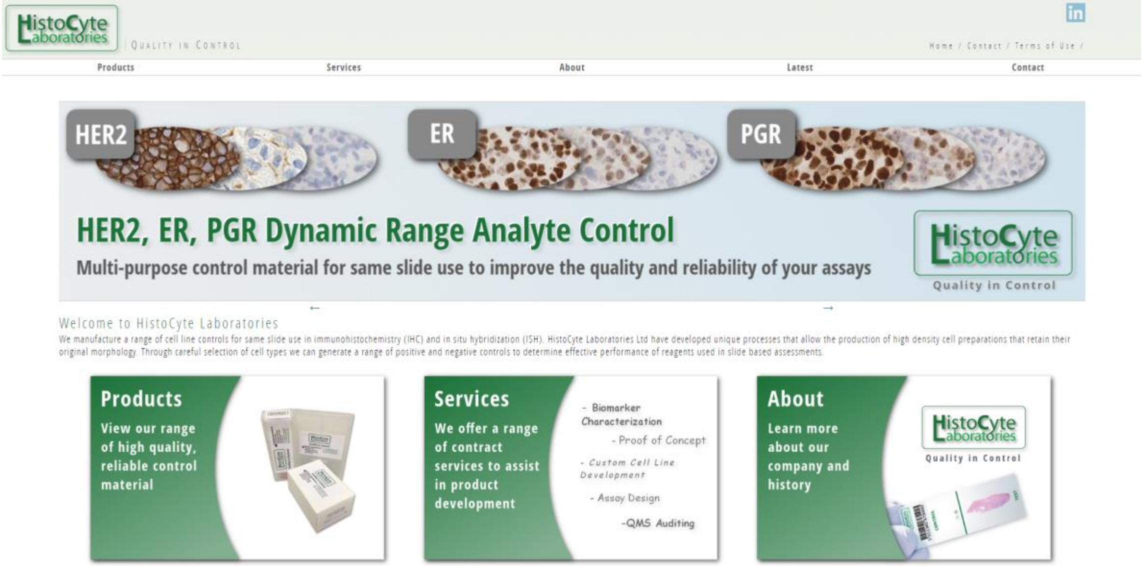
3. Limited access to reference material and/or critical expression levels
   - PD-L1, HER2, ER…

Where to start – how to end…..

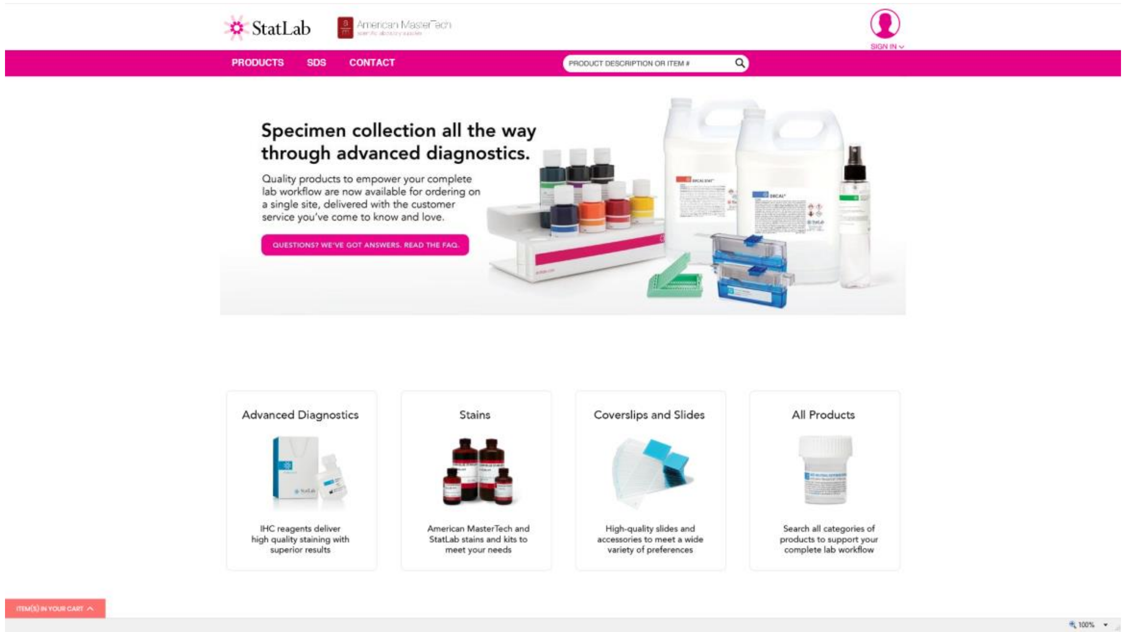
Role of cell lines & histoids for IHC test development

1. Limited access to relevant tissues – rare incidences
   - ALK (lung), ROS1, Myogenin..
3. Limited access to reference material and/or critical expression levels
   - PD-L1, HER2, ER…

Starting help to guide development – validation still required….



www.histocyte.com

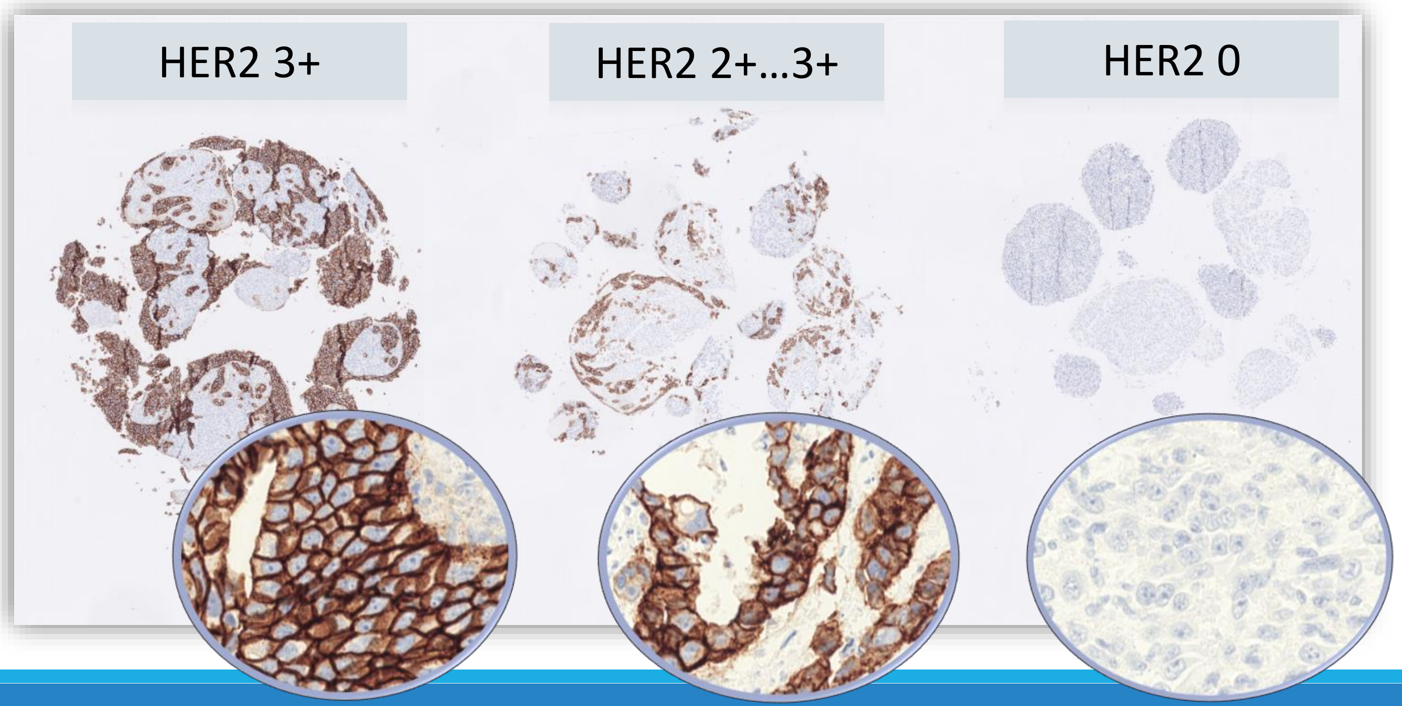


www.statlab.com

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

Histoids / Faux tissue
ALK +/-
HER2, PD-L1 with dynamic range

Histoids / Faux tissue – TruQ IHC controls



Tissue core with IHC 3+ and IHC 2+ almost identical concerning expression levels.

No IHC 1+ tissue

Design seems less adequate for ”precision testing” for HER2 IHC both ”classical” and HER2 low.

www.statlab.com

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

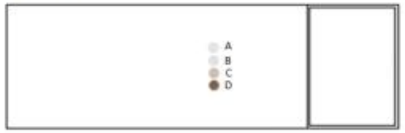
This product is suitable for use in immunohistochemistry and in situ hybridization.

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

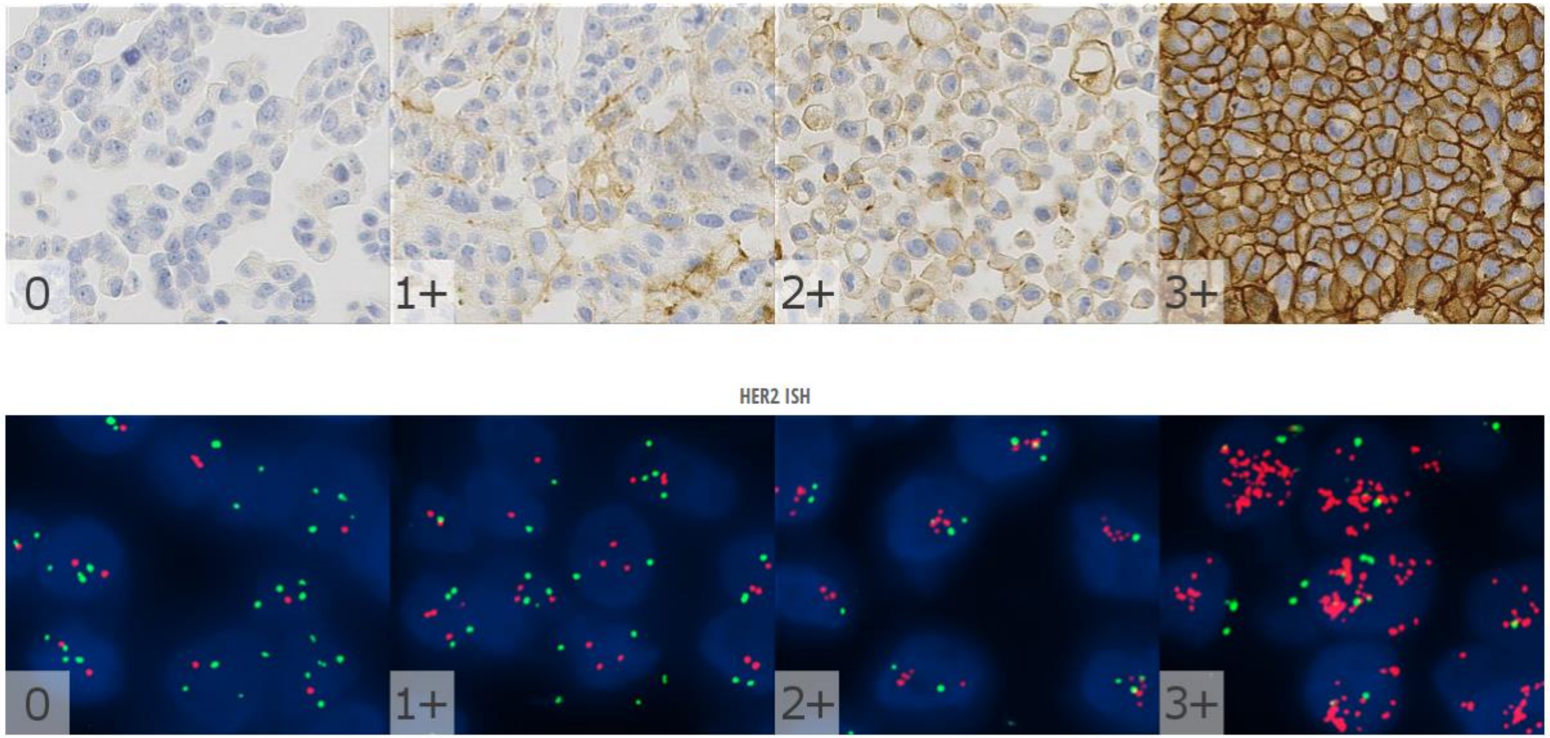
1. The product is intended for research use only.
2. It is the responsibility of the end user to determine suitability with their reagents and procedures within their laboratory.
3. Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those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

TROUBLE SHOOTING

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

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

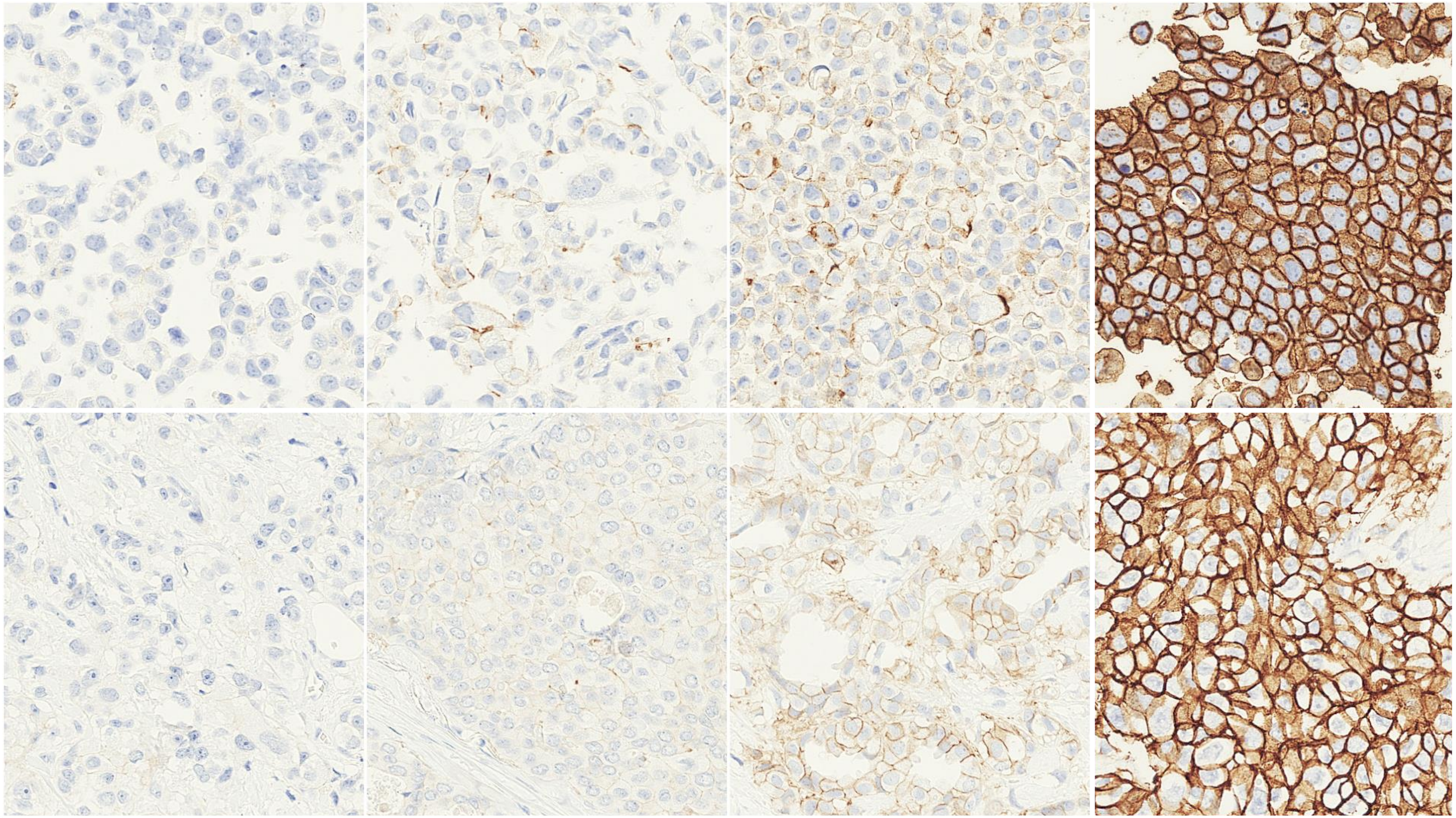
In NordiQC run B34 10% of the participants used cell lines as onslide control



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 insuccessful test?
Impact on section thickness?
Pattern on different assays?

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



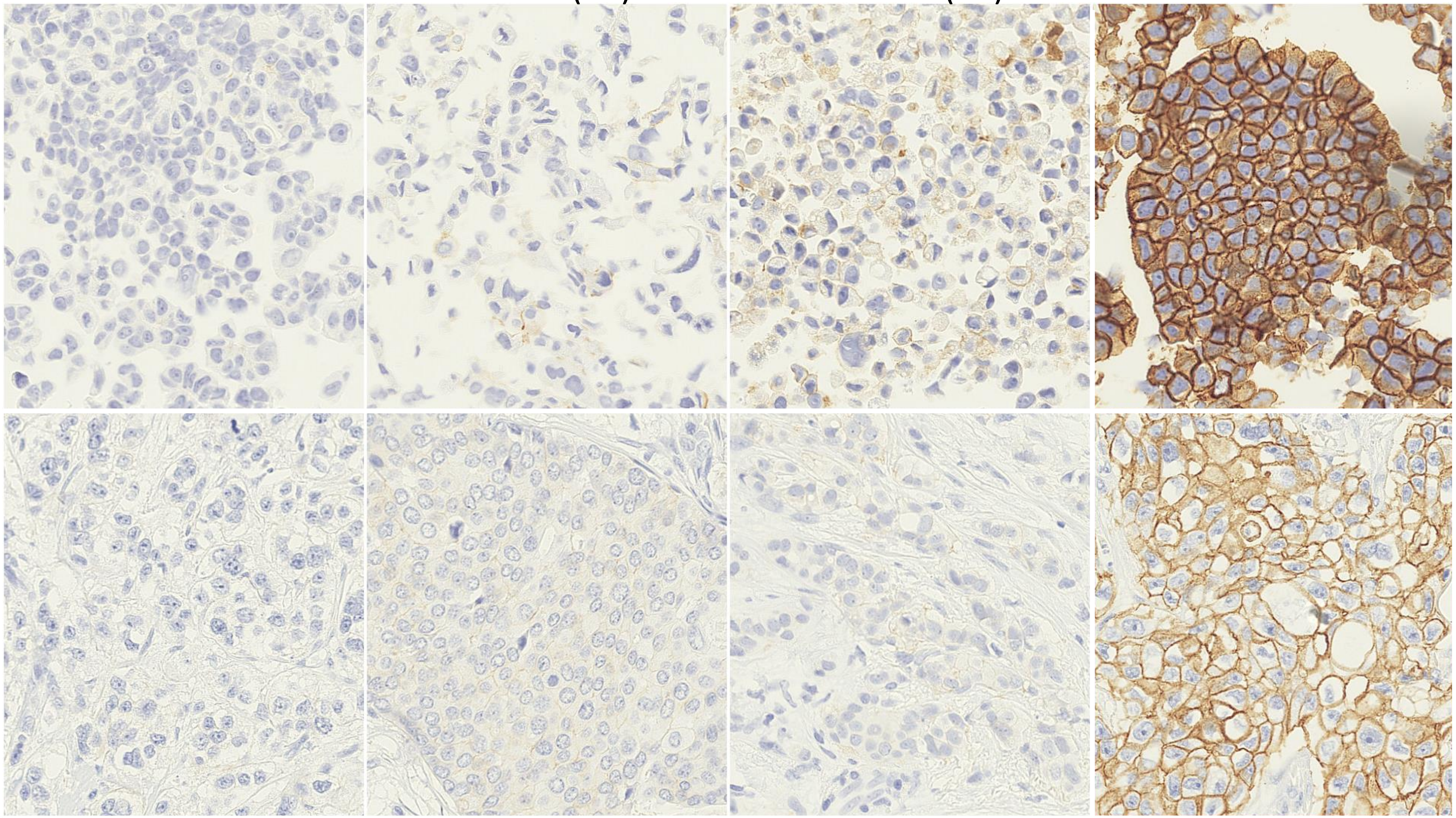
Correlation of IHC for HER2 – (inaccurate) PATHWAY – cell lines and tissues

0 | 0 (1+) | 1+ (2+) | 3+

Cell lines
(HCL028 Histocyte)

Breast carcinomas

NordiQC run B34

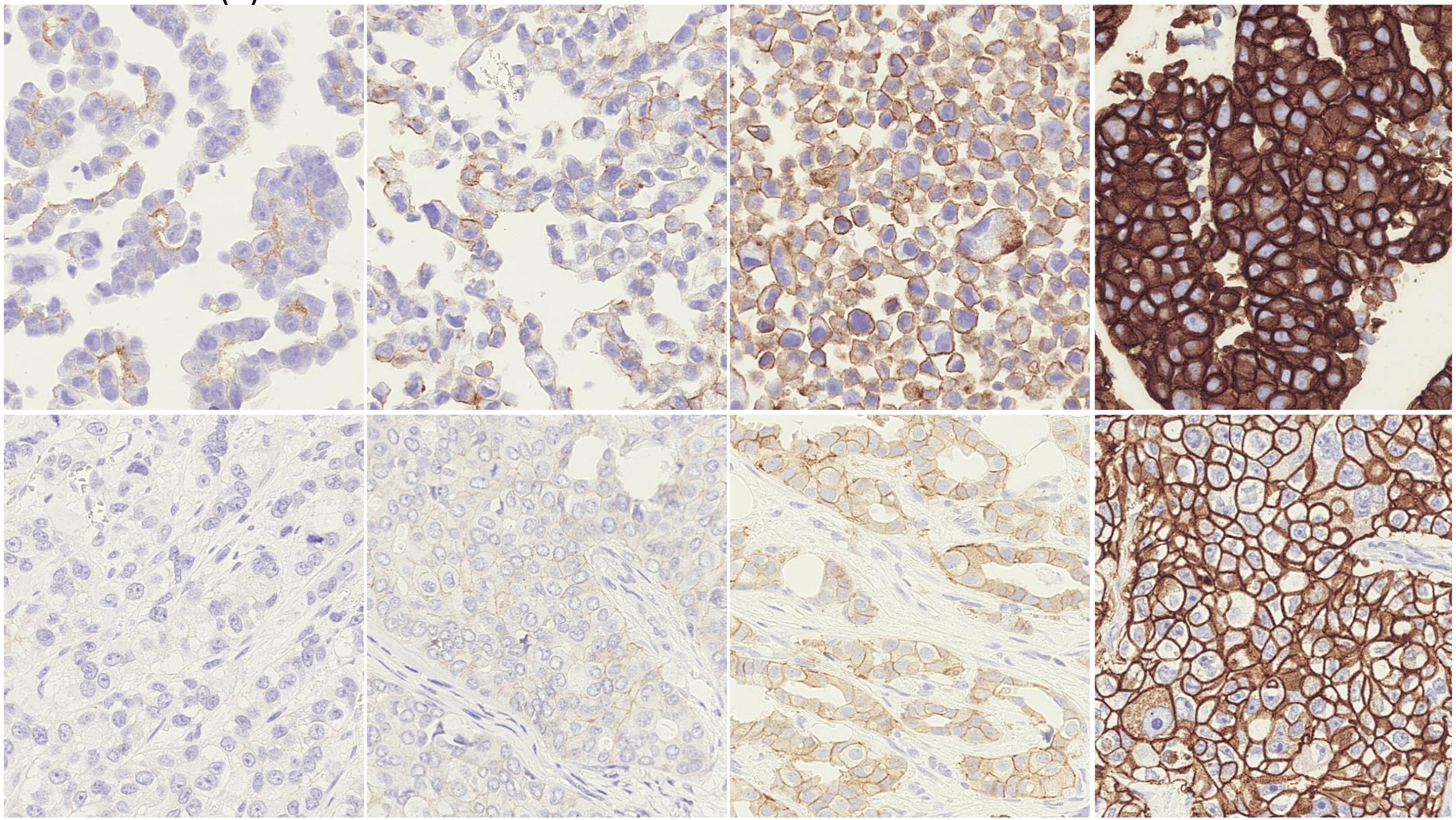


0 | 0 (1+) | 0 (2+ AMP) | 2+ (3+)

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+

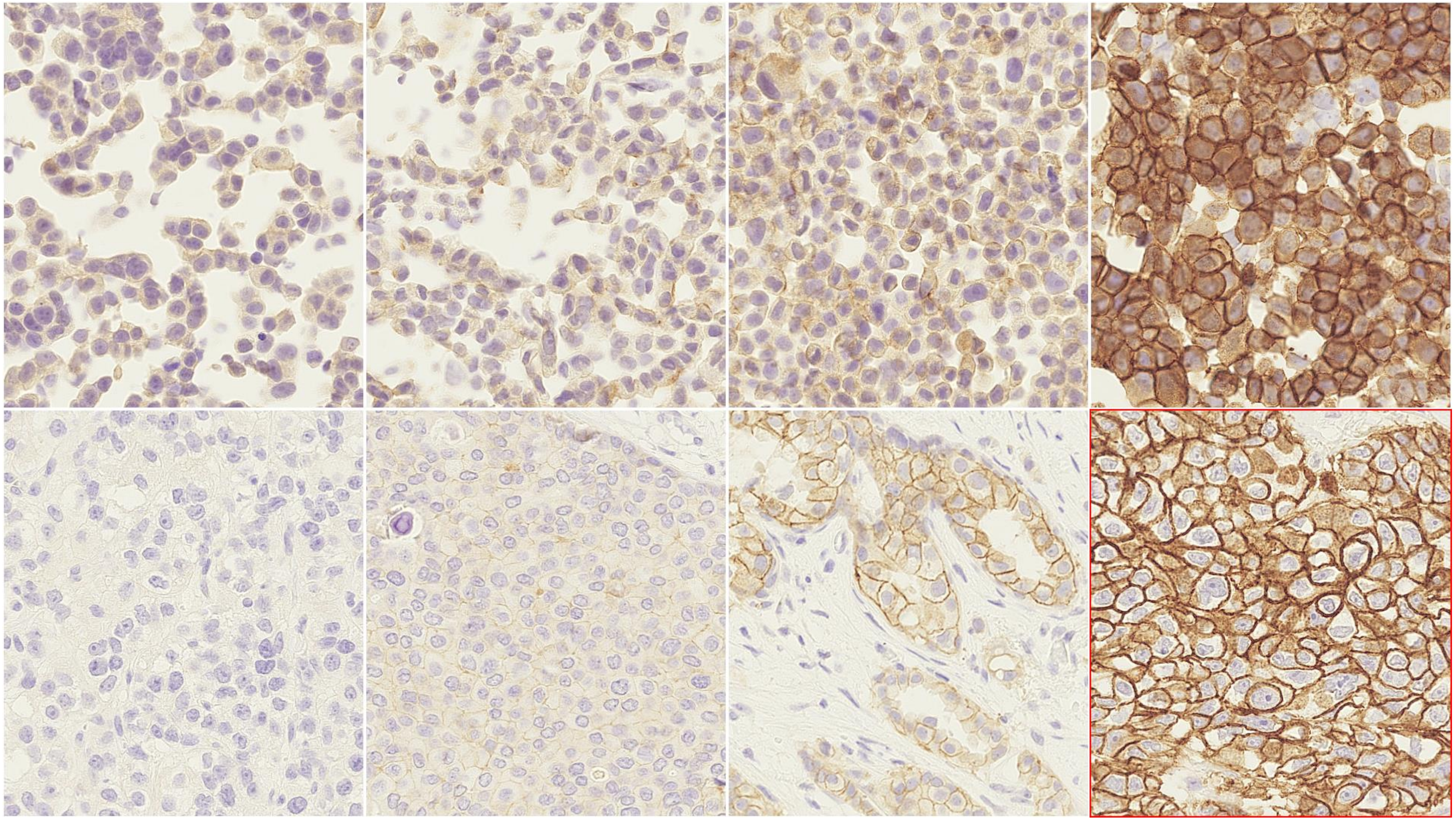
Correlation of IHC for HER2 – SP3 – cell lines and tissues

? (0) ? (1+) ? (2+? 3+

Cell lines
(HCL028 Histocyte)

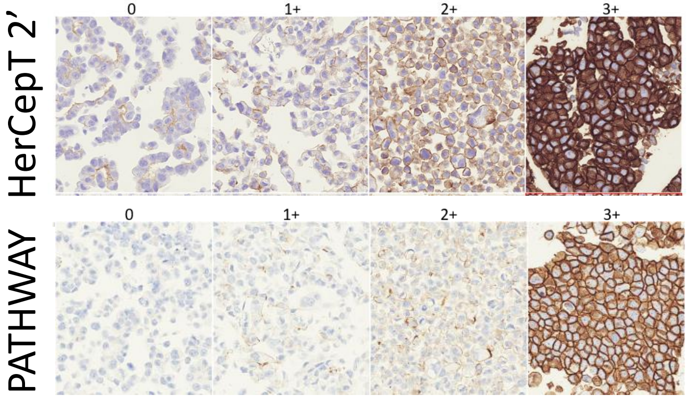
Breast carcinomas

NordiQC run B34



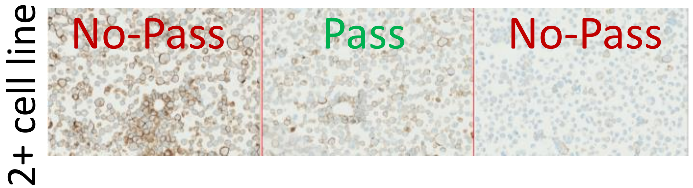
0 1+ 2+ AMP 3+

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

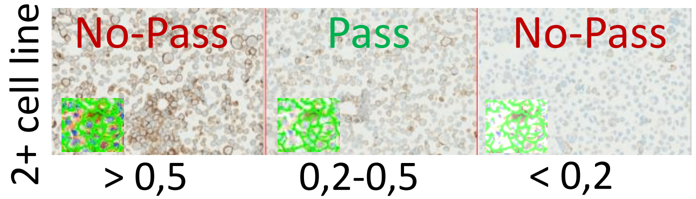


- Need to map staining characteristics for most commonly used IHC assays
  - The different assays will provide different patterns

- Need to identify change in patterns being critical with risk of false negative / false positive results
  - Each assay most likely will have different patterns / tresholds



- 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 insuccessful test



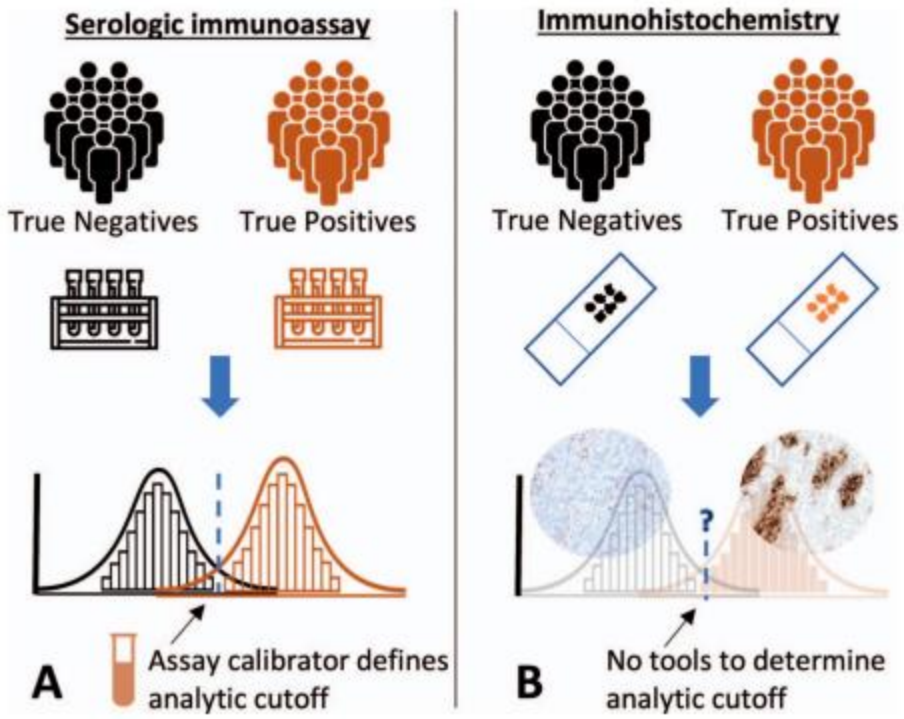
- The DIA/AI QC-scores must be validated for each IHC assay both with focus on expected level and critical levels
  - Large scale testing on e.g. breast carcinomas with the dynamic and critical range of the target analyte
  - Both to identify e.g. ”classical” HER2 overexpression and the novel HER2 low category

Analytical standards – IHC versus clinical chemistry; Calibrators

CA125; 35u/ml

CA-19; 37-40u/ml

….



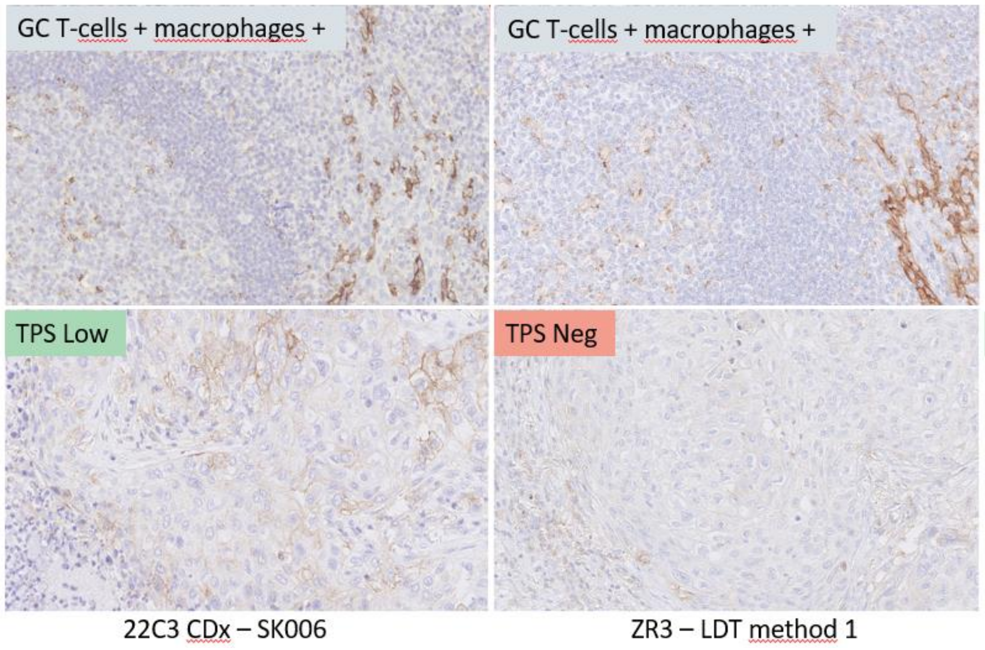
A Consortium for Analytic Standardization in Immunohistochemistry

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)

ICAPCs

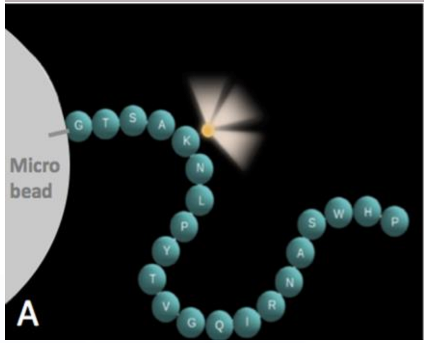
But challenge for more semiquantitative biomarkers as especially HER2 and PD-L1…



Analytical standards – IHC versus clinical chemistry; Calibrators

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

ER, HER2, PD-L1 and p53



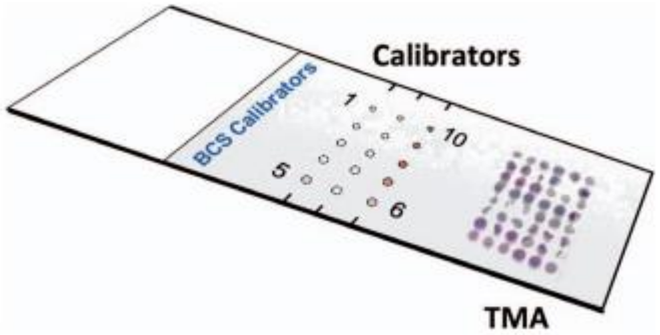


Figure 5. *Illustration of the survey tool for correlating clinical accuracy (from the tissue microarray data) with analytic sensitivity (from the calibrator data). The calibrators are at up to 10 different concentrations, for example levels 1–10. The middle row depicts negative controls. Abbreviations: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

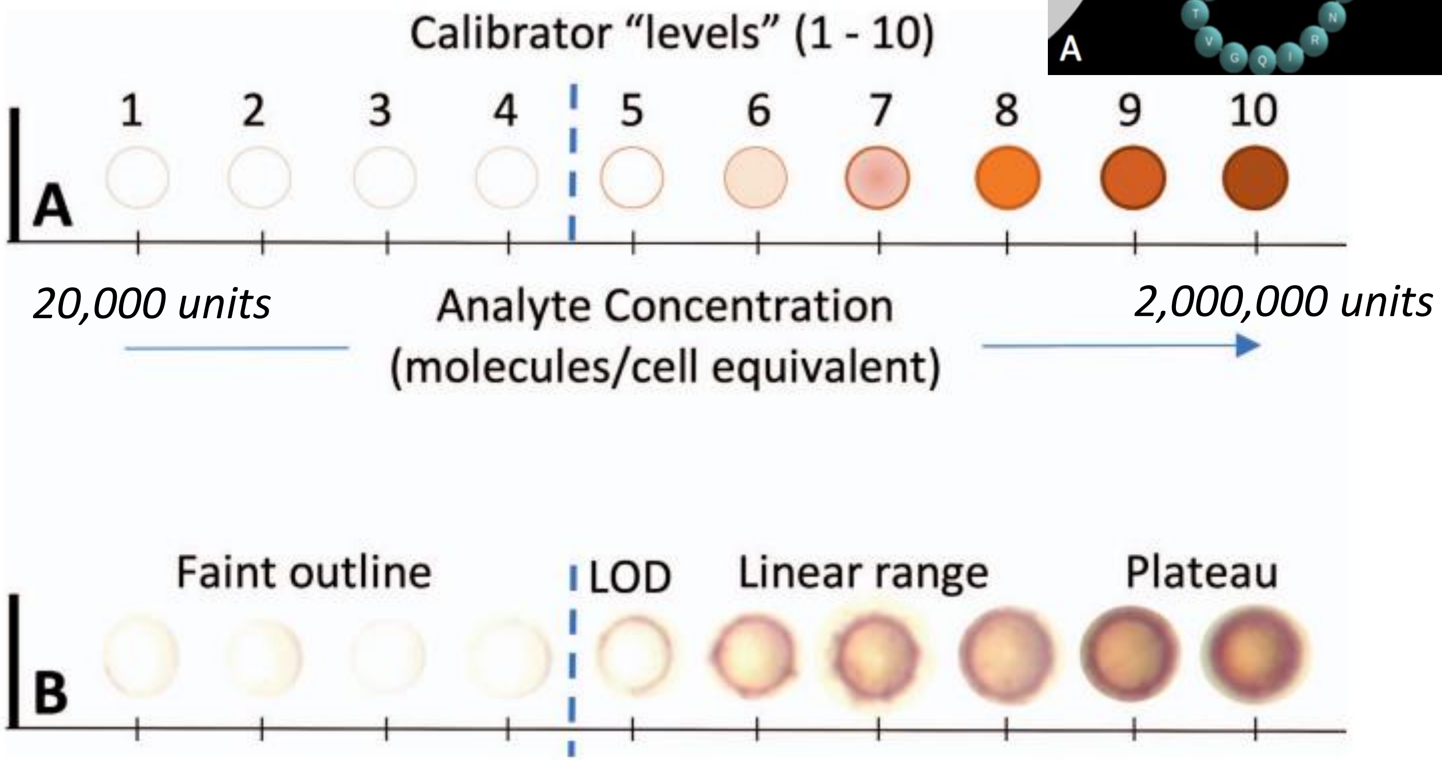


Figure 2. *Illustration of a series of immunohistochemistry calibrators after staining. The numbers refer to calibrator levels, from low (1) to high (10) analyte concentrations. A, The illustration shows that rim staining is stronger than central staining because the analyte is attached to the microbead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

A Consortium for Analytic Standardization in Immunohistochemistry

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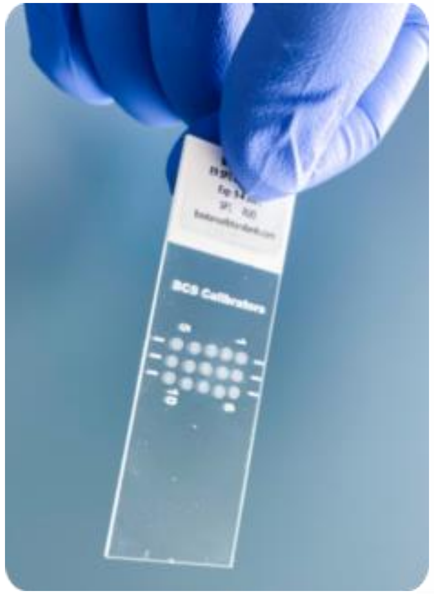
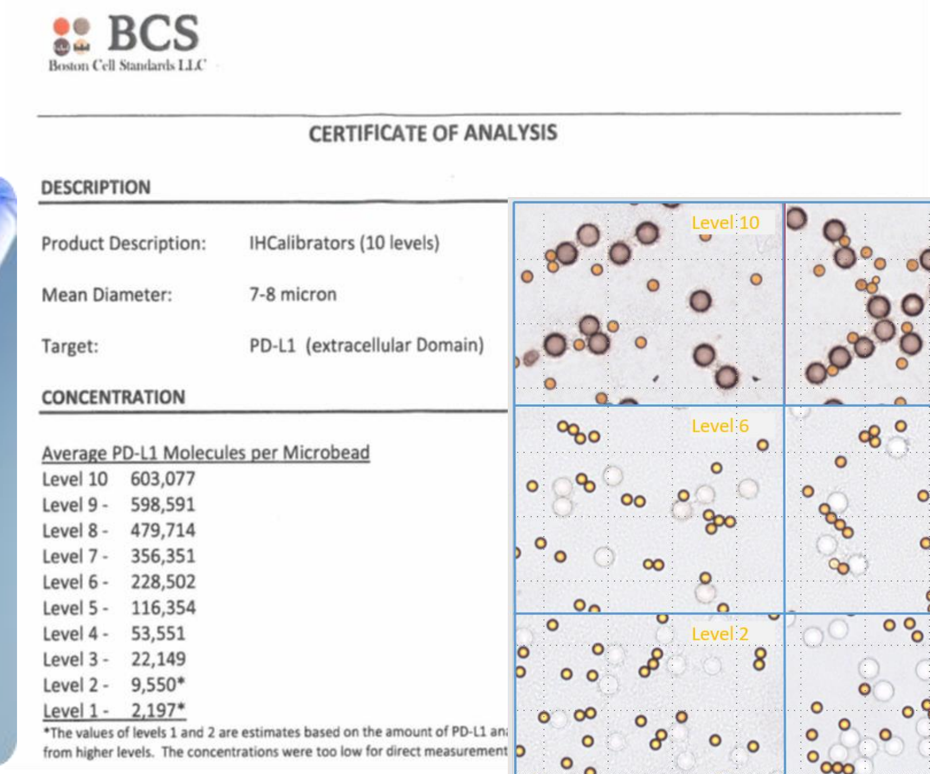
(Arch Pathol Lab Med. doi: 10.5858/arpa.2022-0031-RA)

* LOD; Limit of detection

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





BCS
Boston Cell Standards LLC

CERTIFICATE OF ANALYSIS

DESCRIPTION

Product Description: IHCalibrators (10 levels)

Mean Diameter: 7-8 micron

Target: PD-L1 (extracellular Domain)

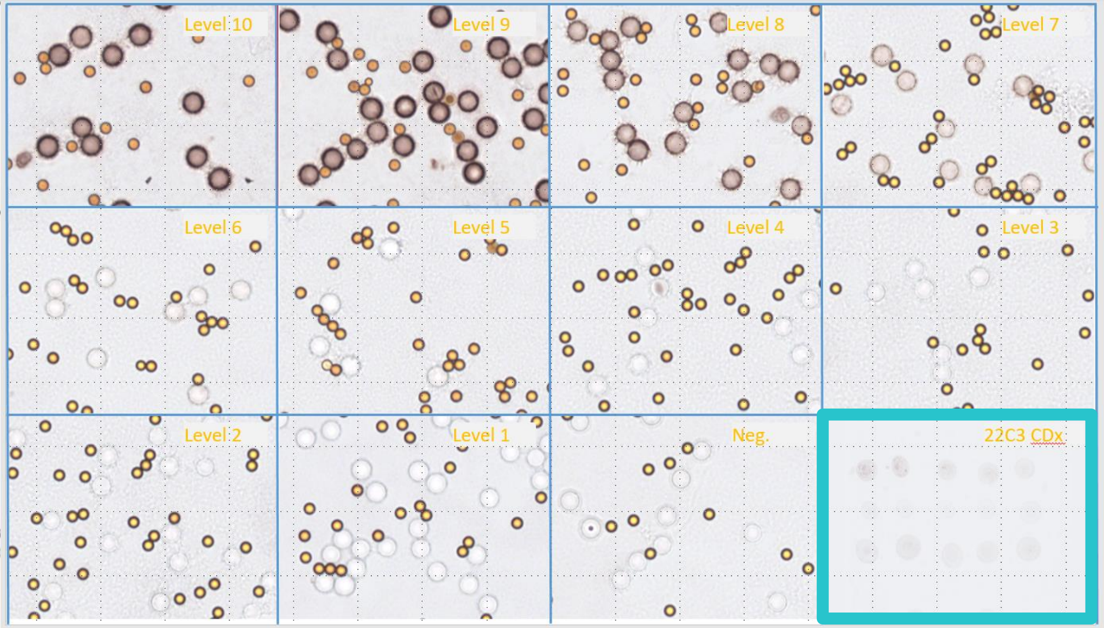
CONCENTRATION

Average PD-L1 Molecules per Microbead

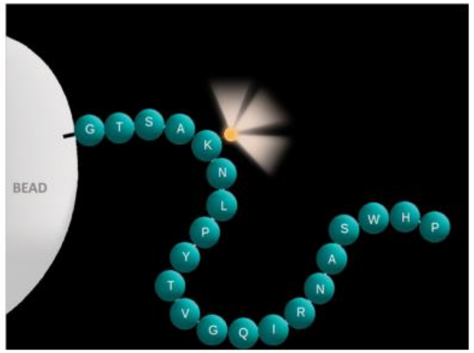
Level 10	603,077
Level 9 -	598,591
Level 8 -	479,714
Level 7 -	356,351
Level 6 -	228,502
Level 5 -	116,354
Level 4 -	53,551
Level 3 -	22,149
Level 2 -	9,550*
Level 1 -	2,197*

*The values of levels 1 and 2 are estimates based on the amount of PD-L1 an
from higher levels. The concentrations were too low for direct measurement

* LOD; Limit of detection / level of analytical sensitiviy



BCS
Boston Cell Standards



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.

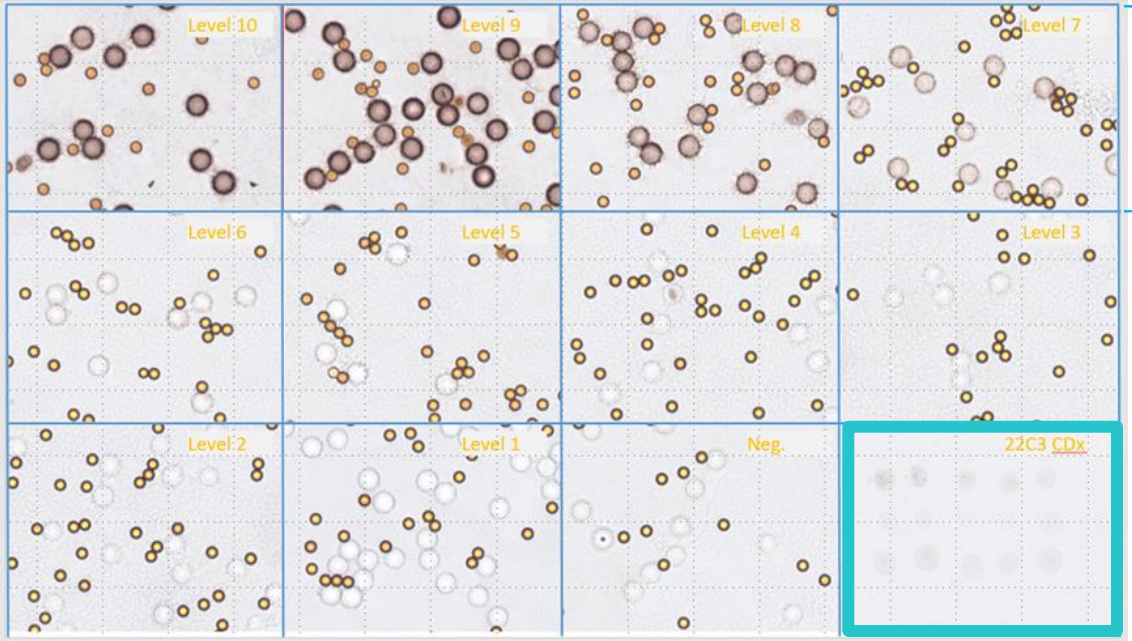
Sompuram, SR, K Vani, AK Schaedle, A Balasubramanian, & SA Bogen. 2018. Quantitative assessment of immunohistochemistry laboratory performance by measuring analytic response curves and limits of detection. *Arch Pathol Lab Med.* 142 (7):851-862.

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

ARTICLE OPEN

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

Seshi R. Sompuram[1], Emina E. Torlakovic[2,3], Nils A. 't Hart[4], Kodela Vani[1] and Steven A. Bogen[1]✉



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*

Mod Pathol. 2022;35(3):326–332.

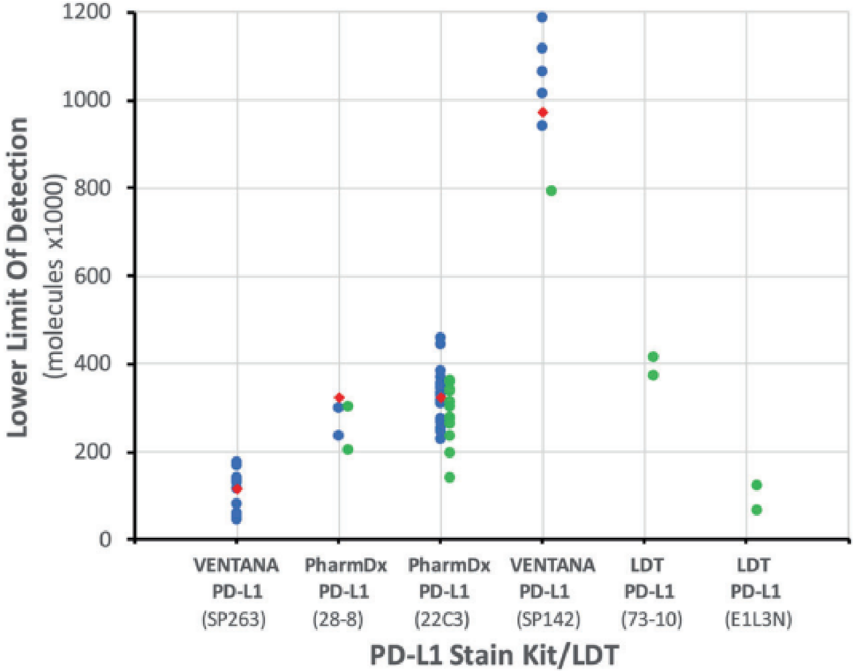
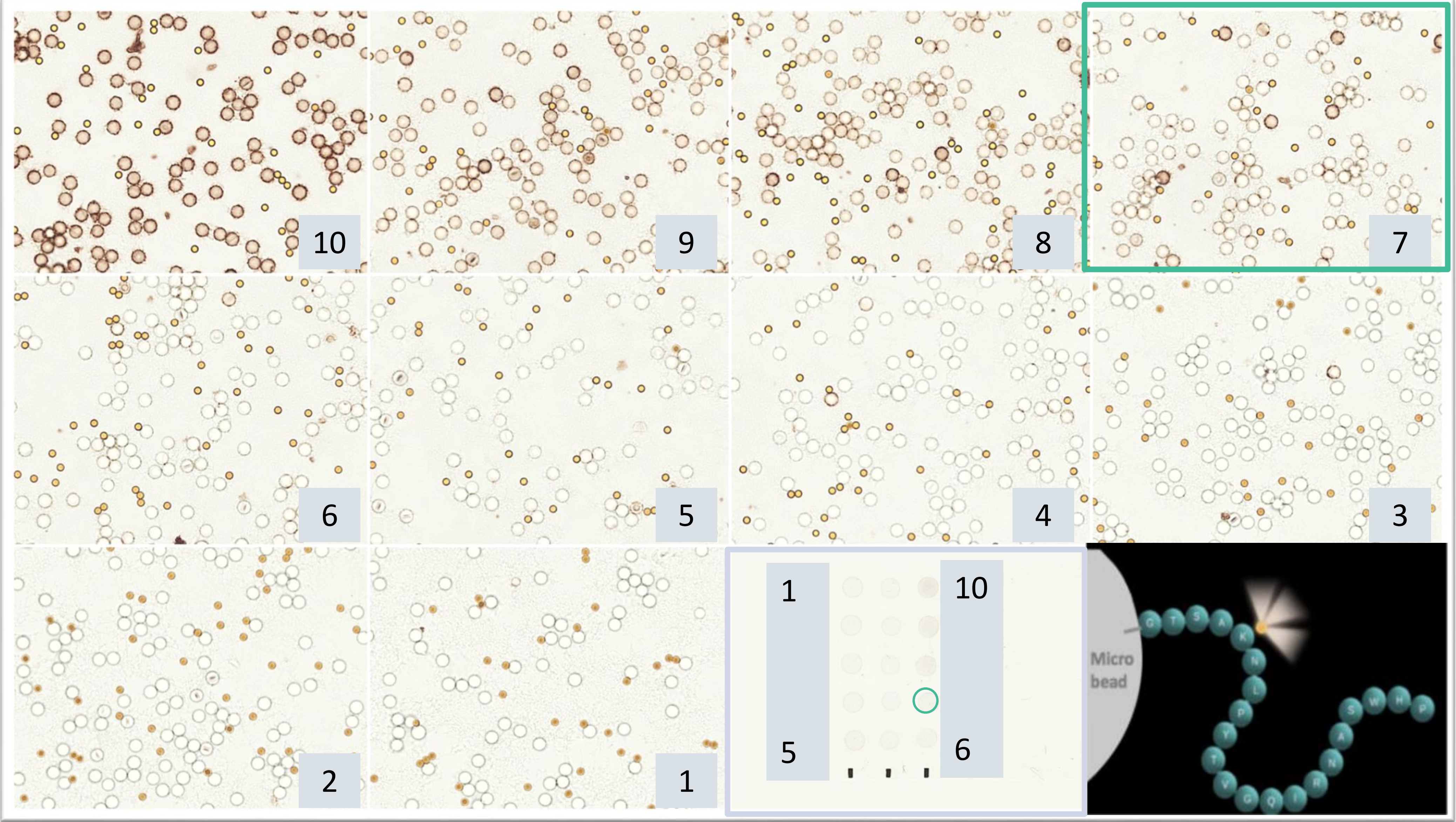


Fig. 2 Lower limit of detection (LOD) of various PD-L1 assays (x axis). Lower numbers (on the y axis) equate to greater sensitivity. Each dot represents a separate IHC laboratory test. Blue dots depict FDA-cleared assays in clinical laboratories, green dots for laboratory-developed tests (LDTs), and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

IHC Calibrator 10 levels HER2 – Boston Cell Standards - PATHWAY

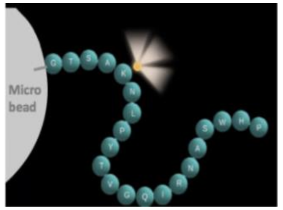


HER2 molecules

pr microbead

10. >2,715,976
9. 2,715,976
8. 2,669,835
7. **<u>1,981,264</u>**
6. 1,274,947
5. 724,800
4. 376,965
3. 206,597
2. 114,315
1. 62,849

Correlation of IHC for HER2 – Microbeads – Accuracy/Precision

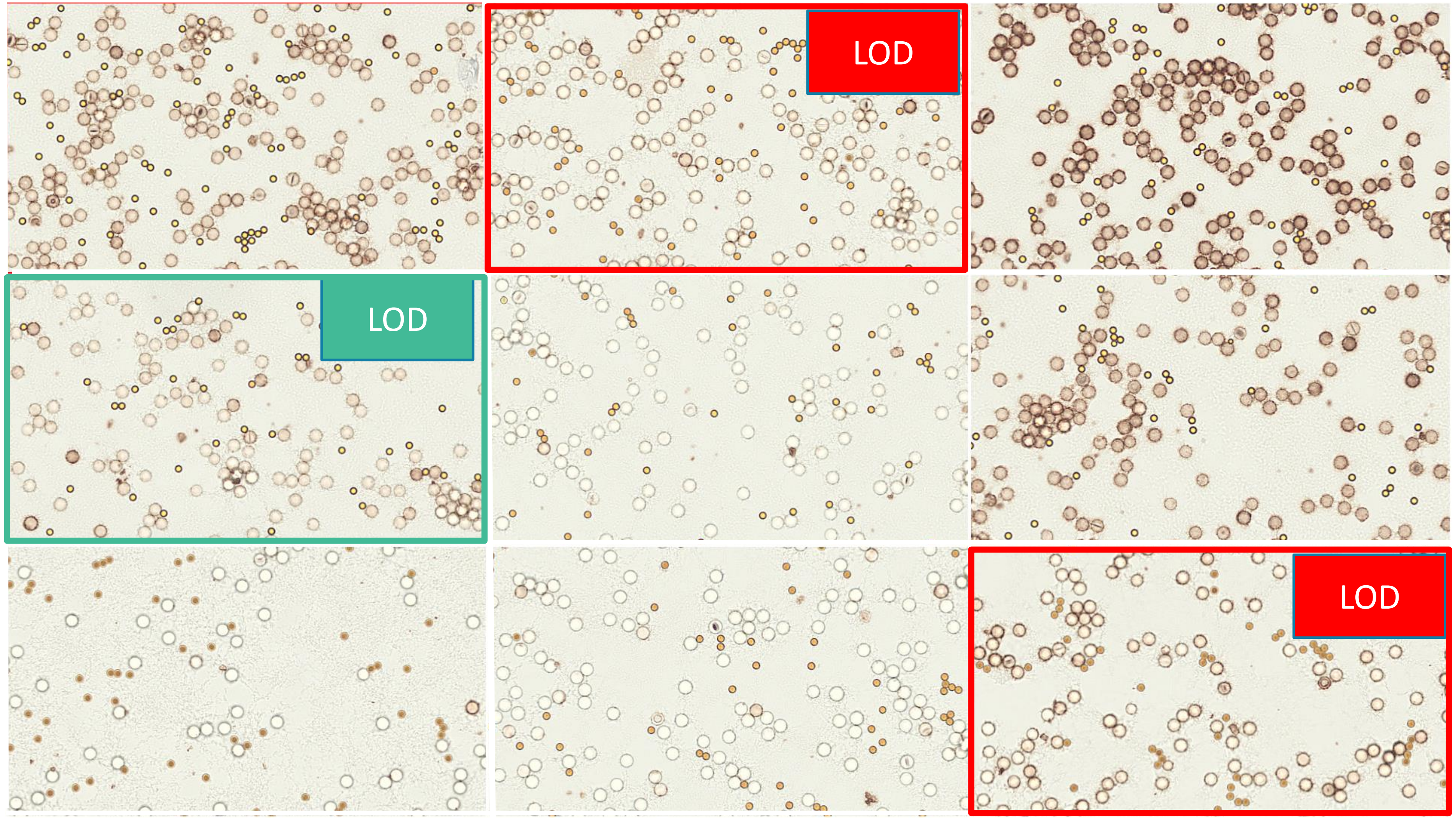


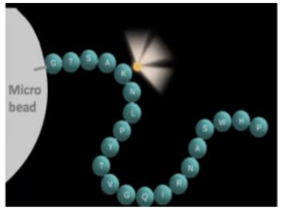
HER2 molecules pr microbead | PATHWAY Standard | PATHWAY – red. HIER & Ab | PATHWAY + OptiView

8. 2,669,835

7. <u>1,981,264</u>

6. 1,274,947





Correlation of IHC for HER2 – Microbeads – Accuracy/Precision

Breast carcinomas

PATHWAY Standard
LOD 1,981,264 HER2 mol.

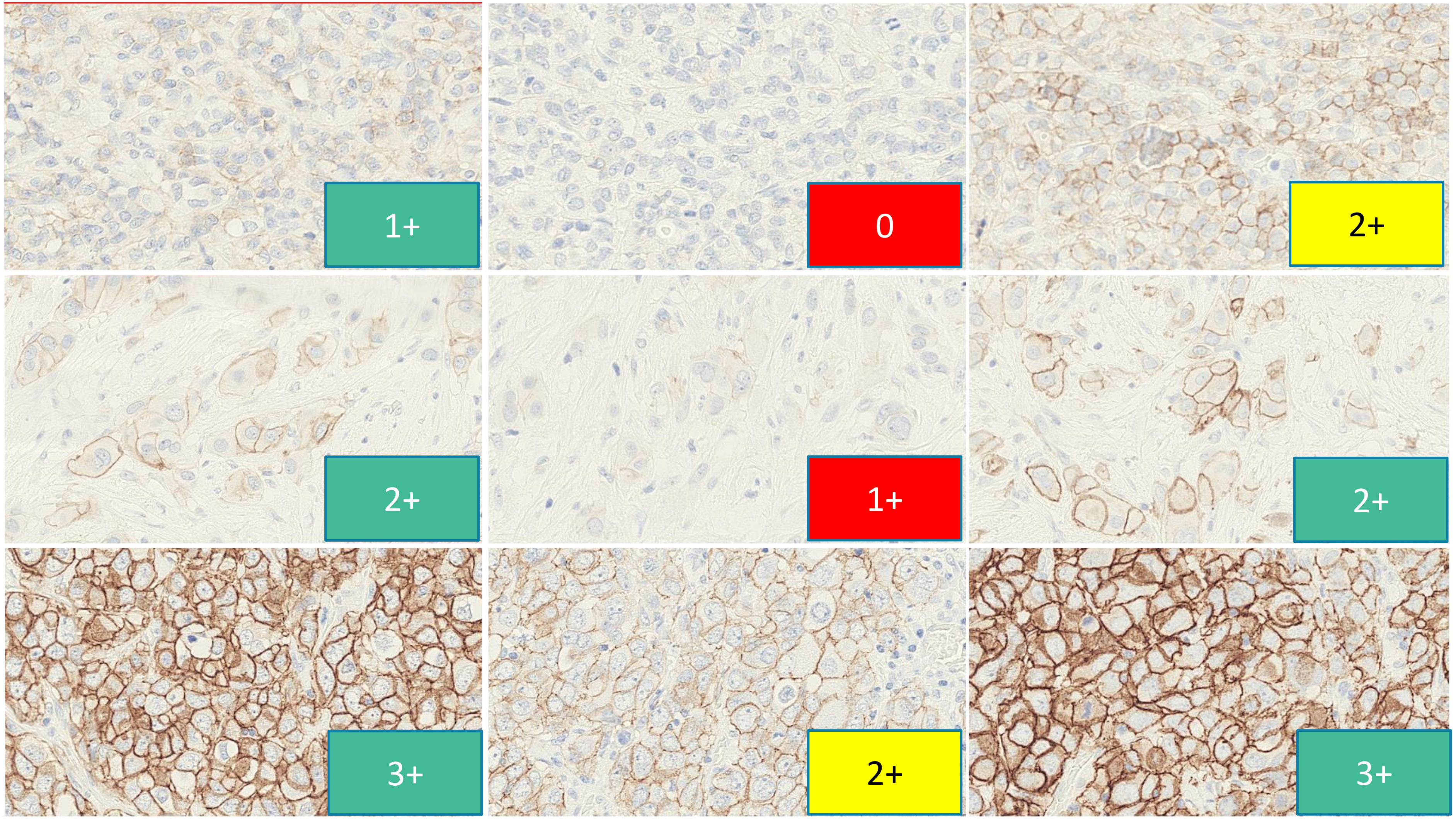
PATHWAY – red. HIER & Ab
LOD 2,669,835 HER2 mol.

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

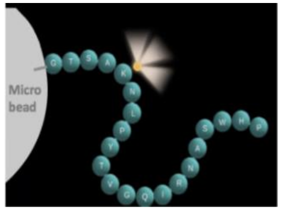
Unamplified 1+

Amplified 2+

Amplified 3+



Correlation of IHC for HER2 – Microbeads – Accuracy/Precision



Breast carcinomas

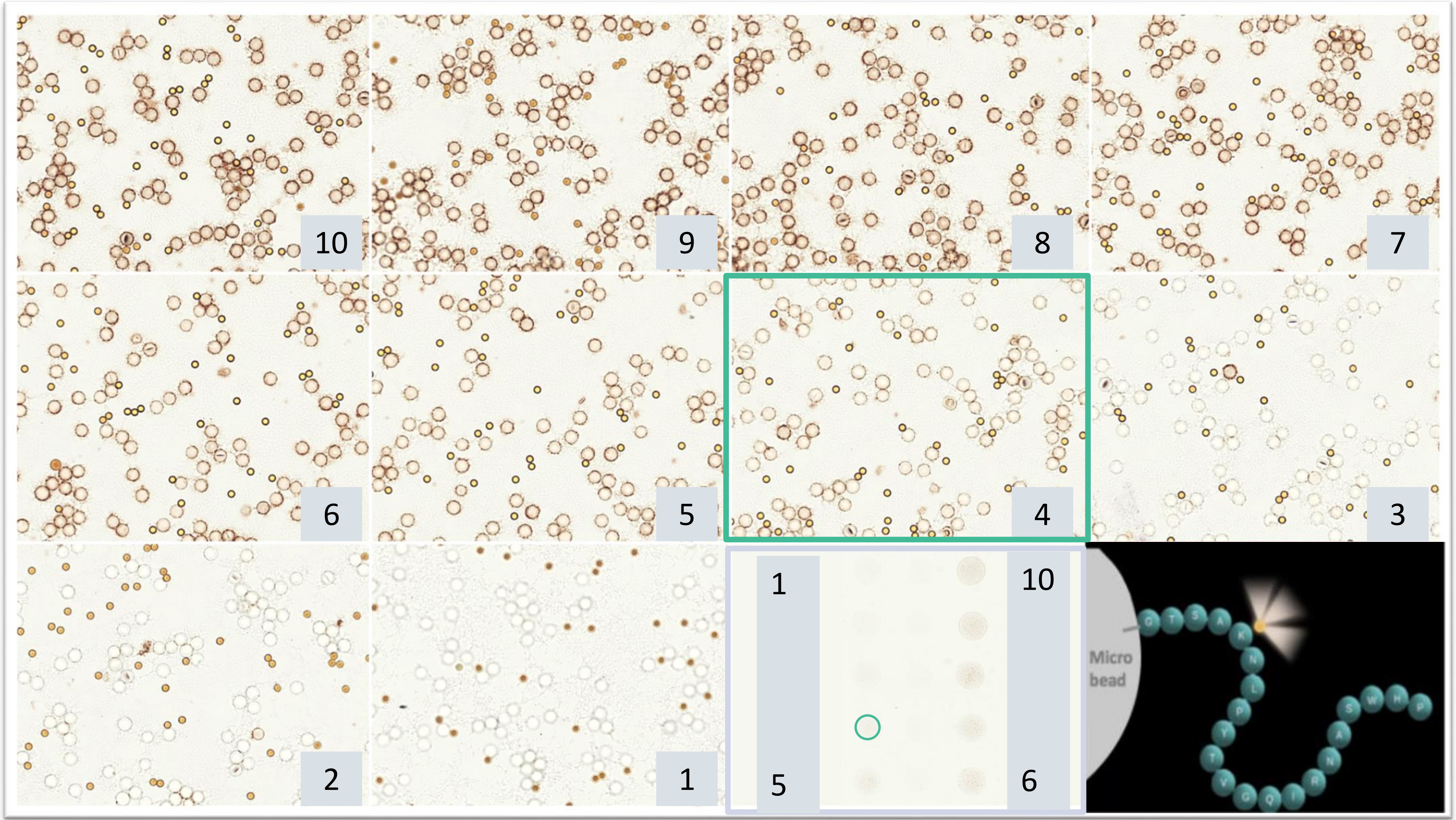
N=15 (NordiQC runs B31, B32, B33)

			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.
HER2 classical	HER2 Low	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

IHC Calibrator 10 levels HER2 – Boston Cell Standards – HercepTest Mo.



HER2 molecules

pr microbead

10. >2,715,976
9. 2,715,976
8. 2,669,835
7. 1,981,264
6. 1,274,947
5. 724,800
4. **376,965**
3. 206,597
2. 114,315
1. 62,849

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, <u>BUT</u> still require extensive documentation and data how to use these

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

Main elements to develop & validate IHC assays

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

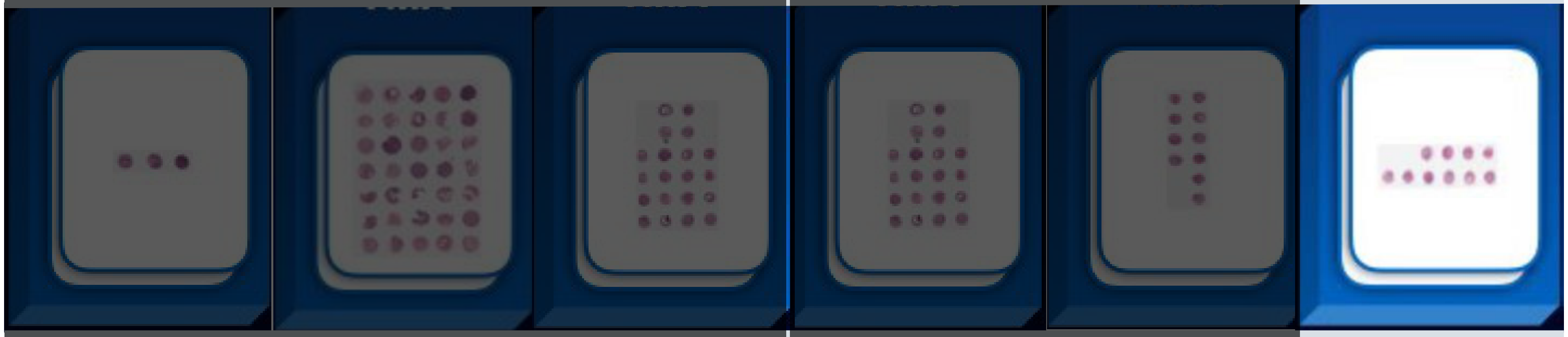
1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
2. Evaluation of robustness of the IHC assay – impact on pre-analytics
3. Evaluation of analytical sensitivity/specificity
4. Identification of IHC performance controls providing information that the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

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 Method of transfer proof
High expression Low expression No expression	With expression No expression		With expression No expression 20/40 of each Type I/II IHC	High expression Low expression No expression + relevant cut-off	

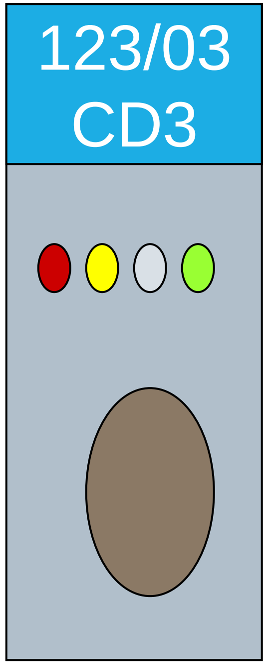


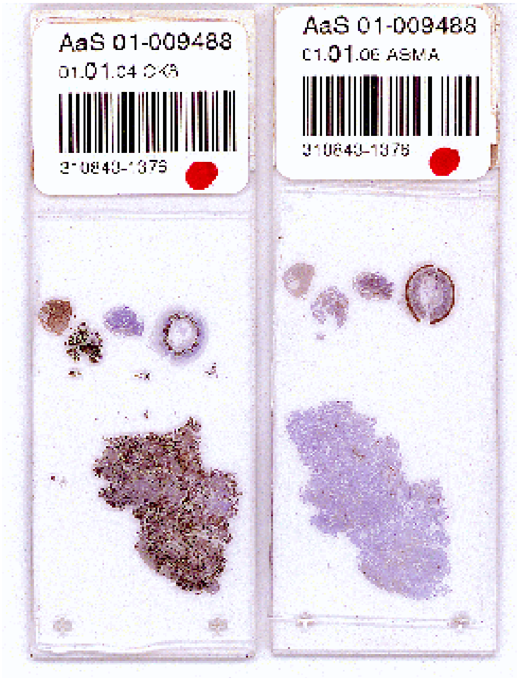
*Immunohistochemical critical assay performance controls

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,† 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‡§*

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†*

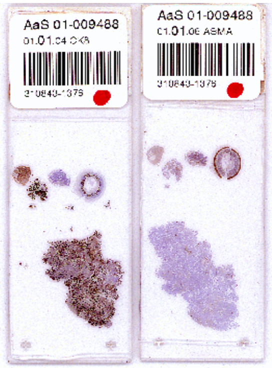
ASCO special articles

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

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

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".



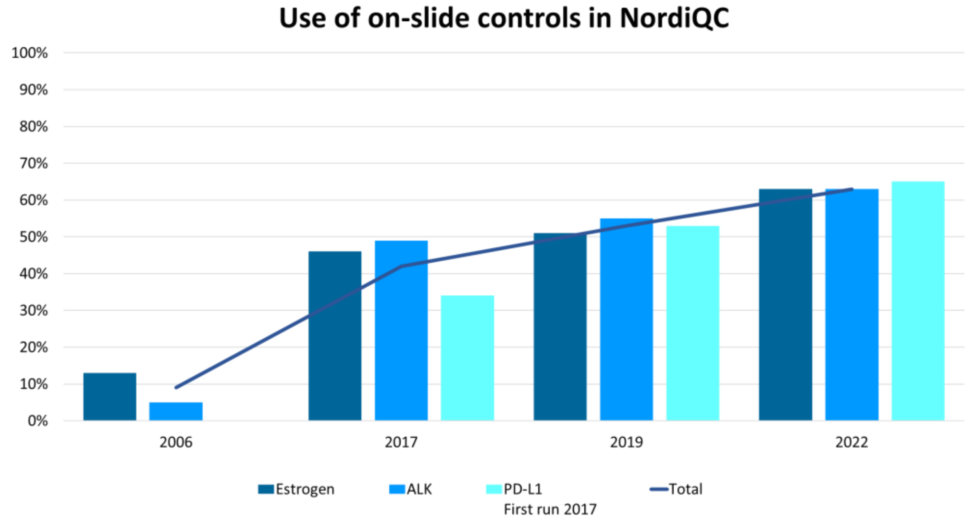


Fig. 5 **Evolution of use of on-slide controls in NordiQC**

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

Application of TMA for QC of diagnostic IHC

2% error rate;

Class I 0,8%
Class II 9,0%

(452/22.234 slides)

(*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†*

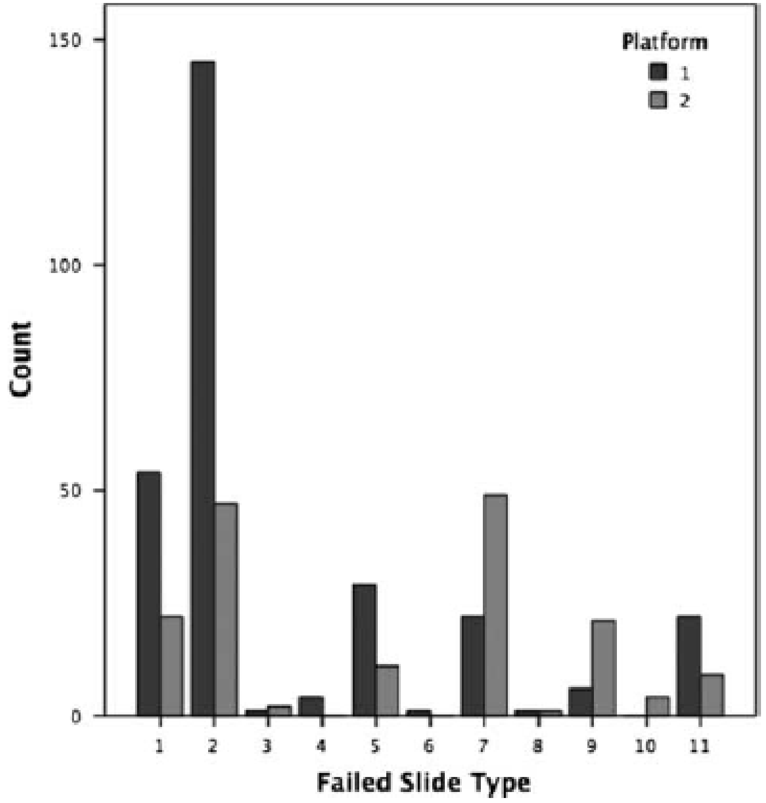


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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.

Application of TMA for QC of diagnostic IHC

A: On-slide controls

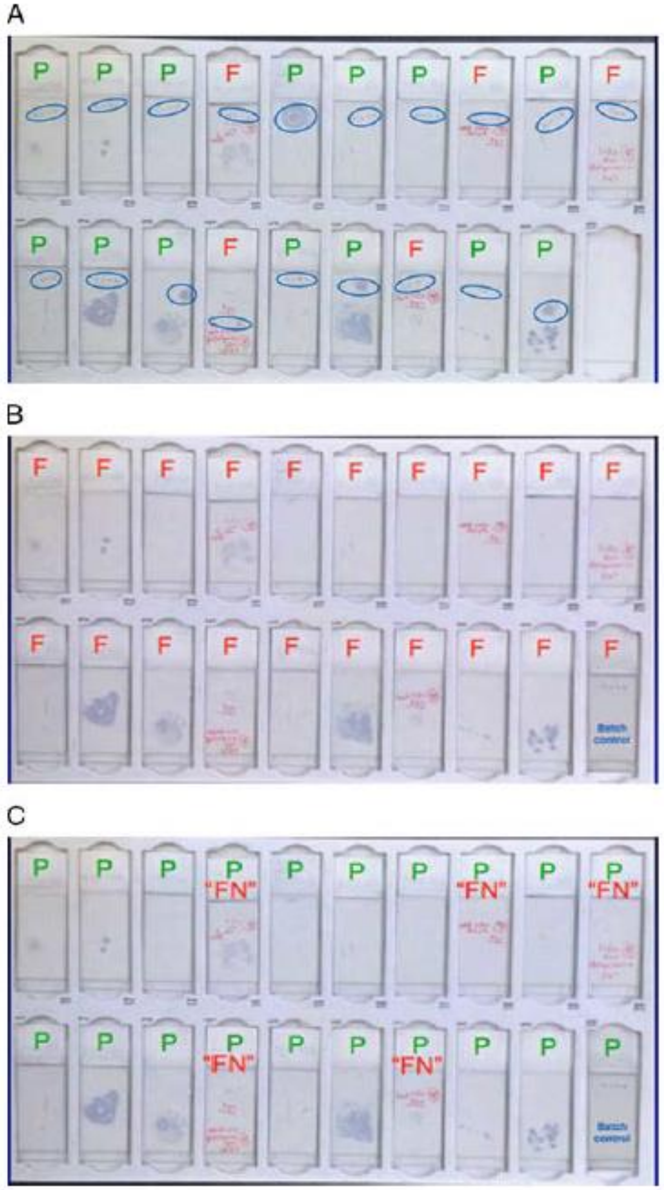
IHC slides stained for ALK (Class II),
same run, same instrument, same protocol
14/19 passed
5/19 failed   (5 x 150 USD)

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

Controls are essential to evaluate IHC results:

- Tissue controls used to calibrate IHC assay
- Tissue controls processed by variables applied in the laboratory is needed to evaluate on robustness
- Tissue controls to evaluate analytical potential and value
- Tissue controls to monitor consistency of IHC assay
- <u>Use of critical tissue controls / ICAPCs with relevant range of target analyte is crucial</u>

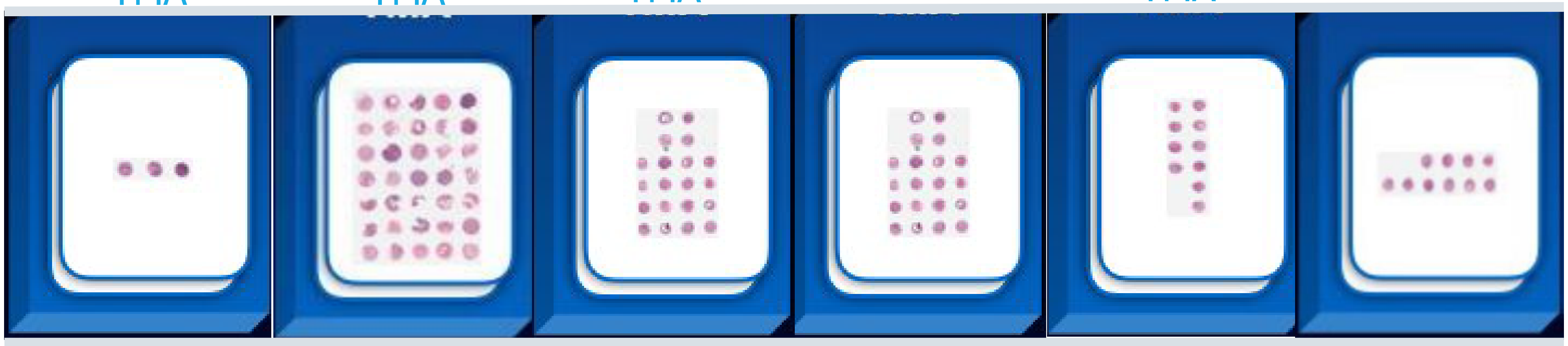
Conclusions

Focus on external tissue controls is central to standardize and optimize IHC:

- On-slide TMA controls are preferable to 1 bacth control
- Internal tissue controls are of limited value
- Need to generate consensus guidelines on ICAPCs for all IHC tests – which tissues, which staining pattern. Interaction of industry, EQA and pathology organisations and societies required.
- Need to identify best practice controls – tissues, beads, cell lines.. – for type 2 IHC

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
			With expression	High expression Low expression	Method of transfer proof
High expression Low expression No expression	With expression No expression		No expression 20/40 of each Type I/II IHC	No expression + relevant cut-off	

*Immunohistochemical critical assay performance controls

Questions and/or comments

Thank You for the attention and.....