



**Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 5-7th 2022**

The Tissue Tool Box

-

IHC Critical Assay Performance Controls

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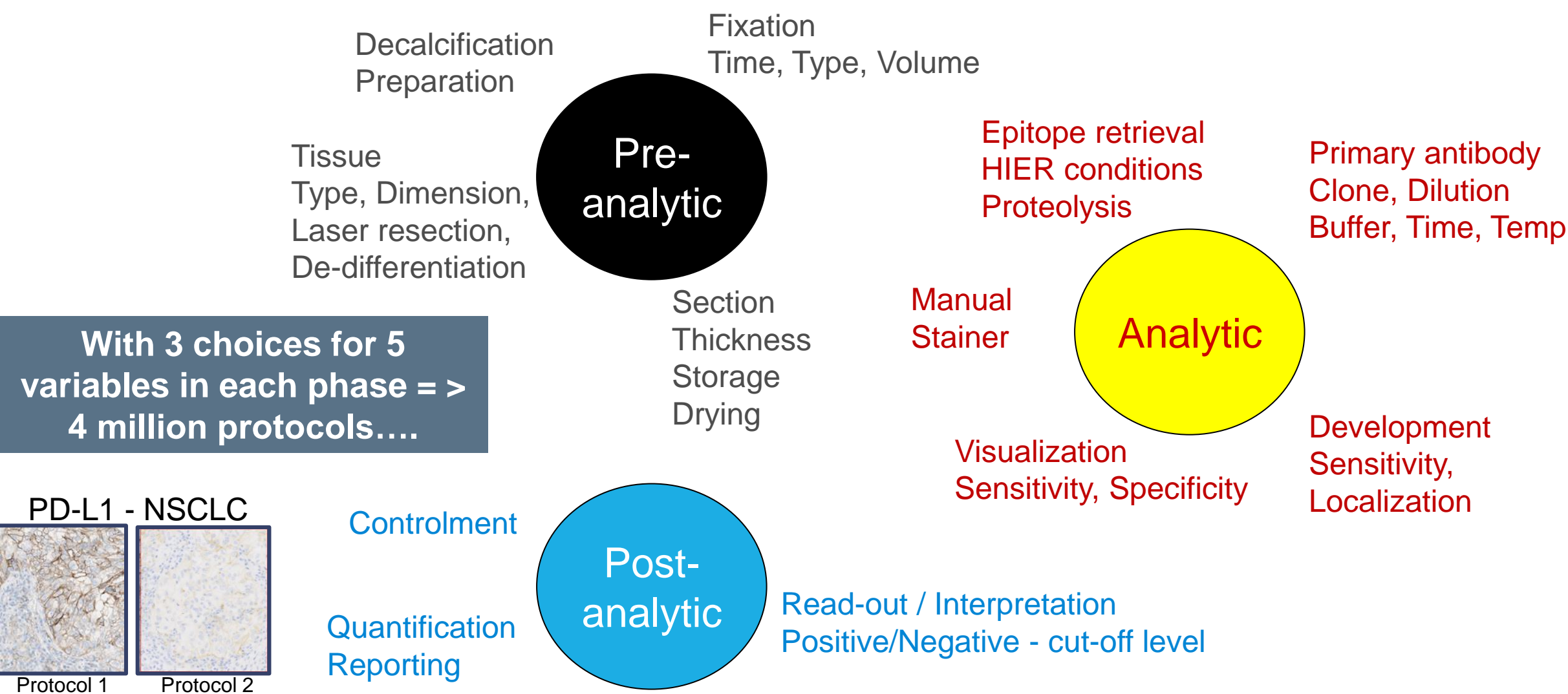


Agenda and focus areas

- 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 !!!!!



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

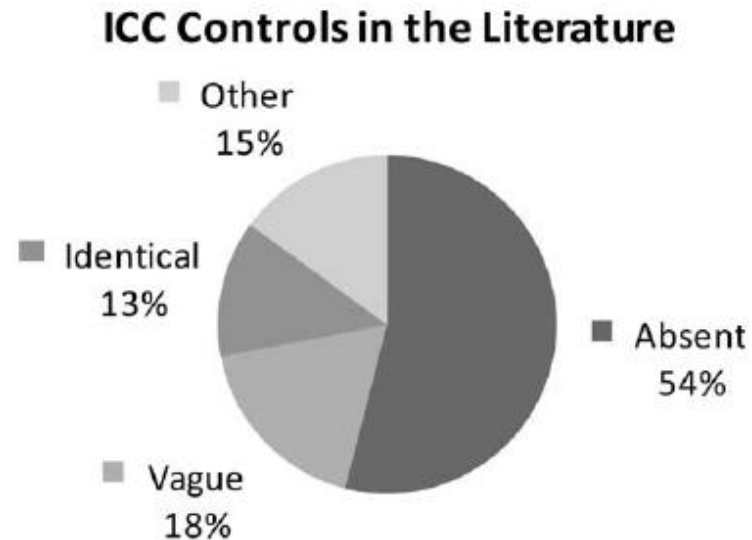


Fig. 1. Description of immunocytochemistry controls in articles reviewed.

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....

IHC controls to guide reliability of data...

PAX8 expression in breast cancer – true or 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...

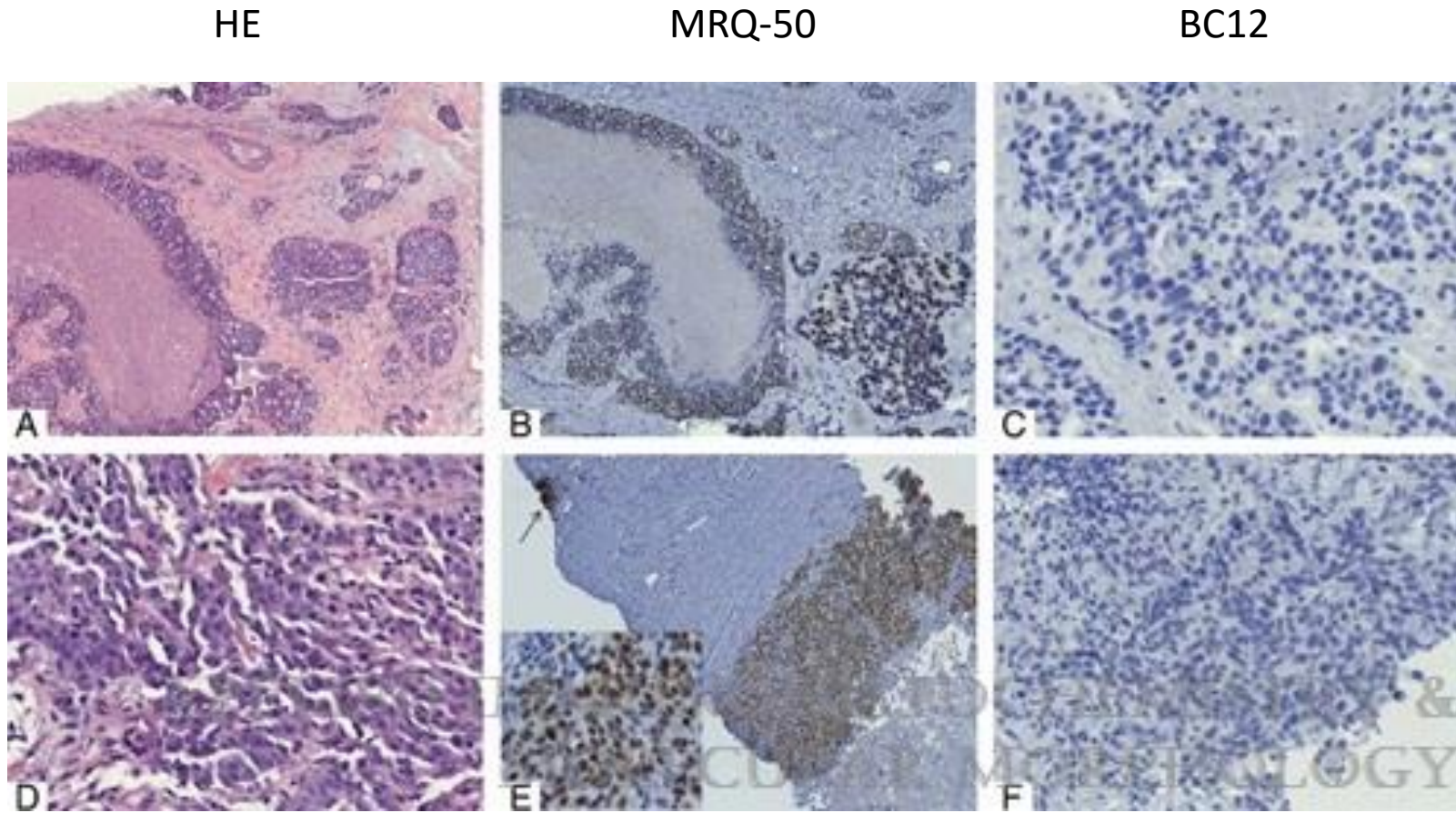


FIGURE 1

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

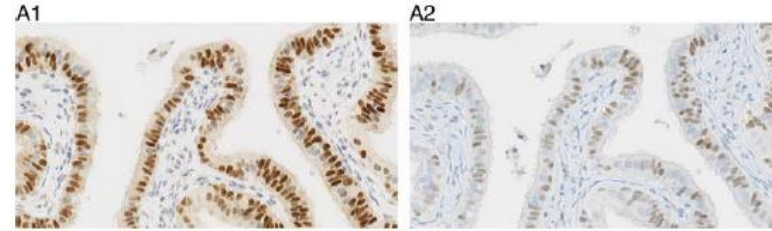
Photomicrographs from 2 breast carcinomas with aberrant PAX8 expression by MRQ-50 clone. On staining with hematoxylin and eosin (A, D) both tumors were high grade with necrosis. Immunohistochemistry for PAX8 with MRQ-50 antibody (B, E) showed nuclear positivity in tumor cells and lymphocytes (arrow).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

IHC controls to guide reliability of data...

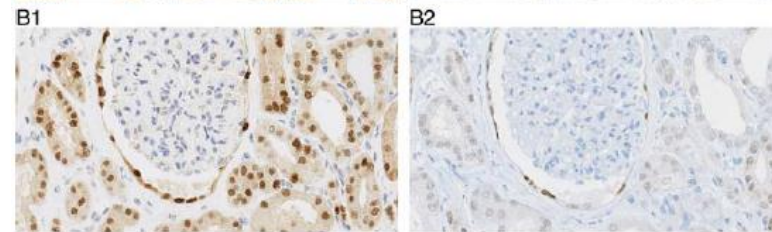
NordiQC Assessments of PAX8 Immunoassays

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

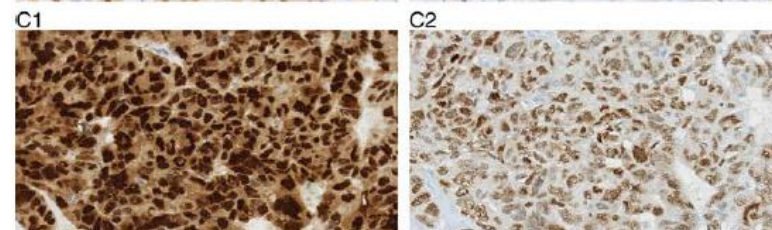
Positive tissue control 1
Fallopian tuba



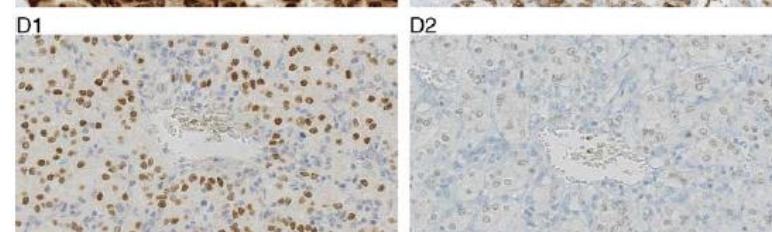
Positive tissue control 2
Kidney



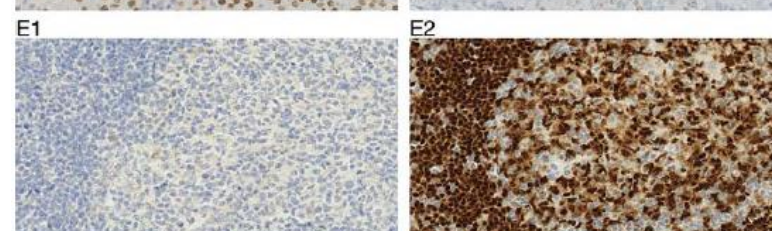
Tumour type 1
Ovarian carc.



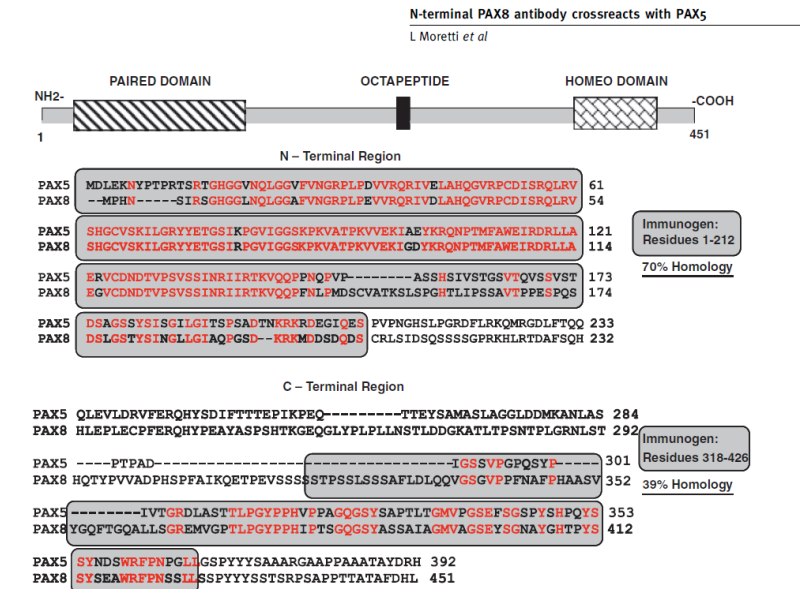
Tumour type 2
Renal cell carc.



Negative tissue control 1
Tonsil



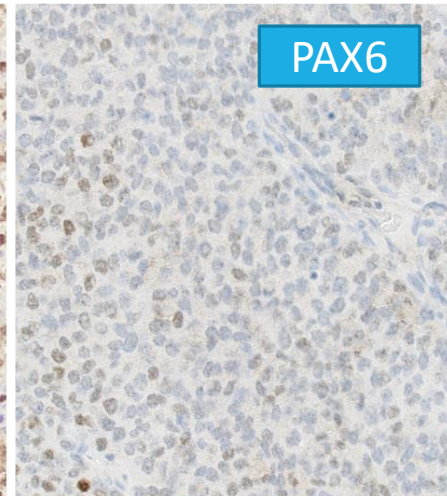
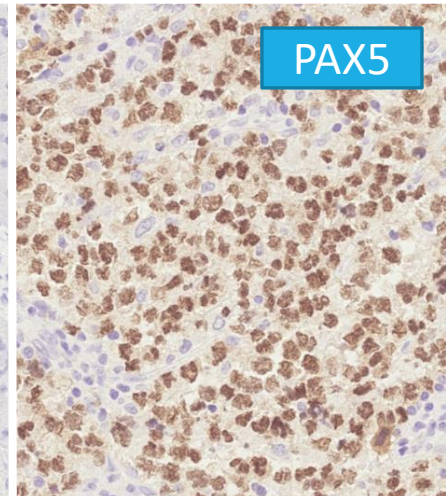
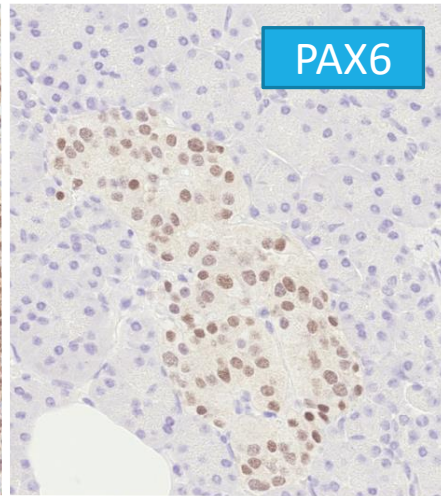
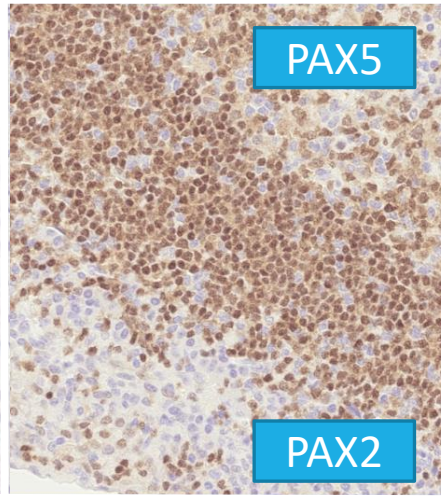
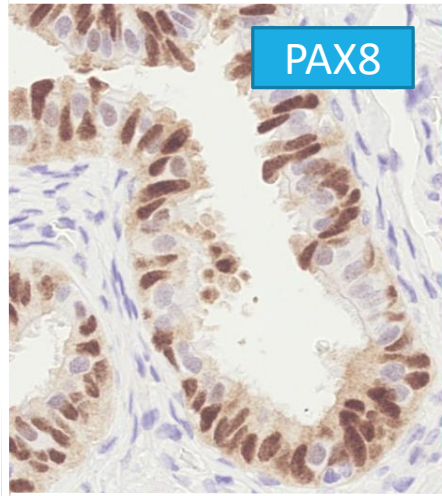
Level of analytical sensitivity



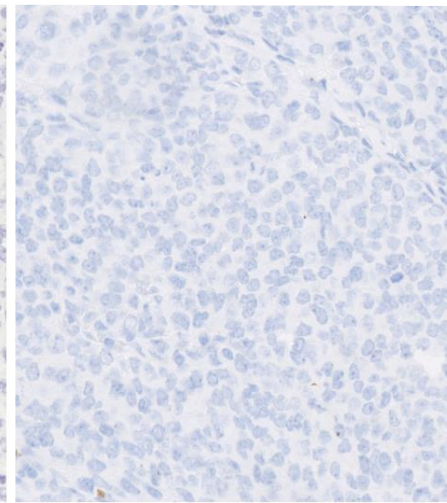
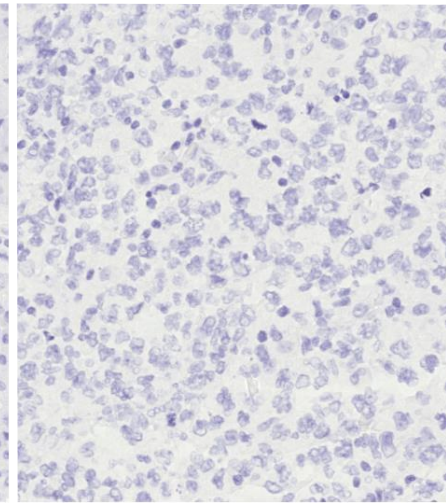
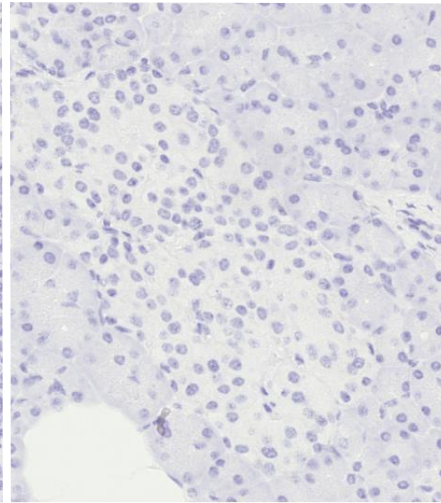
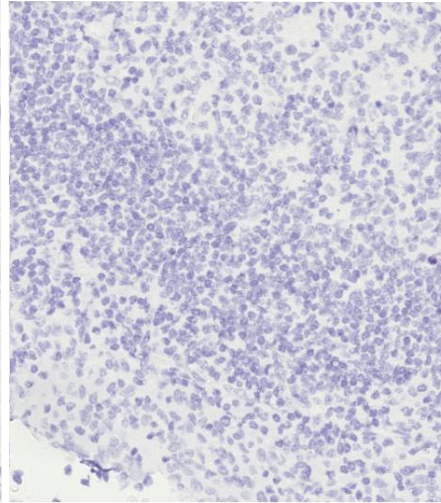
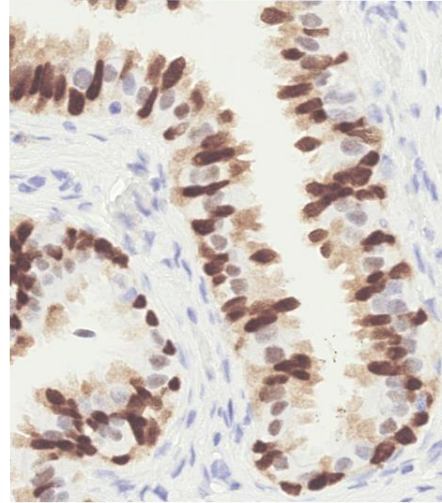
Level of analytical specificity

IHC controls to guide reliability of data...

MRQ-50
(& pAb)



SP348
(& BC12)



Fal. Tube

Tonsil

Pancreas

DLBCL

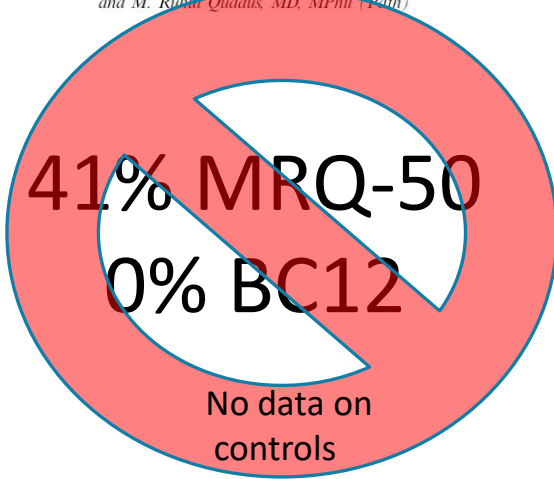
Breast carc.

IHC controls to guide reliability of data...

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

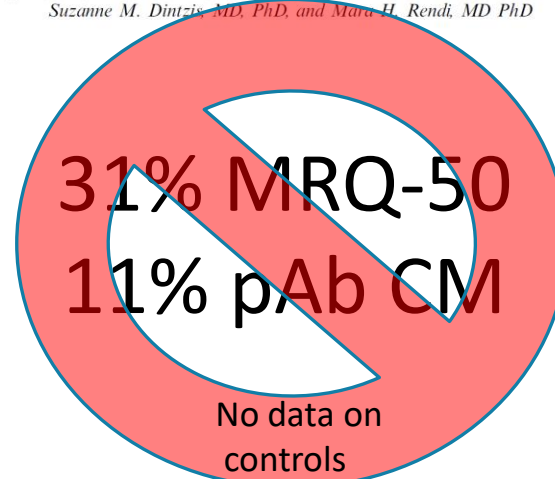
Comparison of PAX8 Expression in Breast Carcinoma Using MRQ50 and BC12 Monoclonal Antibodies

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



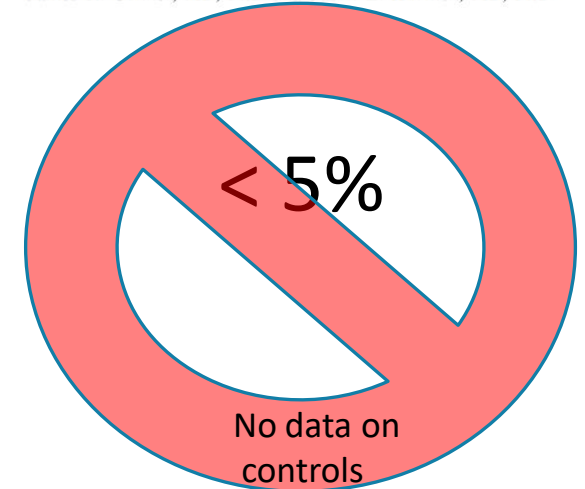
Unexpected PAX8 Immunoreactivity in Metastatic High-grade Breast Cancer

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



KEEP
CALM
AND
PAX

VOBISCUM



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, Oligodontia
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

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,‡‡ Soren 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,† 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‡§*

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 Immunohistochemistry and Molecular Morphology (ISIMM)

International Quality Network for Pathology (IQN-PATH)

Published AIMM 2017 (Jan-April)

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

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and Emina E. Torlakovic, MD, PhD,*†††††
From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
and International Quality Network for Pathology (IQN Path)

Abstract: Technical progress in immunohistochemistry (IHC) as well as the increased utility of IHC for biomarker testing in precision medicine avails us of the opportunity to reassess clinical IHC as a laboratory test and its proper characterization as a special type of immunoassay. IHC, as used in current clinical applications, is a descriptive, qualitative, cell-based, usually nonlinear, in situ protein immunoassay, for which the readout of the results is principally performed by pathologists rather than by the instruments on which the immunoassay is performed. This modus operandi is in contrast to other assays where the instrument also performs the readout of the test result (eg, nephelometry readers, mass spectrometry readers, etc.). The readouts (results) of IHC tests are used either by pathologists for diagnostic purposes or by treating physicians (eg, oncologists) for patient management decisions, the need for further testing or follow-up. This paper highlights the distinction between the

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

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

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

In the era of precision medicine, biomarker testing using immunohistochemistry (IHC) has not only become more precise but also more complex. Precision medicine requires precision results, which can only come about from precision testing. Because of increasing reliance on

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine – Part 2: Immunohistochemistry Test Performance Characteristics

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Antonio Marchetti, MD, PhD,††† Keith Miller, FIBMS,*** J. Han van Krieken, MD, PhD,†††
Søren Nielsen, BMS,§§§||| Paul E. Swanson, MD,§§§ Mogens Vyberg, MD,§§§|||
Xiaoge Zhou, MD,§§§**** Clive R. Taylor, MD,††††† and
From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
and International Quality Network for Pathology (IQN Path)

Abstract: All laboratory tests have test performance characteristics (TPCs), whether or not they are explicitly known to the laboratorian or the pathologist. TPCs are thus also an integral characteristic of immunohistochemistry (IHC) tests and other in situ, cell-based molecular assays such as DNA or RNA in situ hybridization or aptamer-based testing. Because of their descriptive, in situ, cell-based nature, IHC tests have a limited repertoire of appropriate TPCs. Although only a few TPCs are relevant to IHC, proper selection of informative TPCs is nonetheless essential for the development of and adherence to appropriate quality assurance measures in the IHC laboratory. This paper describes the TPCs that are relevant to IHC testing and emphasizes the role of TPCs in the validation of IHC tests.

This is part 2 of the 4-part series "Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine."

Key Words: biomarkers, quality assurance, quality control, validation, immunohistochemistry, test performance characteristics

(Appl Immunohistochem Mol Morphol 2017;25:79-85)

Historically, immunohistochemistry (IHC) has for all practical purposes been considered a "special stain" similar to traditional histochemical preparations; how-

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine. Part 3: Technical Validation of Immunohistochemistry (IHC) Assays in Clinical IHC Laboratories

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Paul E. Swanson, MD,§§§ Mogens Vyberg, MD,§§§||| Xiaoge Zhou, MD,§§§****
and Clive R. Taylor, MD,†††††
From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
and International Quality Network for Pathology (IQN Path)

Abstract: Validation of immunohistochemistry (IHC) assays is a subject that is of great importance to clinical practice as well as basic research and clinical trials. When applied to clinical practice and focused on patient safety, validation of IHC assays creates objective evidence that IHC assays used for patient care are "fit-for-purpose." Validation of IHC assays needs to be properly informed by and modeled to assess the purpose of the IHC assay, which will further determine what sphere of validation is required, as well as the scope, type, and tier of technical validation. These concepts will be defined in this review, part 3 of the 4-part series "Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine."

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

(Appl Immunohistochem Mol Morphol 2017;25:151-159)

In the last decade, the development of precision medicine and the high throughput discovery methods that support it have led to increasing use of selective biomarkers for diagnosis, prognosis, and prediction of response to targeted therapies.¹⁻⁷ This has also led to increasingly stringent criteria for establishing and monitoring of test performance characteristics in biomarker testing, and has improved processes for validating methods that are used to detect and measure these biomarkers.⁸ The American Association for Cancer Research (AACR), Food and Drug Administration (FDA), and National Cancer Institute (NCI) formed the AACR-FDA-NCI Cancer Biomarkers Collaborative to accelerate the translation of novel cancer therapeutics into the clinic.⁹ The AACR-FDA-NCI consensus recommendations were designed to advance the use of biomarkers in cancer drug development, the harmonization of biomarker validation

Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4: Tissue Tools for Quality Assurance in Immunohistochemistry

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Manfred Dietel, MD, PhD,* Glenn D. Francis, MBBS, FRCPA, MBA, FFSc (RCPA),§§§***††
Regan Fulton, MD, PhD,†† C. Blake Gilks, MD,§§§ Jacqueline A. Hall, PhD,|||
Jason L. Hornick, MD, PhD,§§§ Merdol Ibrahim, PhD,*** Antonio Marchetti, MD, PhD,†††††
Keith Miller, FIBMS,*** J. Han van Krieken, MD, PhD,§§§ Søren Nielsen, BMS,|||§§§
Paul E. Swanson, MD,§§§||| Clive R. Taylor, MD,§§§ Mogens Vyberg, MD,|||§§§
Xiaoge Zhou, MD,††††† Emina E. Torlakovic, MD, PhD,§§§||| and
From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
and International Quality Network for Pathology (IQN Path)

Abstract: The numbers of diagnostic, prognostic, and predictive immunohistochemistry (IHC) tests are increasing; the implementation and validation of new IHC tests, revalidation of existing tests, as well as the on-going need for daily quality assurance monitoring present significant challenges to clinical laboratories. There is a need for proper quality tools, specifically tissue tools that will enable laboratories to successfully carry out these processes. This paper clarifies, through the lens of laboratory tissue tools, how validation, verification, and revalidation of IHC tests can be performed in order to develop and maintain high-quality "fit-for-purpose" IHC testing in the era of precision medicine. This is the final part of the 4-part series "Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine."

Key Words: immunohistochemistry, quality tools, tissue tools, test development, quality assurance, biomarker, validation
(Appl Immunohistochem Mol Morphol 2016;00:000-000)

Before the decision to implement a new immunohistochemistry (IHC) test is made, several considerations relevant to test development and maintenance need to be contemplated (see parts 1 to 3 of the Evolution series). To introduce a new IHC test, a series of steps must be followed that require careful planning, from test development through to on-going quality monitoring. For this process to be successful, proper tissue tools, which are a cornerstone of quality for the modern day clinical

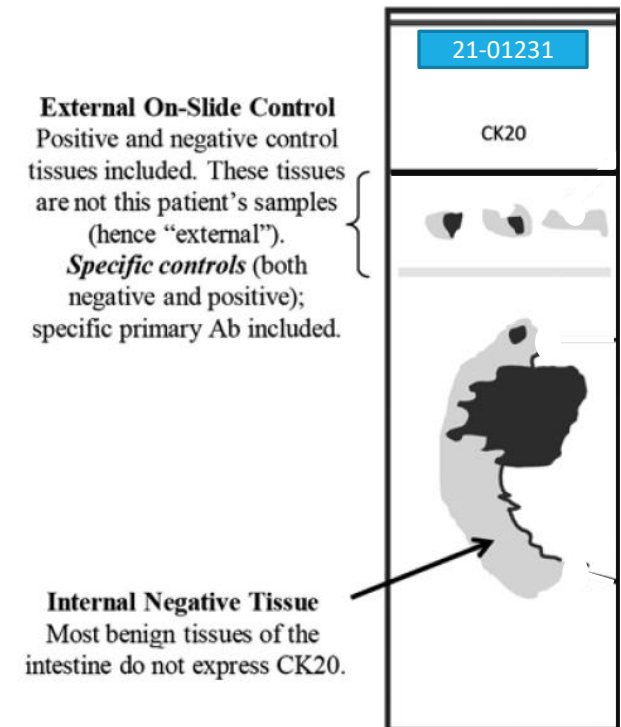
Main elements to develop & validate IHC assays

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

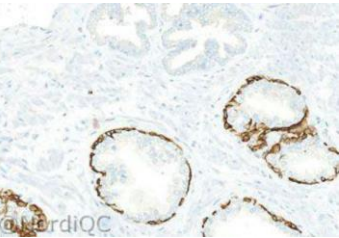
- Reagent and tissue controls are necessary for the validation of immunohistochemical staining results.
- Tissue controls are the most valuable tool to monitor the specificity and sensitivity for IHC
 - Internal positive and negative tissue control
 - Cells/structures within the patient material
 - External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



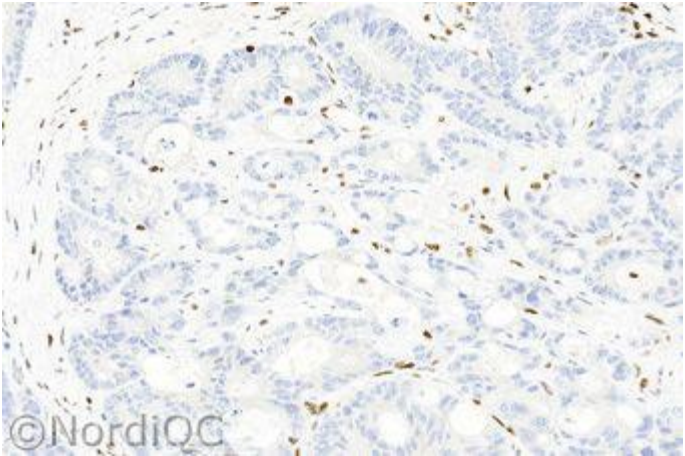
How to use internal tissue controls

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
SMAD4/Dpc4	Ubiquitously expressed tumor suppressor Ag that is inactivated in about 55% of pancreatic adenocarcinomas	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control
PTEN	Ubiquitously expressed; loss of expression is associated with carcinogenesis, cancer progression, and drug resistance	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 patient relevant material / target evaluated



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

Limitations of internal tissue controls

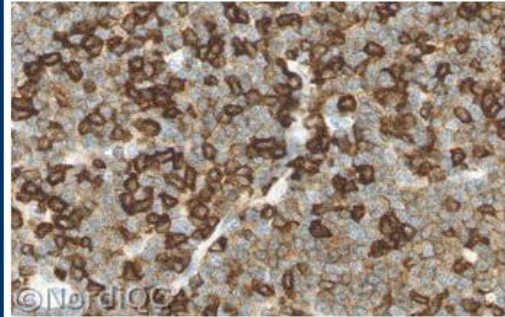


Fig. 4a. Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

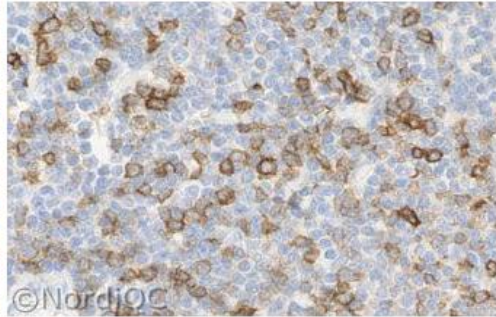


Fig. 4b. Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

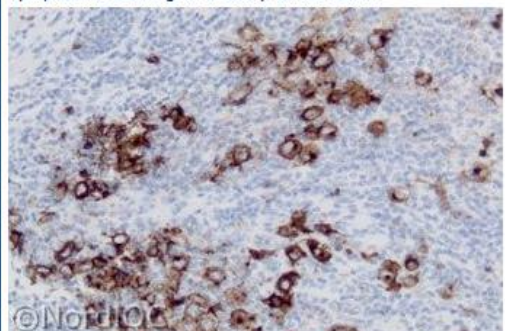


Fig. 2a. Optimal CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

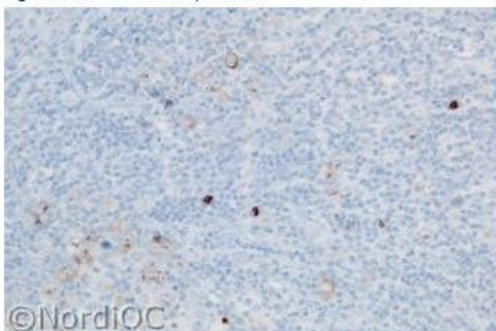


Fig. 2b. CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a.

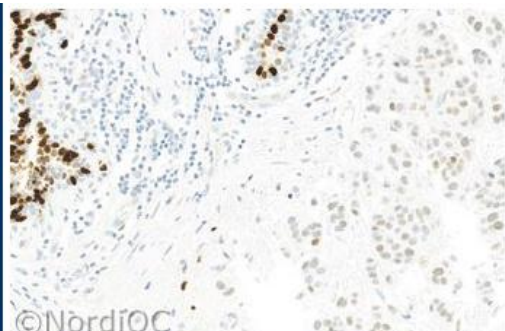


Fig. 3a. Optimal ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

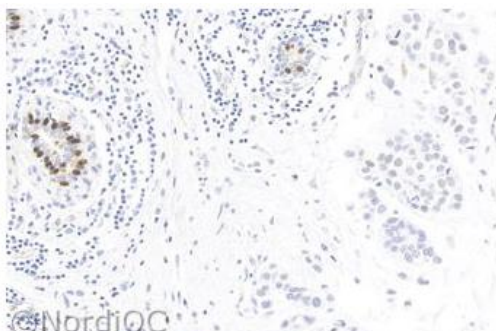
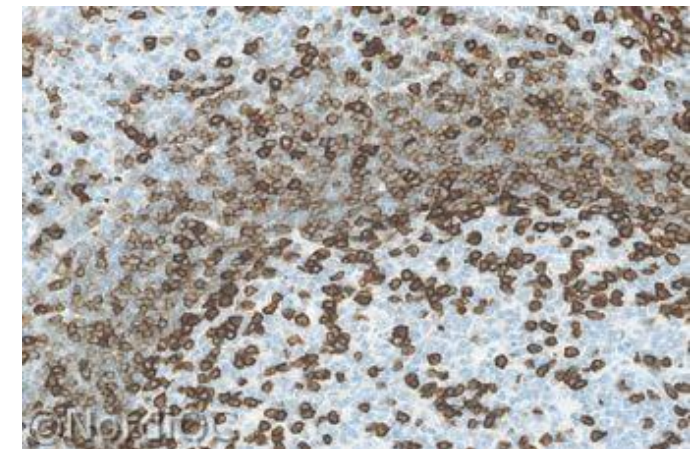


Fig. 3b. Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. Only dispersed neoplastic cells show an equivocal staining.

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



Critical tissue controls = ICAPCs

IHC Critical Assay Performance Controls (ICAPCs)

are basically human positive control tissues with

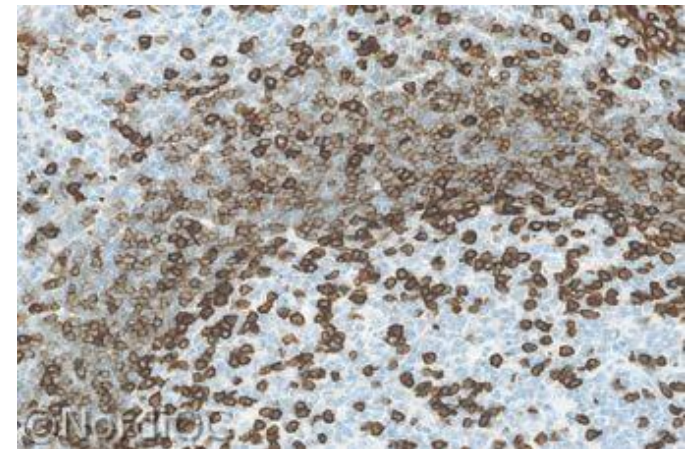
- clinical relevant range of target analyte (antigen) – especially with low limit detection
- well characterized expression pattern – preferable normal tissues
- predictable levels and specified cellular and architectural localization

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,† 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‡§*



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


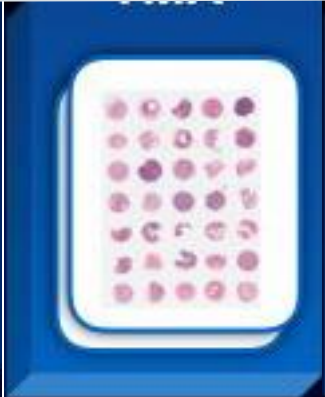




Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification	Range of relevant expression levels 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		20/40 of each Type I/II IHC	+ relevant cut-off	



*Immunohistochemical critical assay performance controls

External tissue control tool box

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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

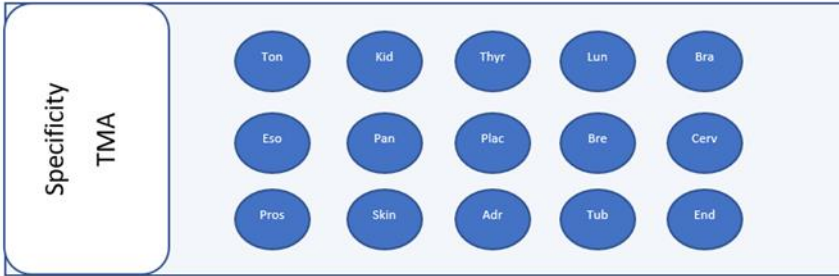
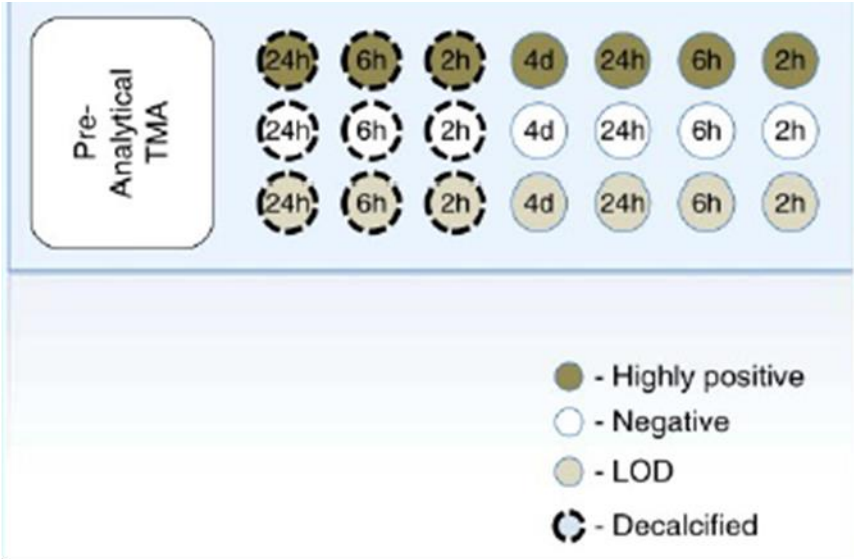
Maps Ab reaction
pattern
Test performance
characteristics

Fixation time
Fixative(s)
Decalcification

High expression
Low expression
No expression

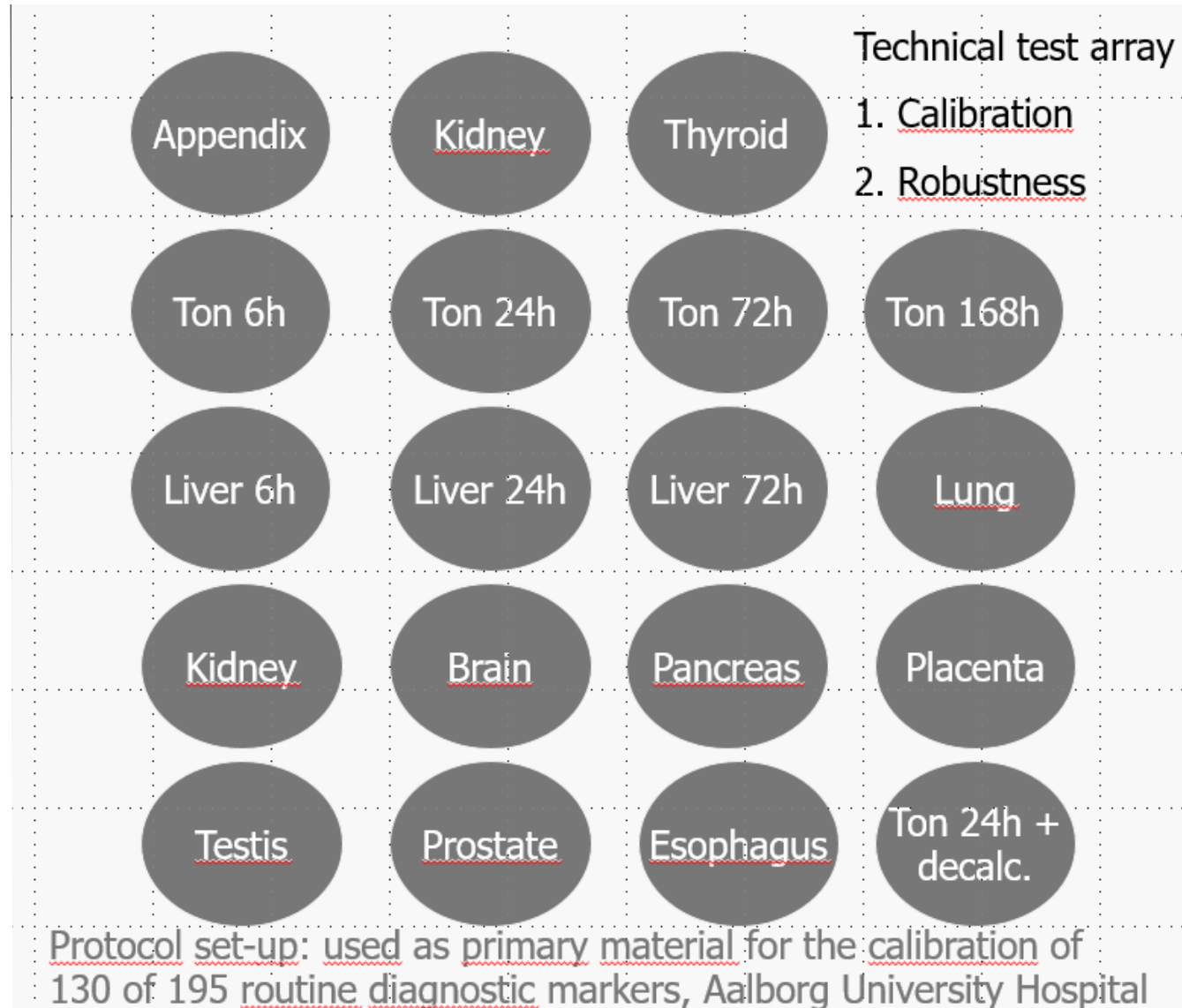
With expression

No expression



*Immunohistochemical critical assay performance controls

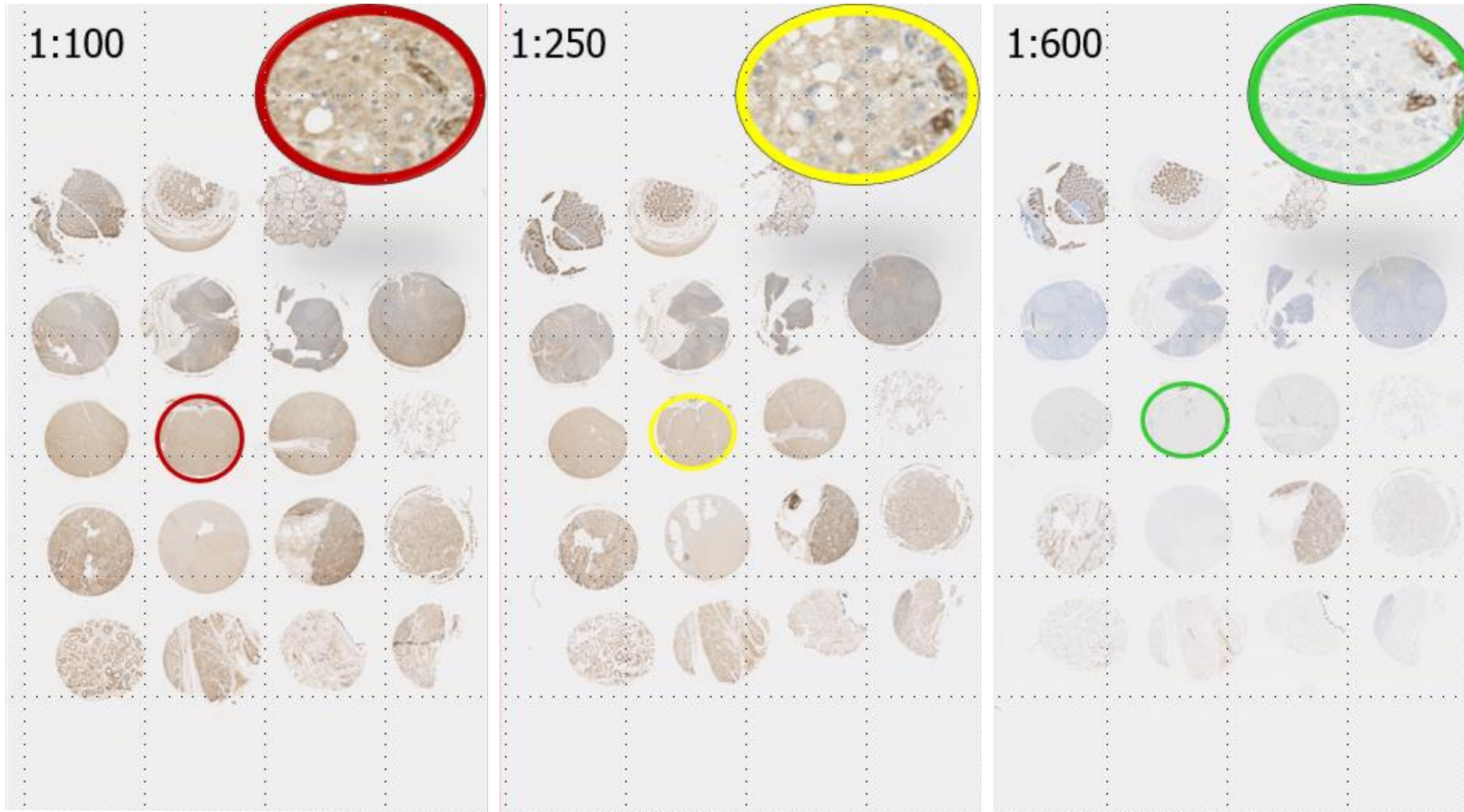
"Poor mans" specificity and pre-analytical TMAs



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

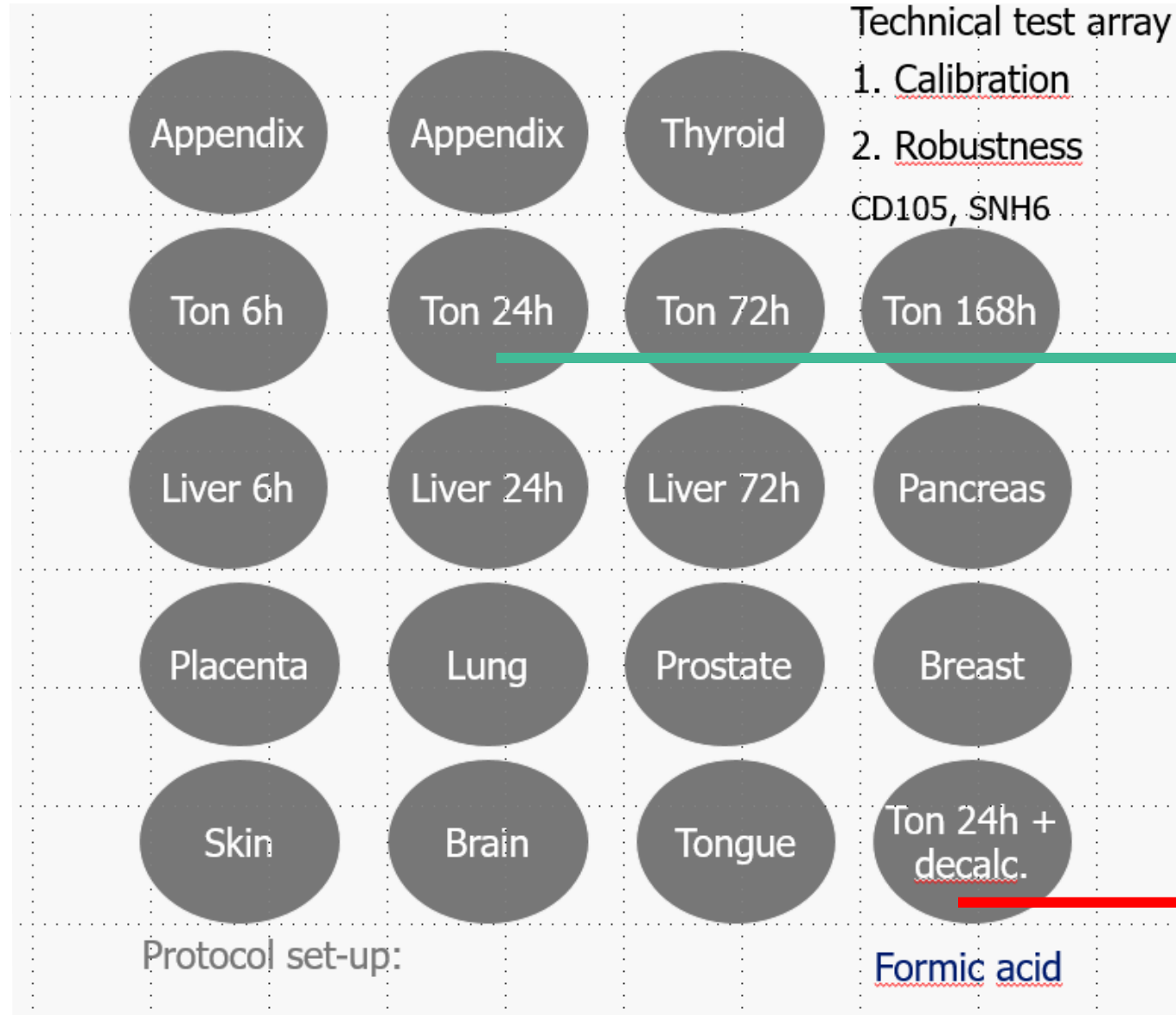


EPCAM calibration

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

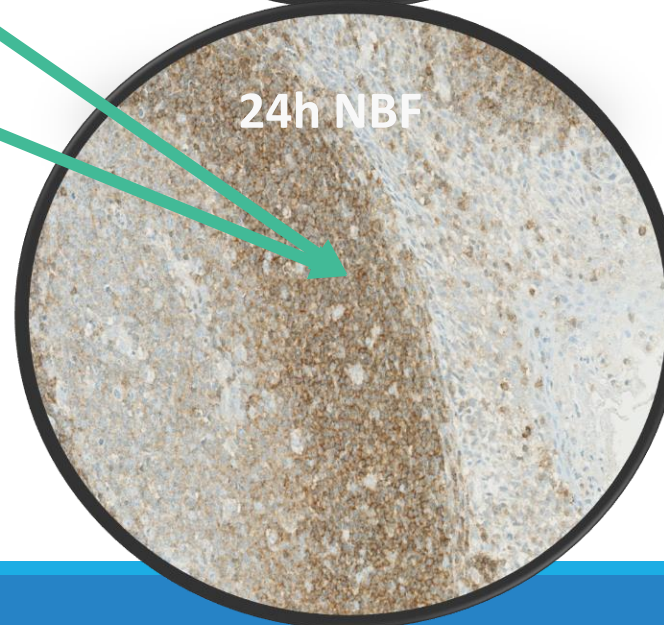
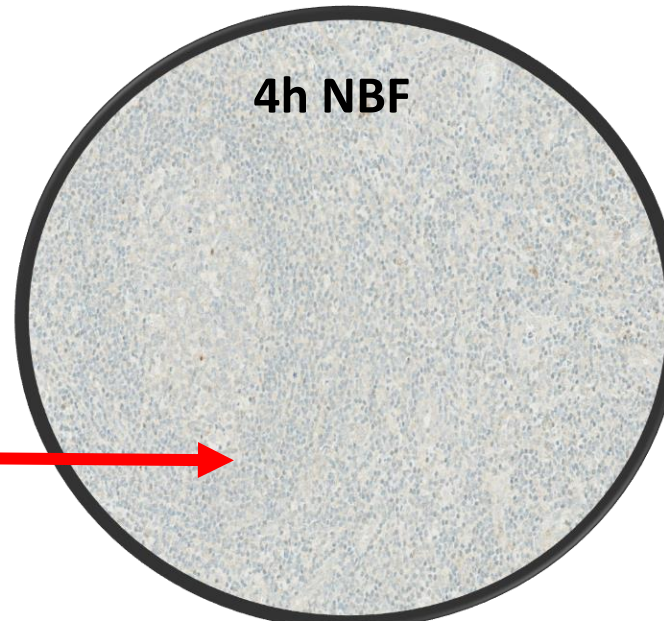
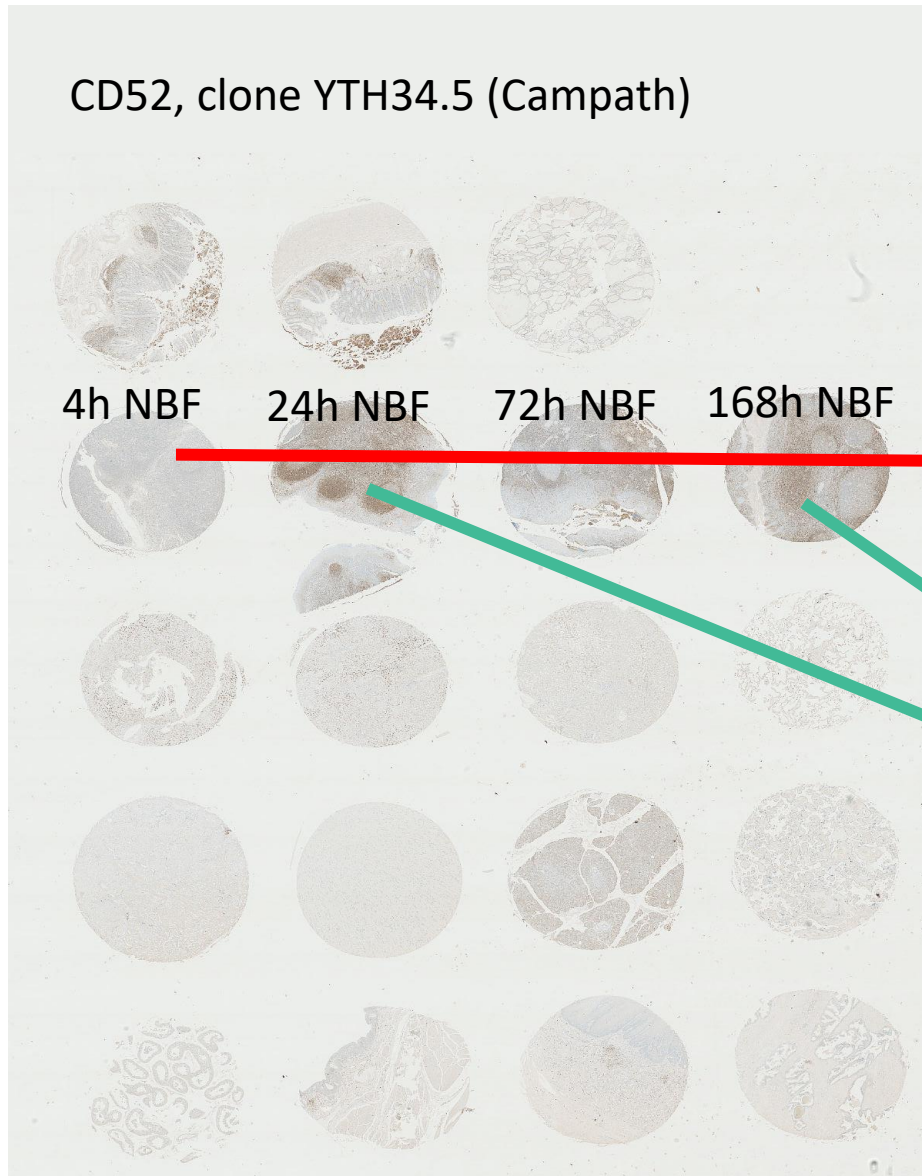


CD105 calibration

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



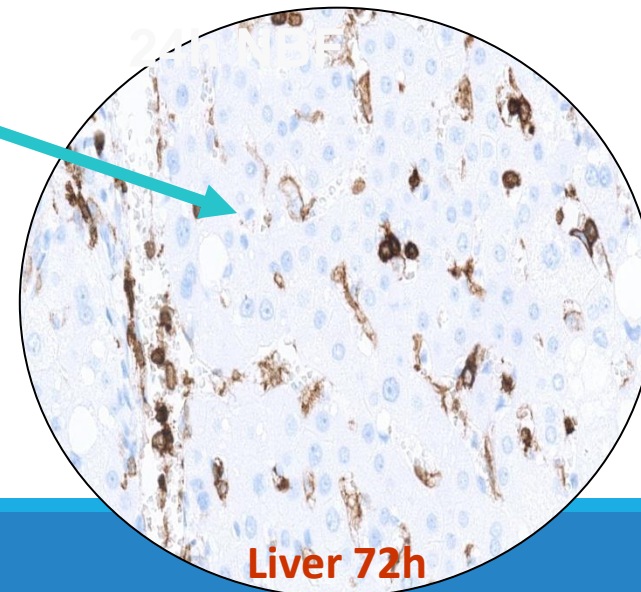
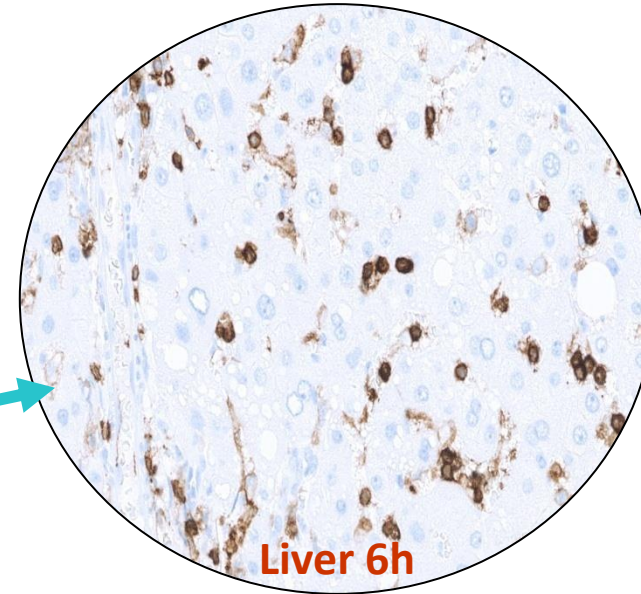
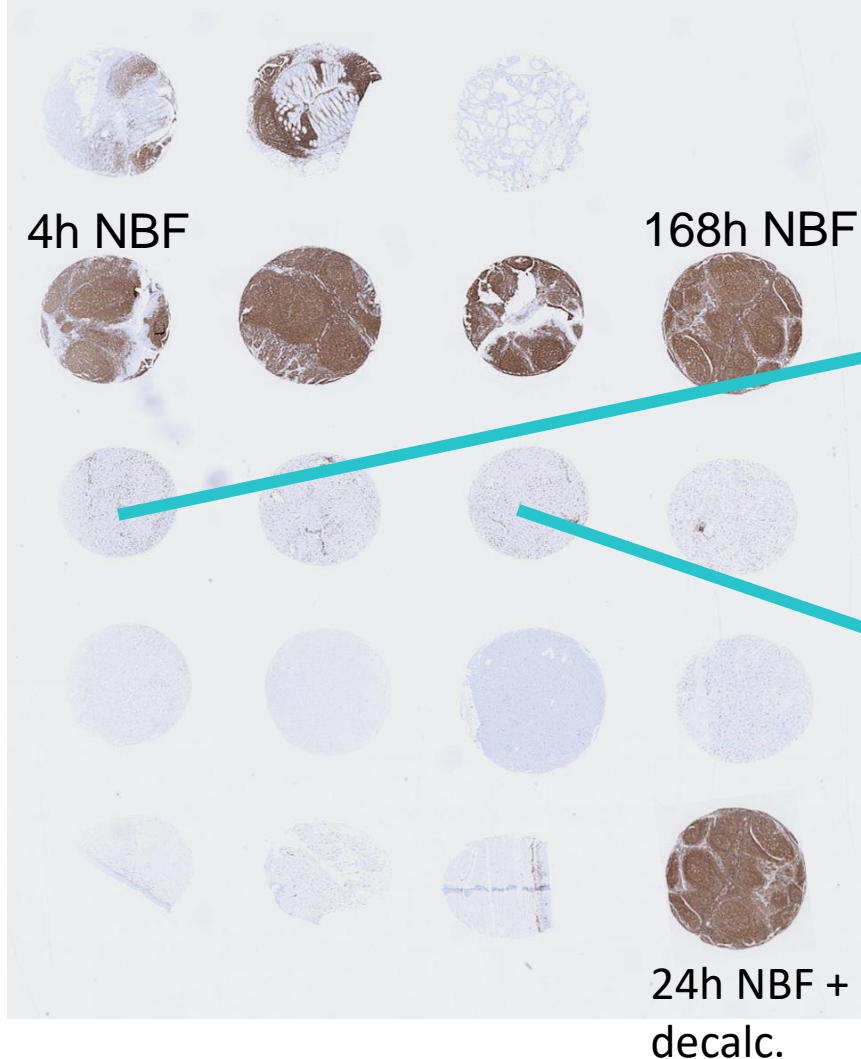
CD52 calibration

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, (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

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

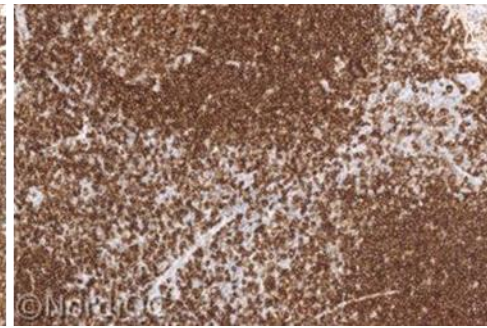
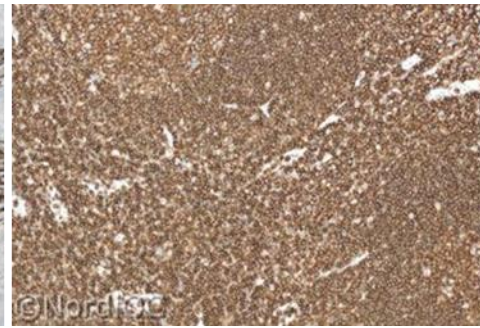
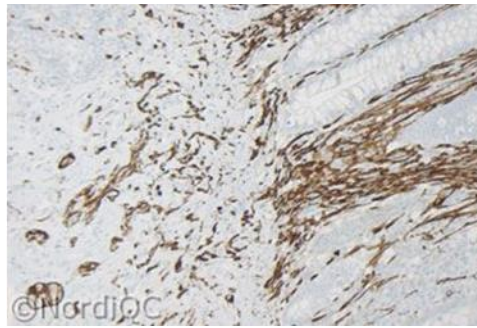
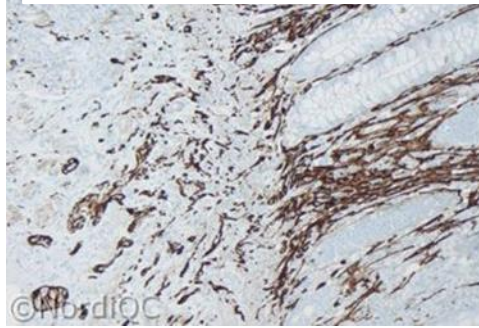
Which tissues / cellular structures show the clinical relevant range of the target analyte with focus on required low level of demonstration – **CRITICAL CONTROLS - ICAPCs?**

Fit For Purpose; the selection....

CD56

CD45

Appendix



Tonsil

Test A

Test B

Test A

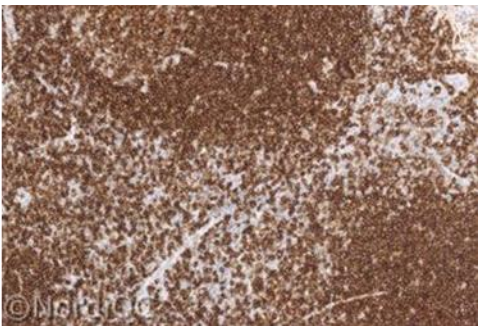
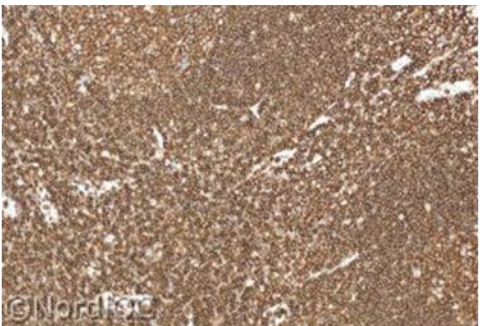
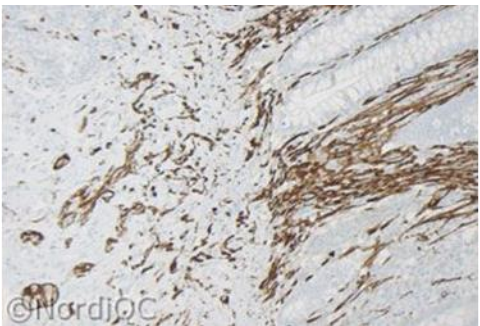
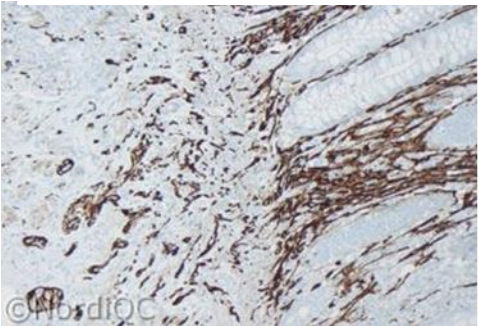
Test B

Fit For Purpose; the selection....

CD56

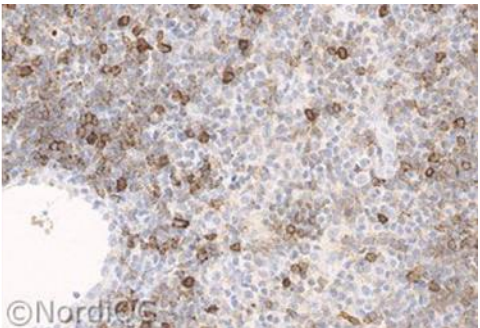
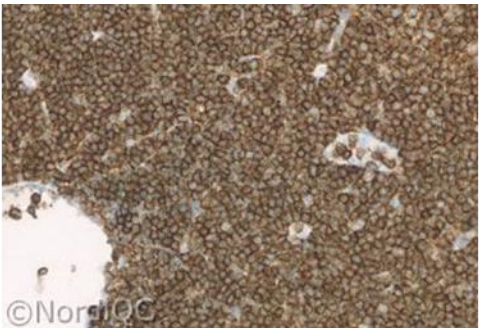
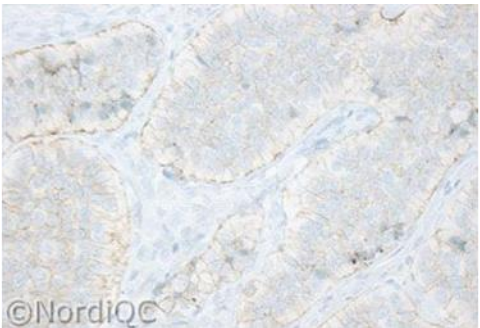
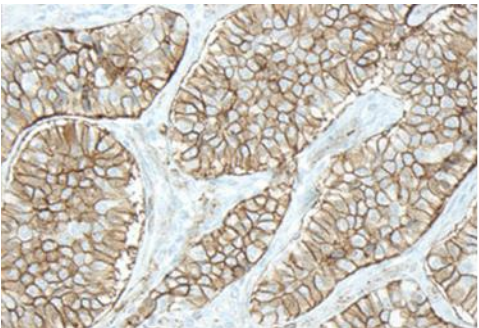
CD45

Appendix



Tonsil

NET



B-CLL

Test A

Test B

Test A

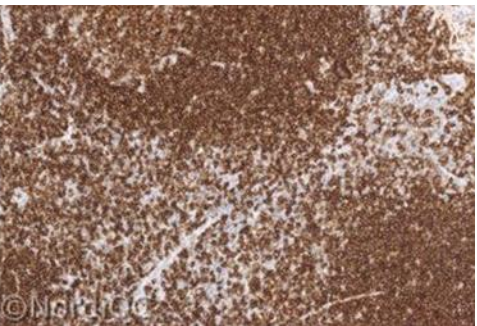
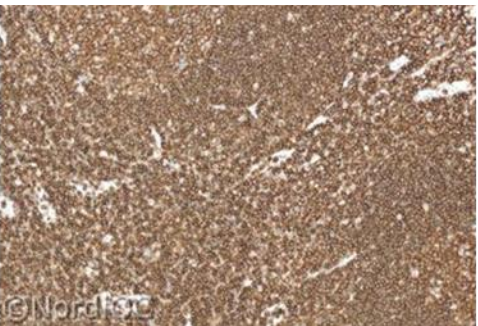
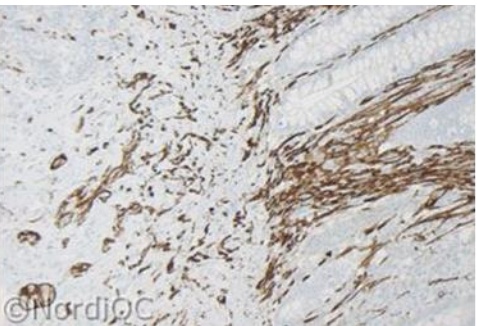
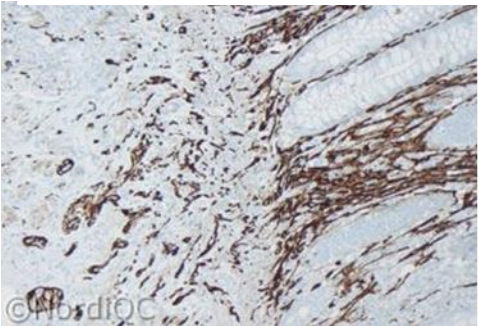
Test B

Fit For Purpose; the selection....

CD56

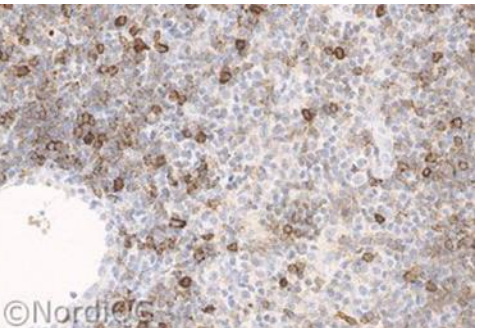
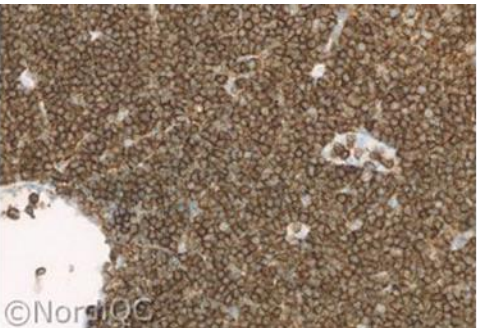
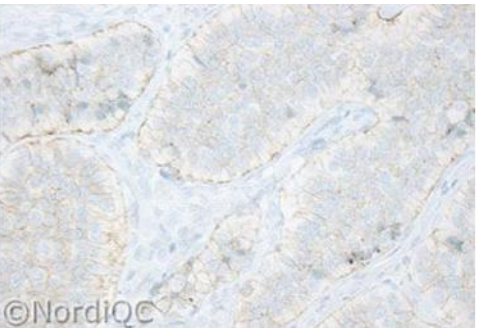
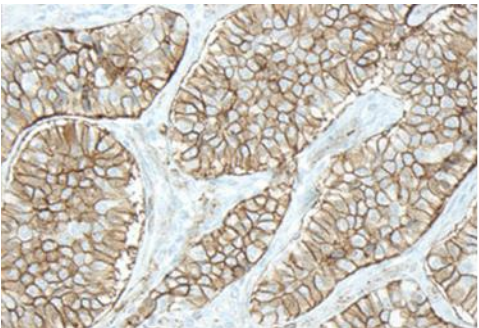
CD45

Appendix



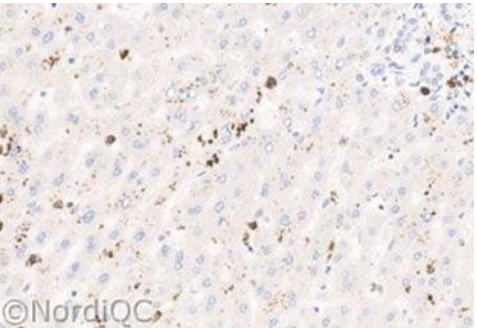
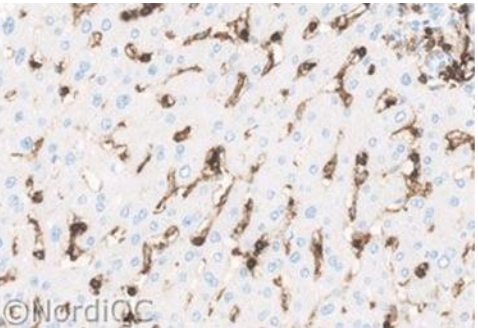
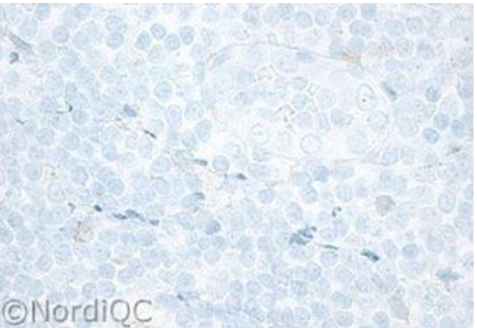
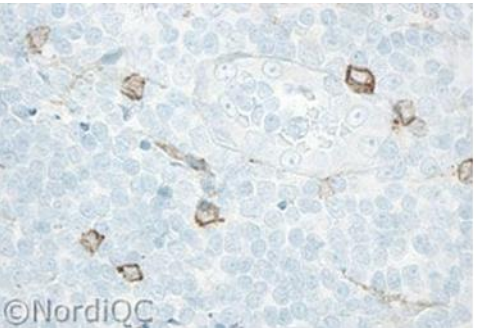
Tonsil

NET



B-CLL

Tonsil



Liver

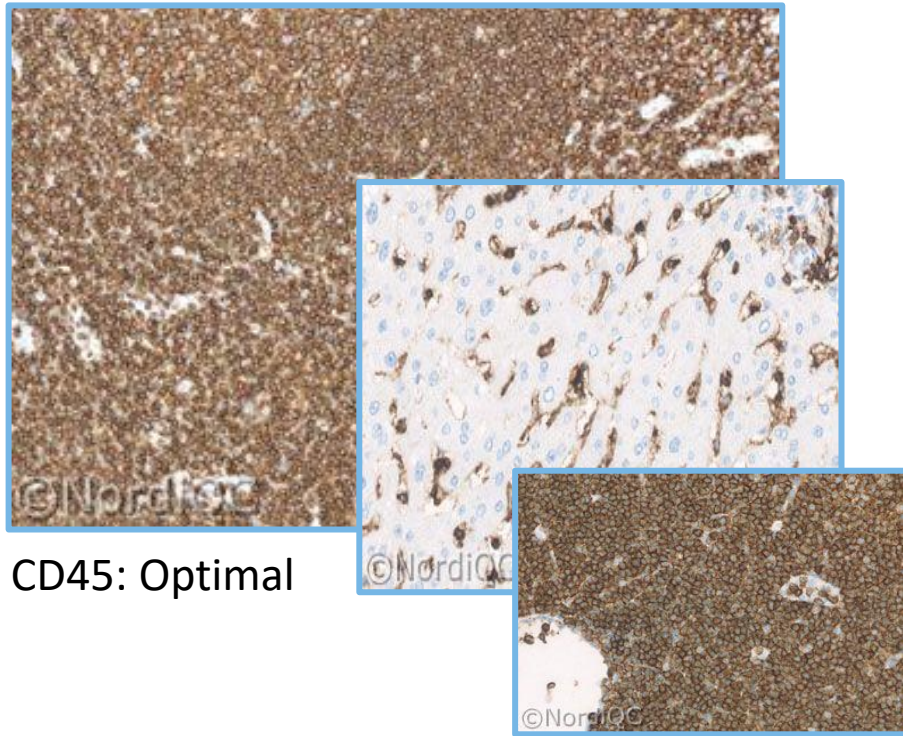
Test A

Test B

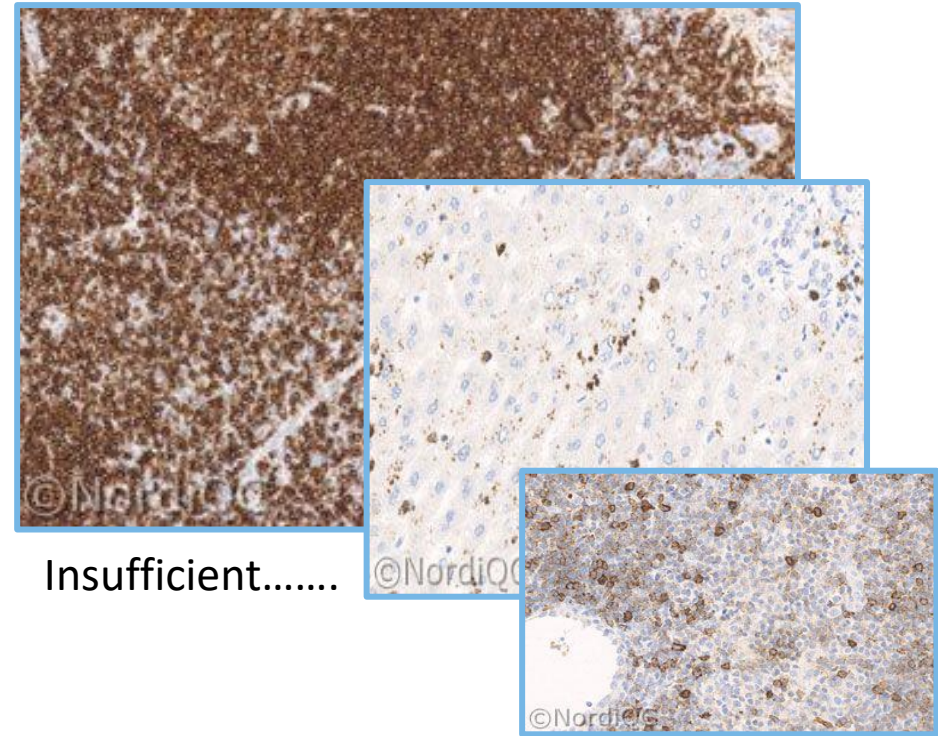
Test A

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 sensitivity in an optimally calibrated IHC assay

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

REVIEW ARTICLE

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

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

asco special articles

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

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

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

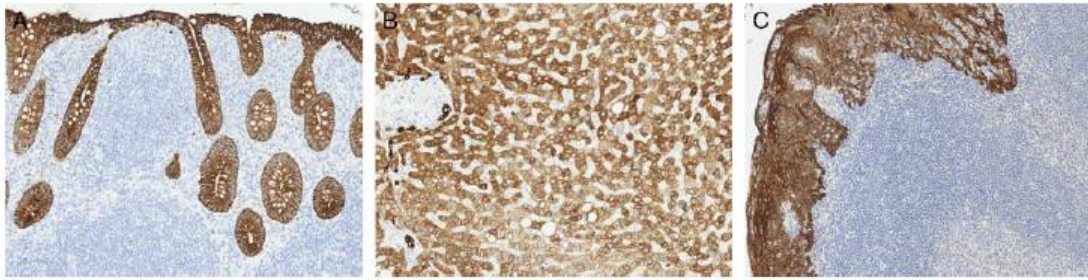


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining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

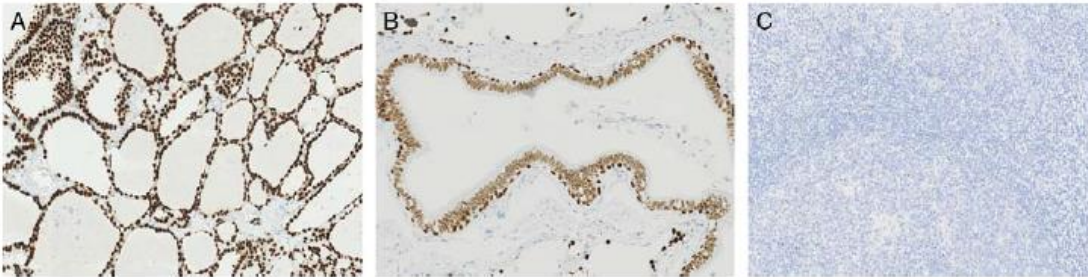


FIGURE 7. TTF-1 iCAPC. A, Thyroid: virtually all epithelial cells must show a strong nuclear staining reaction. B, Lung: virtually all pneumocytes and basal cells of terminal bronchi must show a moderate to strong nuclear staining reaction. Columnar epithelial cells of terminal bronchi must show an at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

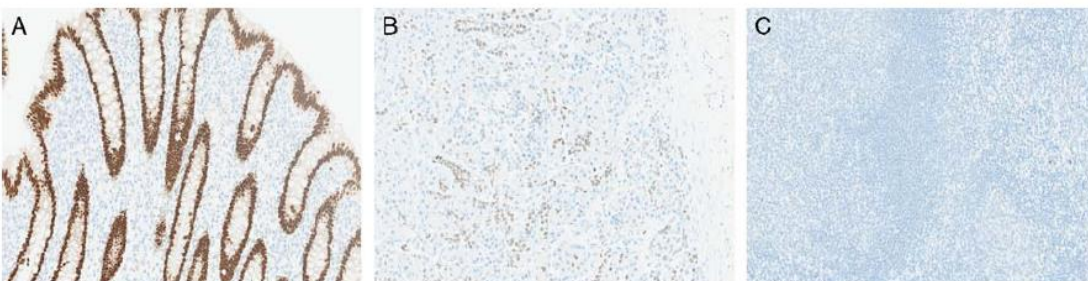


FIGURE 8. CDX-2 iCAPC. A, Appendix: virtually all epithelial cells must show a strong nuclear staining reaction. A weak cytoplasmic staining reaction in addition to strong nuclear staining is often present. B, Pancreas: the majority of epithelial cells of intercalated ducts must show a weak to moderate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

NordiQC IHC tissue control atlas – open from 05.2022



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


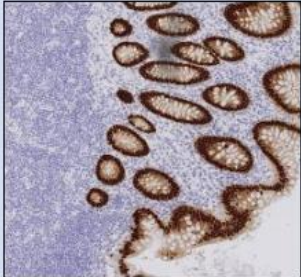
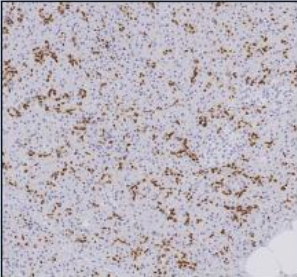

Tissues

Purpose

Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas – open from 05.2022

<div> Info ▾ Modules ▾ Assessments Protocols Controls Events ▾ SN</div>			
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	<p>All epithelial cells must show a strong nuclear staining reaction.</p> <p><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></p>	<p>The vast majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction.</p>	<p>No staining reaction should be seen.</p> <p><i>Note, dispersed lymphocytes can show a faint nuclear staining reaction.</i></p>
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge
Back			

Available for NordiQC participants

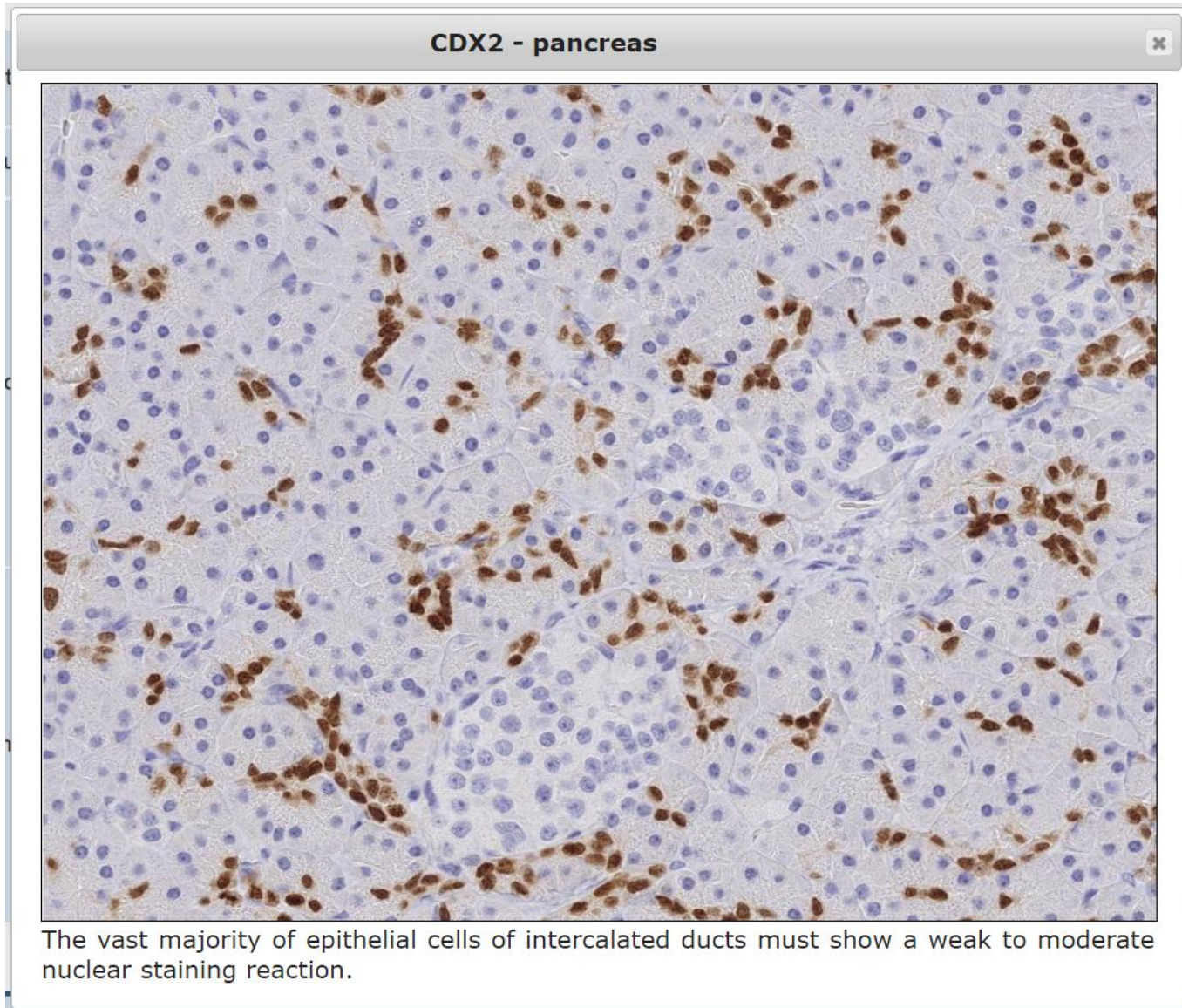
Tissues

Purpose

Reaction patterns

Online scans accessible

NordiQC IHC tissue control atlas – open from 05.2022



Available for NordiQC participants

Tissues

Purpose

Reaction patterns

Online scans accessible

Main elements to develop & validate IHC assays



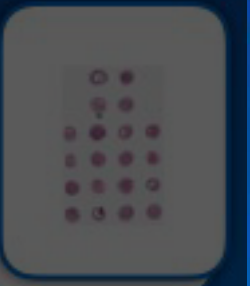

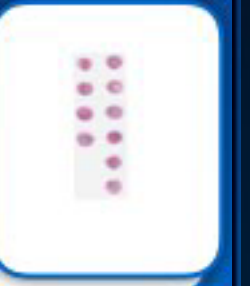

The journey from an antibody to a diagnostic IHC assay with a specific purpose

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
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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

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



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.

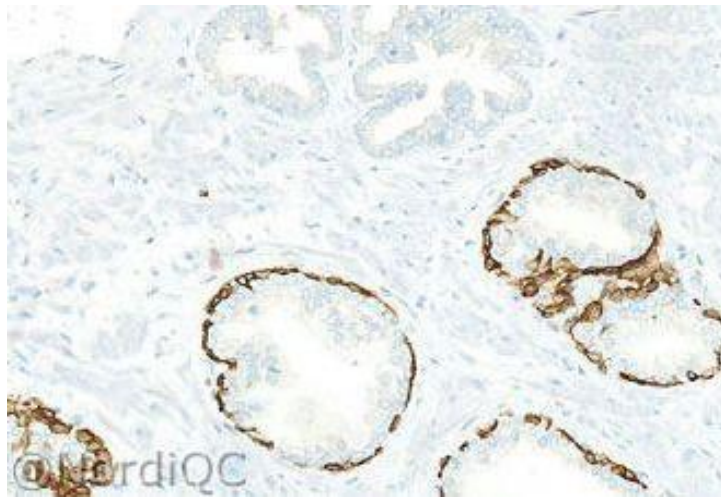
Number perhaps less important compared to use of tissue with full range of expression patterns reflecting the diagnostic use and purpose of test

Identification of purpose of the test

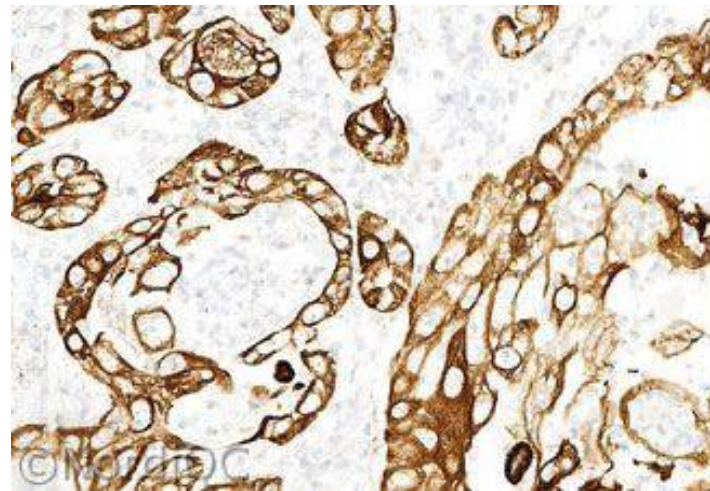
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

IHC for CK5

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



Prostate sample



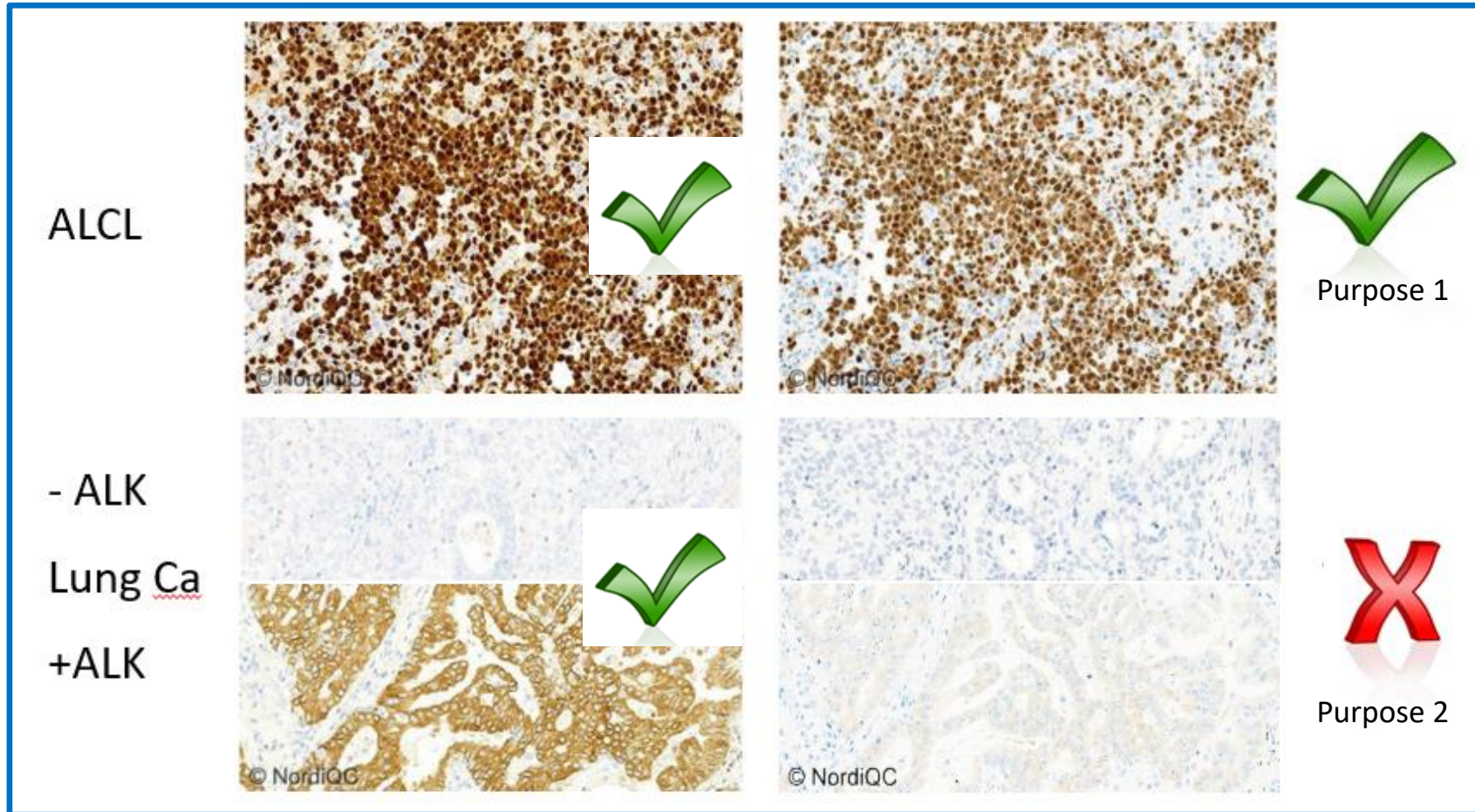
Lung sample

Same protocol applied for 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



IHC method 1

IHC method 2

IHC for ALK

Typically **high** antigen
expression level

Typically **low** antigen
expression level

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

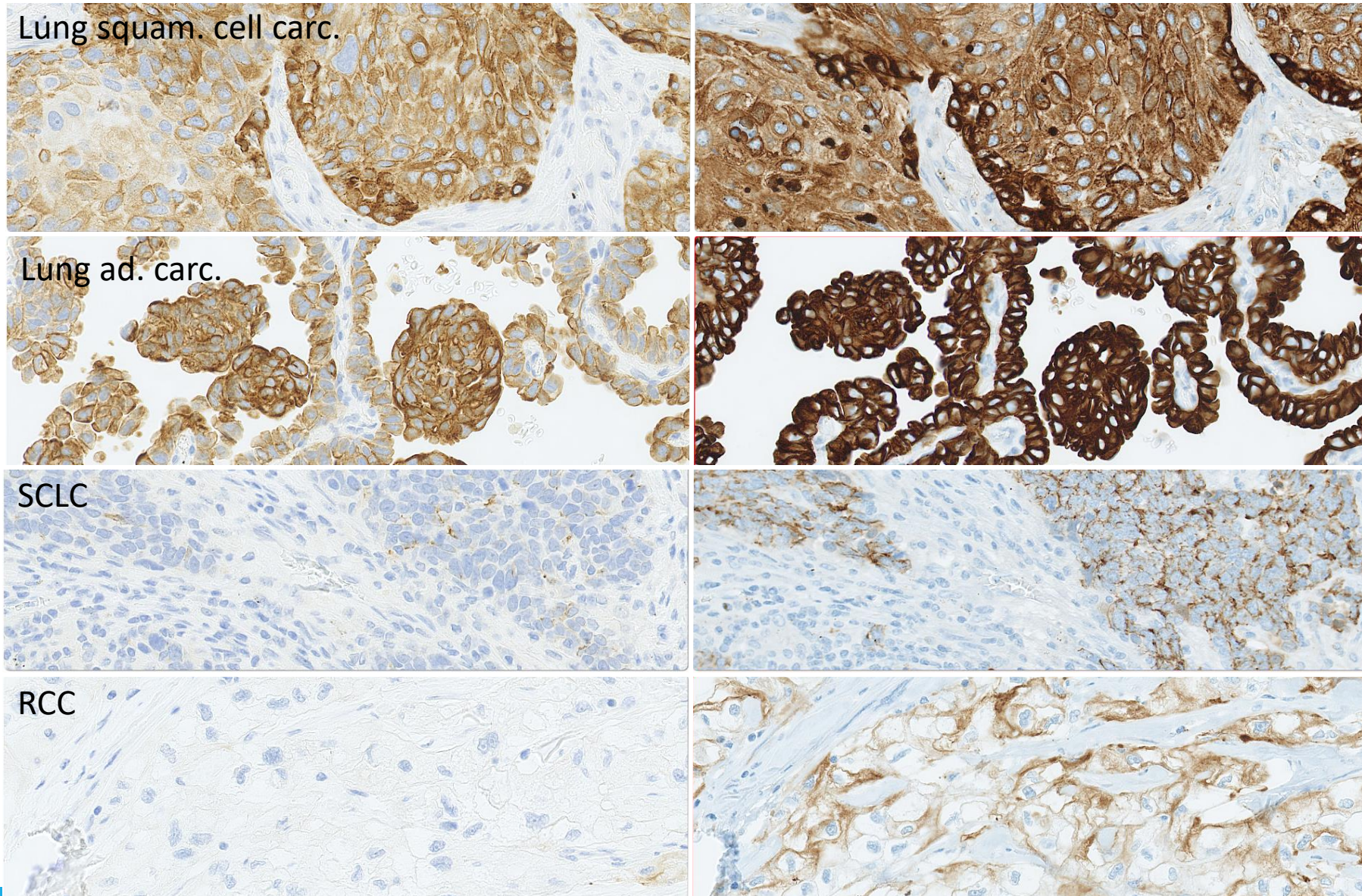
E Torlakovic et al. AIMM 2017;25:4-11
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 1

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

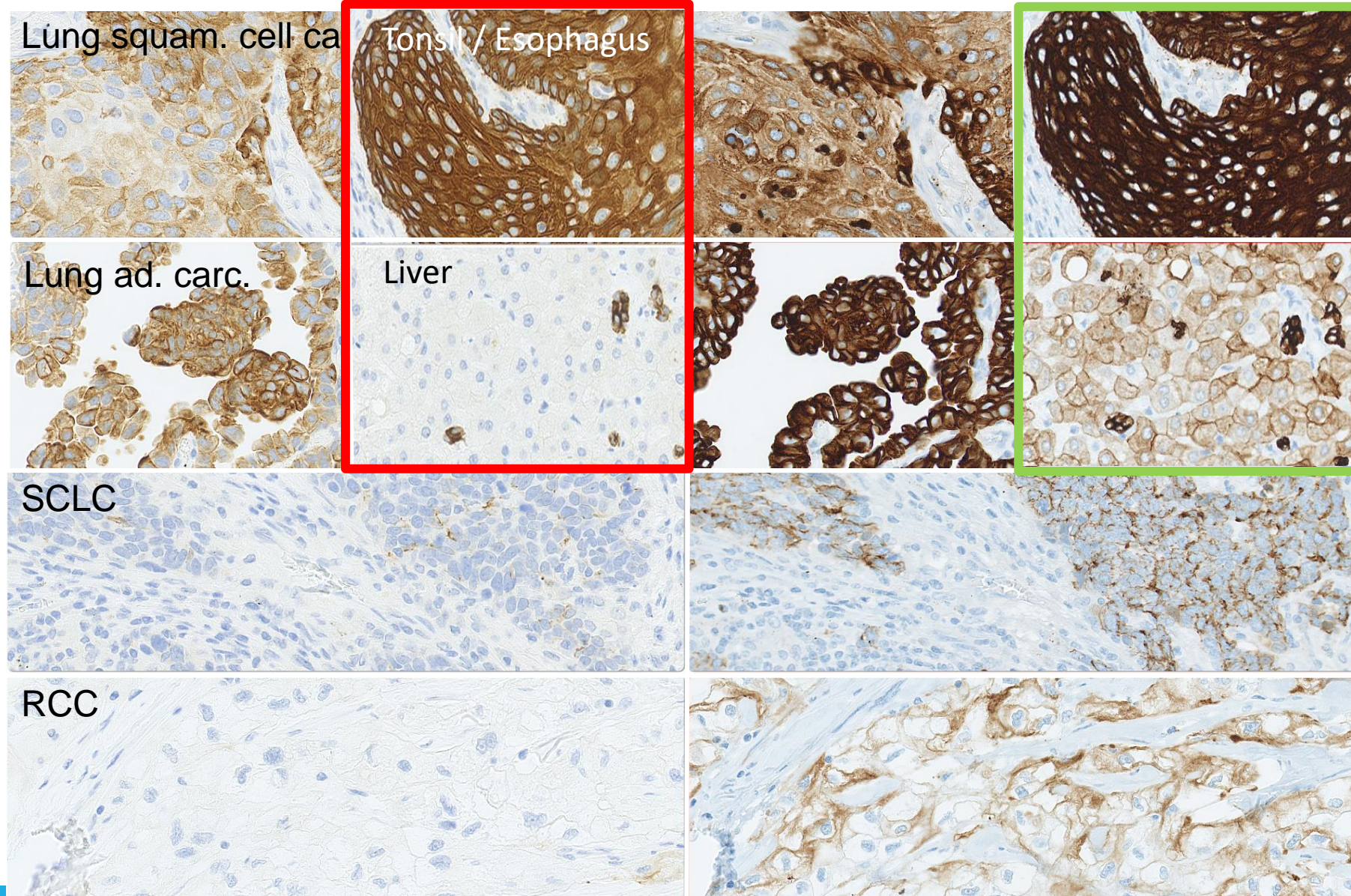
	Purpose* I	Purpose II	Comments
ALK	ALCL	Lung adenocarcinoma with ALK mutation	
CD34	Dermatofibrosarcoma protuberans	Stem cells / leukemia	Different pre-anal
CD56	Neuroendocrine differentiation	Lymphoma classification	
CD117	GIST	Stem cells / leukemia	Different pre-anal
CK5	PIN versus prostate cancer	Lung squamous cell carc vs adenocarcinoma	
CK-PAN	CUP*	Sentinel node status - carcinoma	
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	

* CUP= Cancer Unknown Primary

Use of samples for technical / analytical validation of IHC



Use of samples for technical / analytical validation of IHC



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

	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

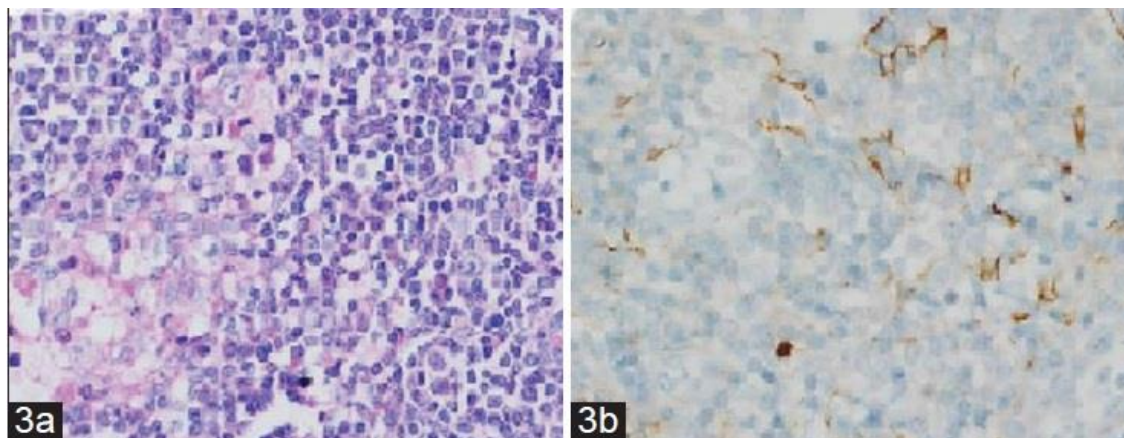
Identification of purpose of the test



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

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

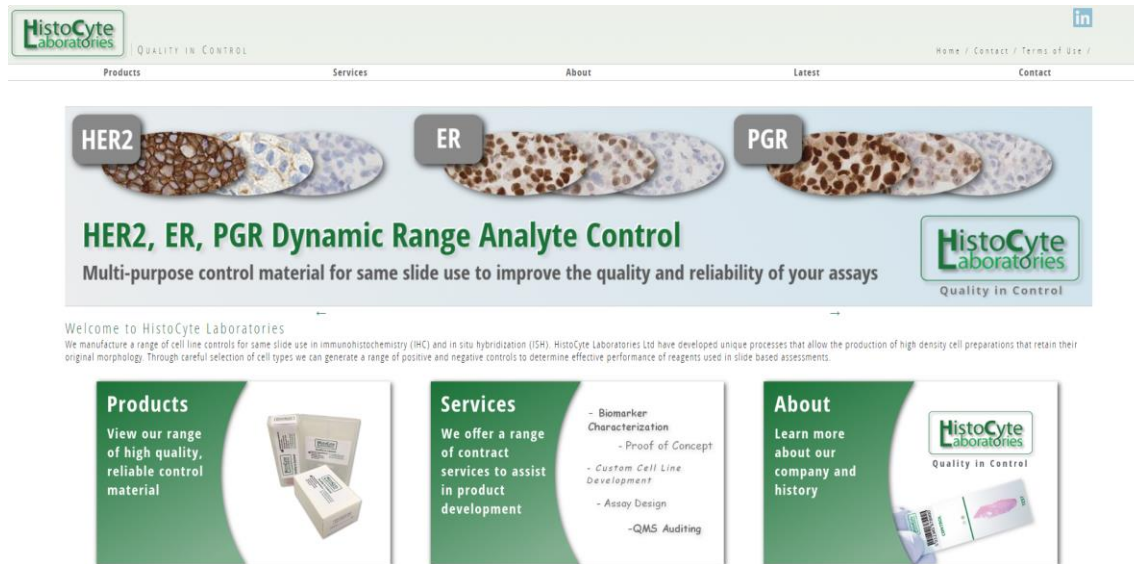


Where to start – how to end.....

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....

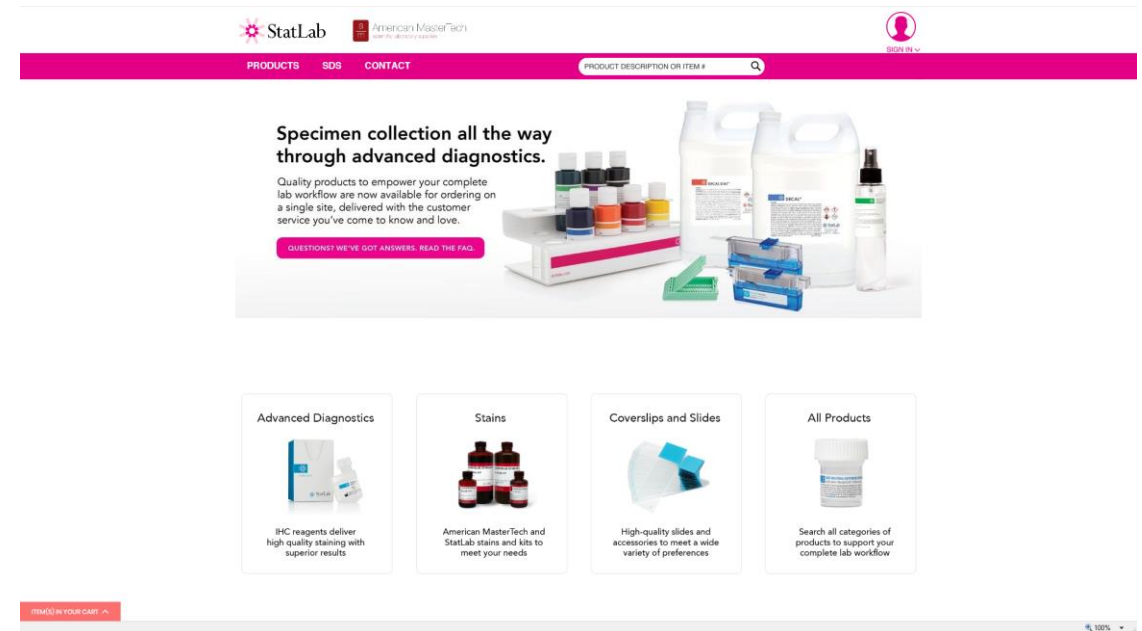


www.histocyte.com

Cell lines

ALK and ROS1 being +/-

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



www.statlab.com

Histoids / Faux tissue

ALK +/-

HER2, PD-L1 with dynamic range

Role of cell lines & histoids 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

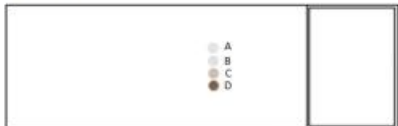
Cell line B: Breast adenocarcinoma

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

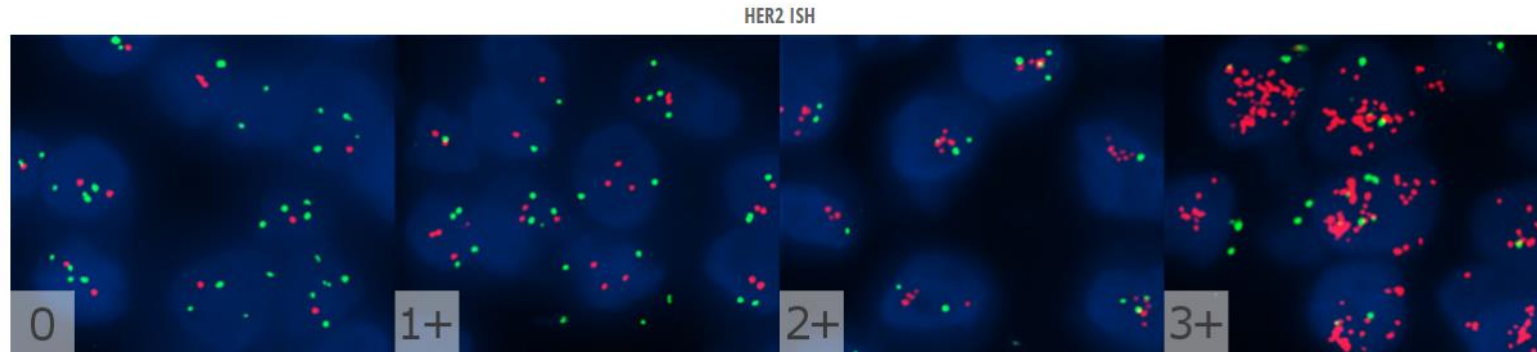
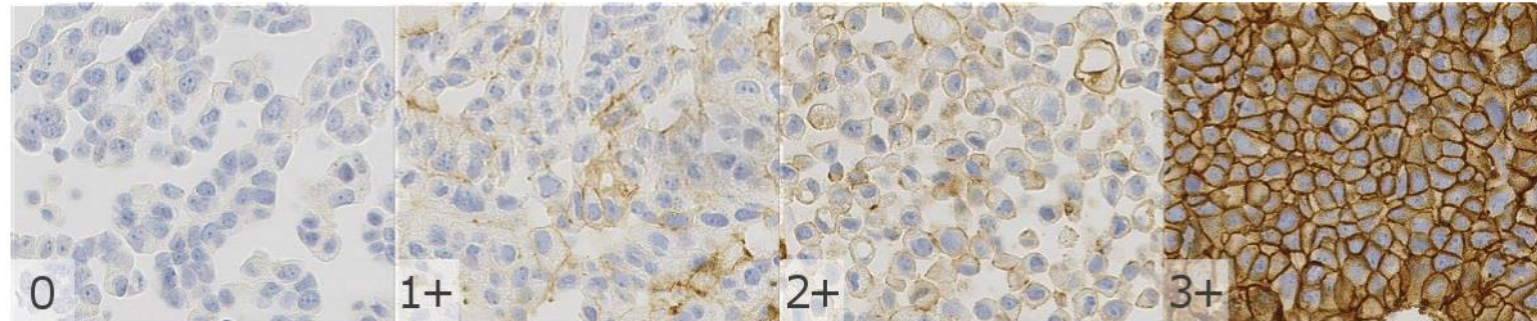
1. The product is intended for research use only.
2. It is the responsibility of the end user to determine suitability with their reagents and procedures within their laboratory.
3. Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those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



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

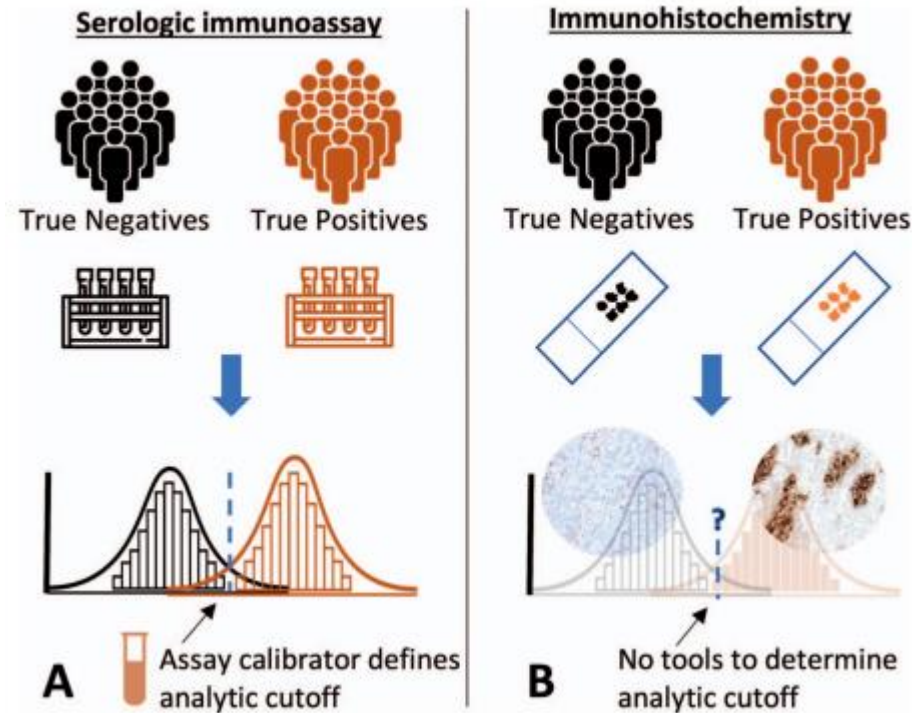
Tissue and cell line expression robustness (too fragile or too stabile)?
What expression levels characterizes a successful vs unsuccessful test?
Impact on section thickness?

Analytical standards – IHC versus clinical chemistry

CA125; 35u/ml

CA-19; 37-40u/ml

....



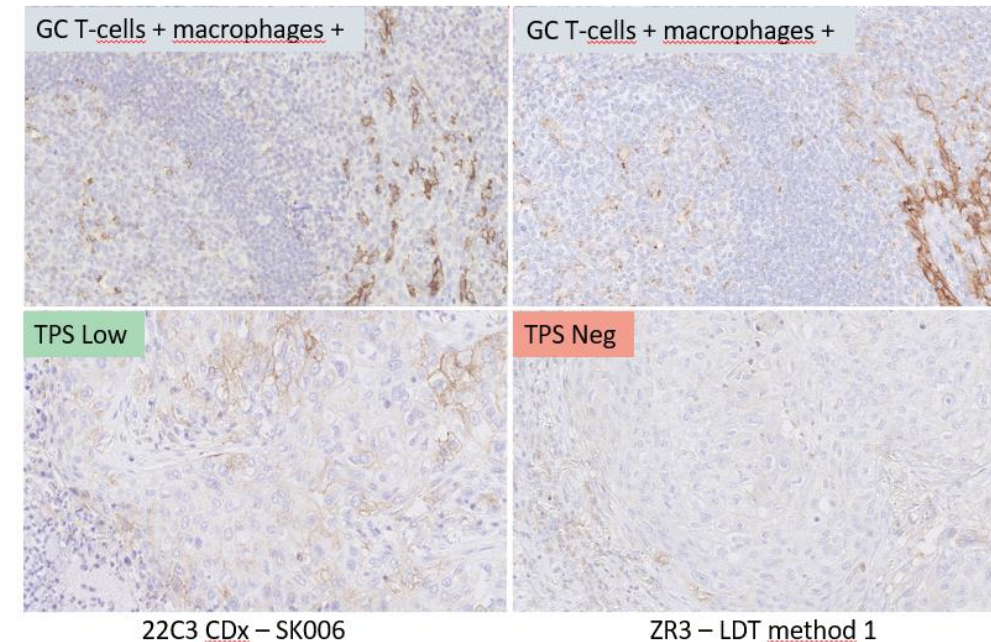
ICAPCs

But challenge for
more semiquantitative
biomarkers as HER2,
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;
Emina E. Torlakovic, MD, PhD

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



Reference materials for IHC; Calibrators – LOD*

R&D
PD-L1 IHC Calibrators
(Extra & Intracellular)
Exp: 6-1-2020
bostoncellstandards.com

BCS
Boston Cell Standards LLC

CERTIFICATE OF ANALYSIS

DESCRIPTION

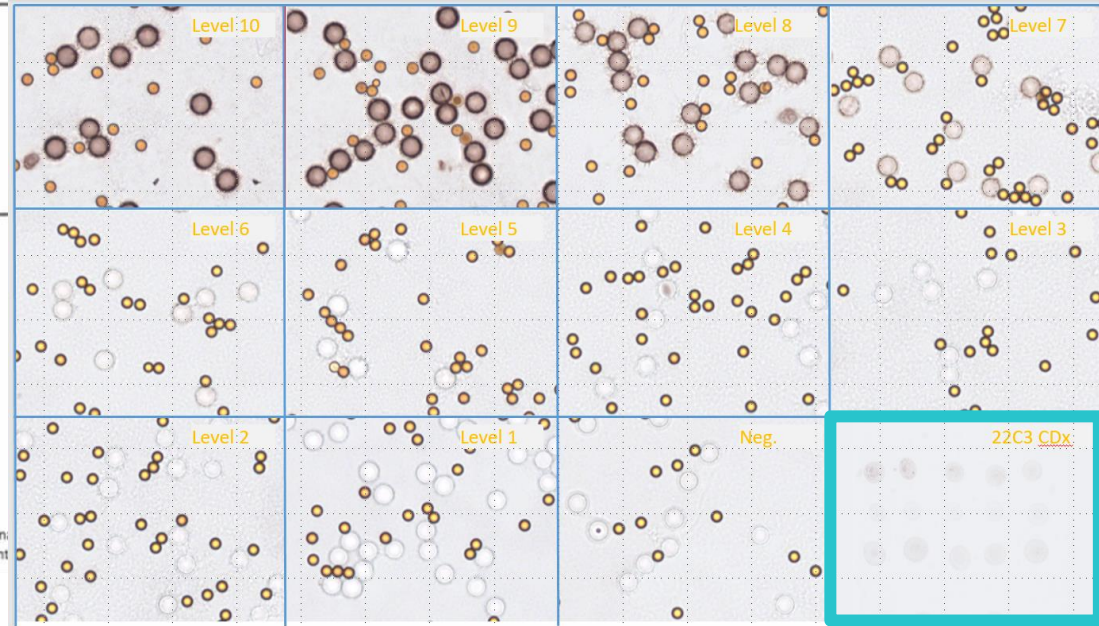
Product Description: IHC Calibrators (10 levels)
Mean Diameter: 7-8 micron
Target: PD-L1 (extracellular Domain)

CONCENTRATION

Average PD-L1 Molecules per Microbead

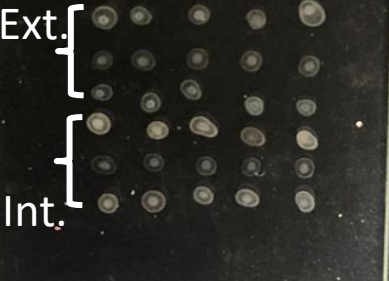
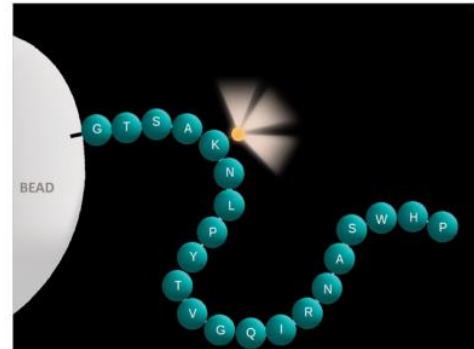
Level 10	603,077
Level 9	598,591
Level 8	479,714
Level 7	356,351
Level 6	228,502
Level 5	116,354
Level 4	53,551
Level 3	22,149
Level 2	9,550*
Level 1	2,197*

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



* LOD; Limit of detection / level of analytical sensitivity

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 materials for IHC; Calibrators – LOD

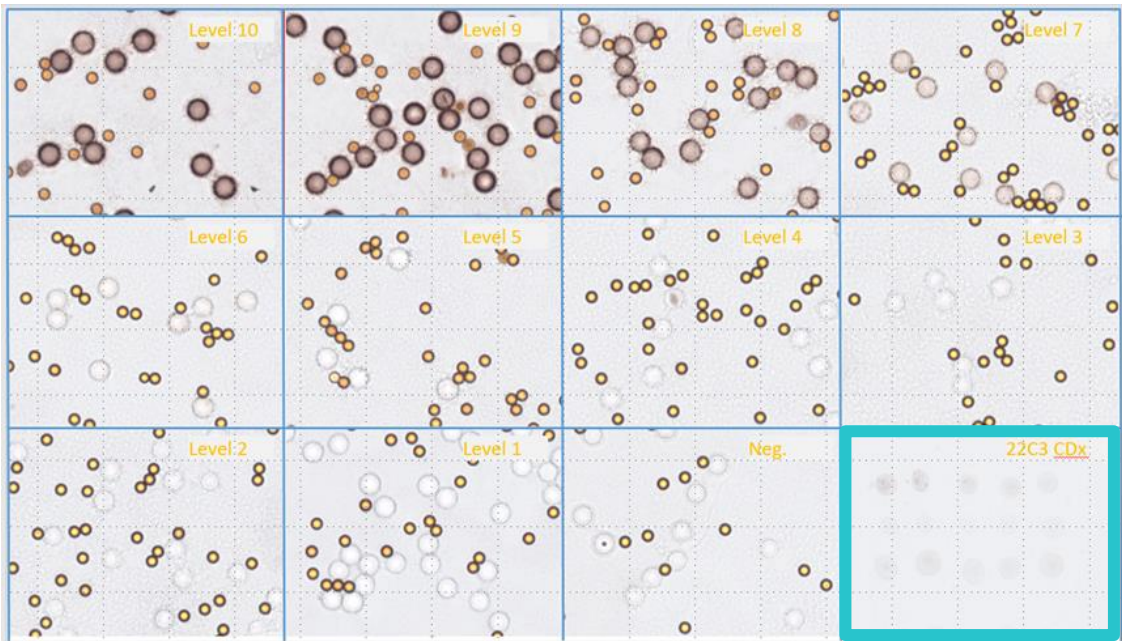
ARTICLE OPEN

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✉

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22C3 LOD
356.351 mol.
pr microbead

Mod Pathol. 2022;35(3):326–332.

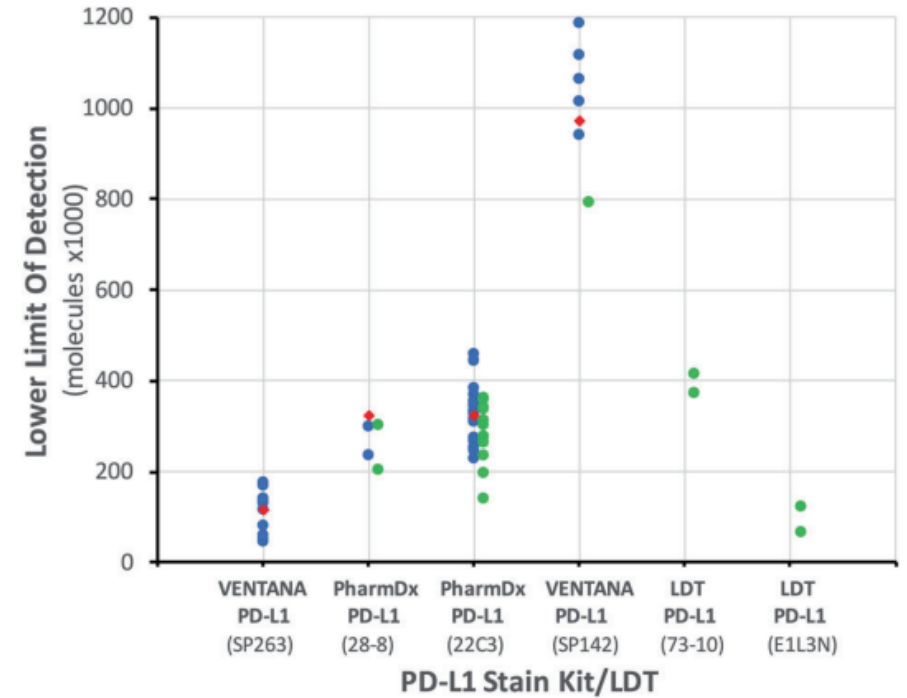
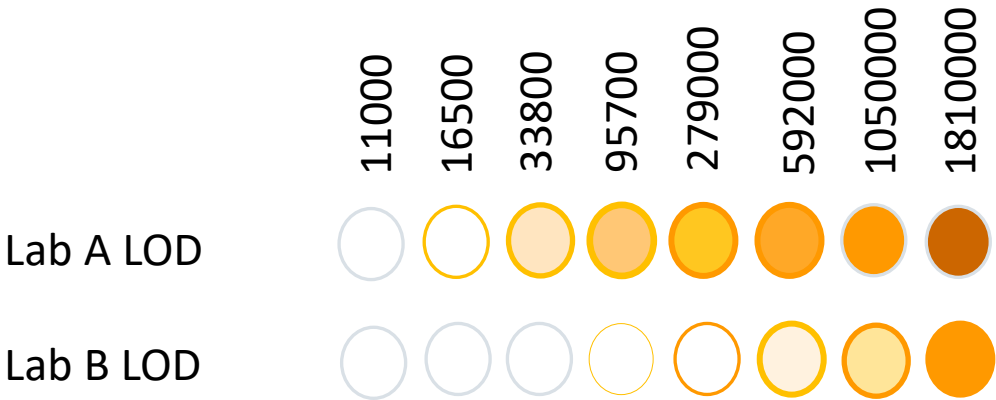
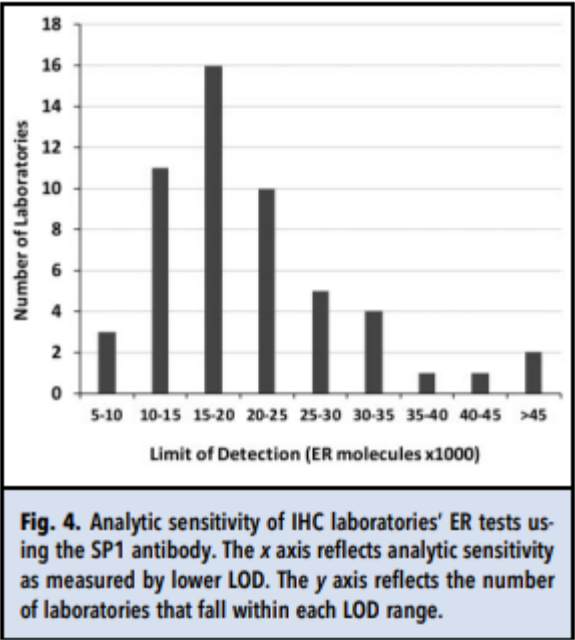
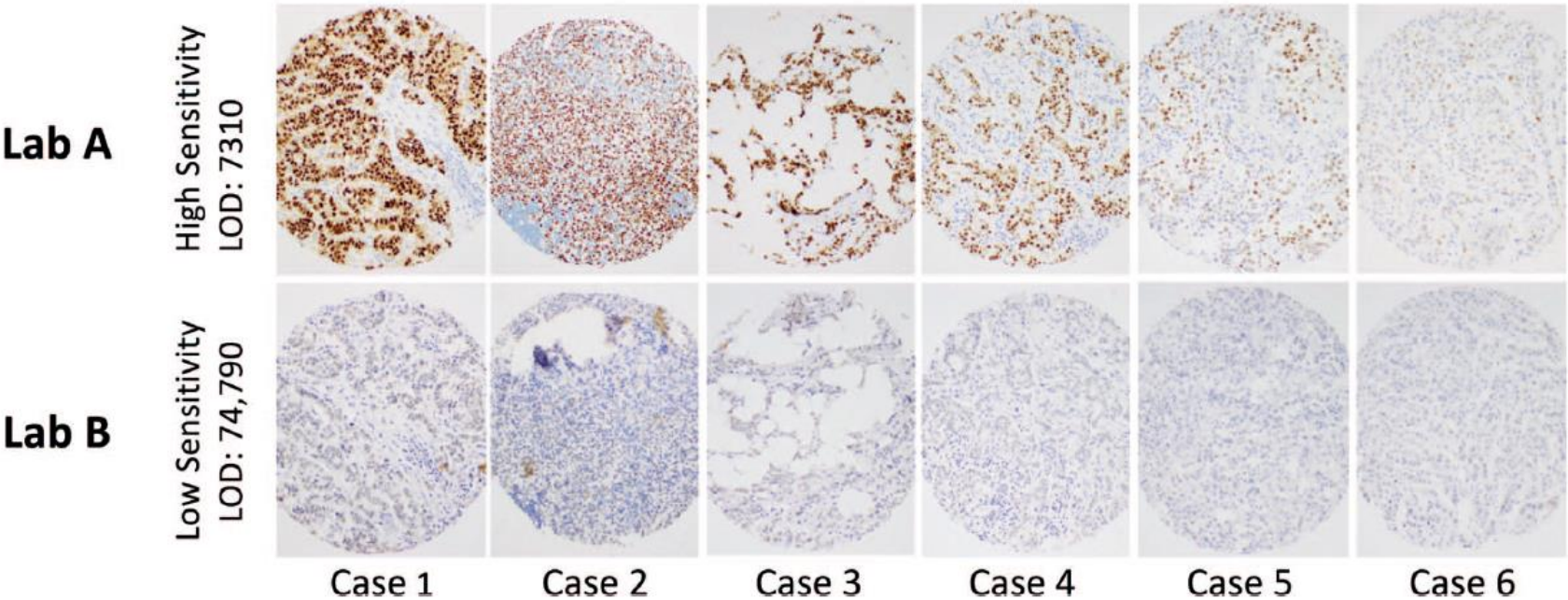


Fig. 2 Lower limit of detection (LOD) of various PD-L1 assays (x axis). Lower numbers (on the y axis) equate to greater sensitivity. Each dot represents a separate IHC laboratory test. Blue dots depict FDA-cleared assays in clinical laboratories, green dots for laboratory-developed tests (LDTs), and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

Reference materials for IHC; Calibrators – ER



Clinical Chemistry 67:5
763-771 (2021)

Clinical Immunology

Development and Validation of Measurement
Traceability for In Situ Immunoassays

Emina E. Torlakovic,^{a,b,c} Seshi R. Sompuram,^d Kodela Vani,^d Lili Wang,^e Anika K. Schaedle,^d
Paul C. DeRose,^e and Steven A. Bogen^{d,*}

Analytical standards – IHC versus clinical chemistry

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

ER, HER2, PD-L1 and p53

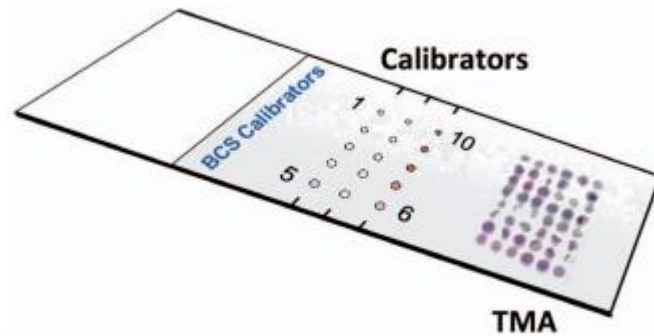


Figure 5. Illustration of the survey tool for correlating clinical accuracy (from the tissue microarray data) with analytic sensitivity (from the calibrator data). The calibrators are at up to 10 different concentrations, for example levels 1–10. The middle row depicts negative controls. Abbreviations: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

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

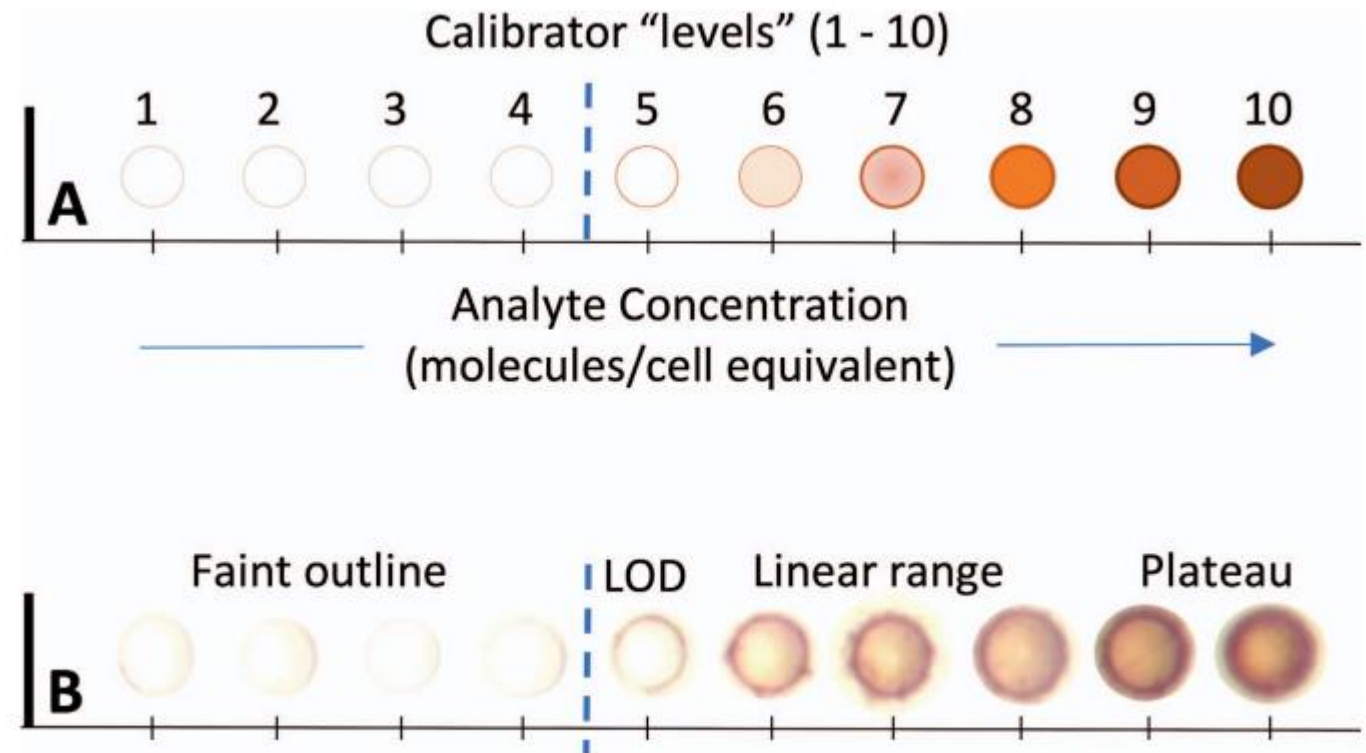
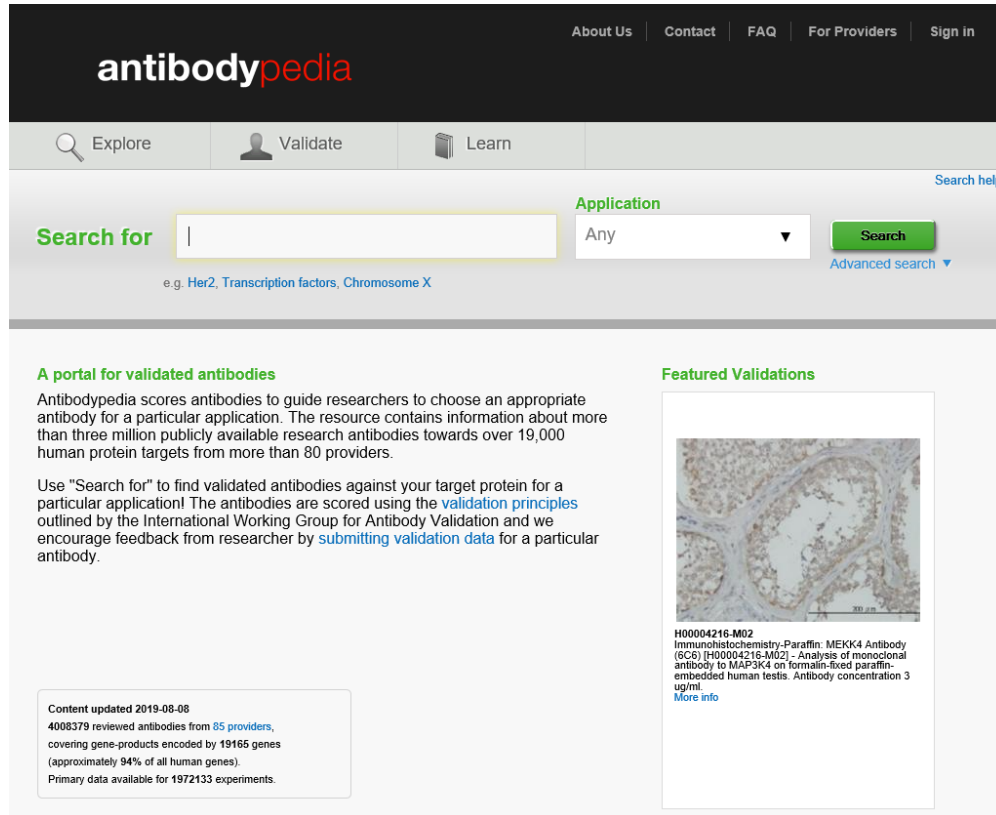


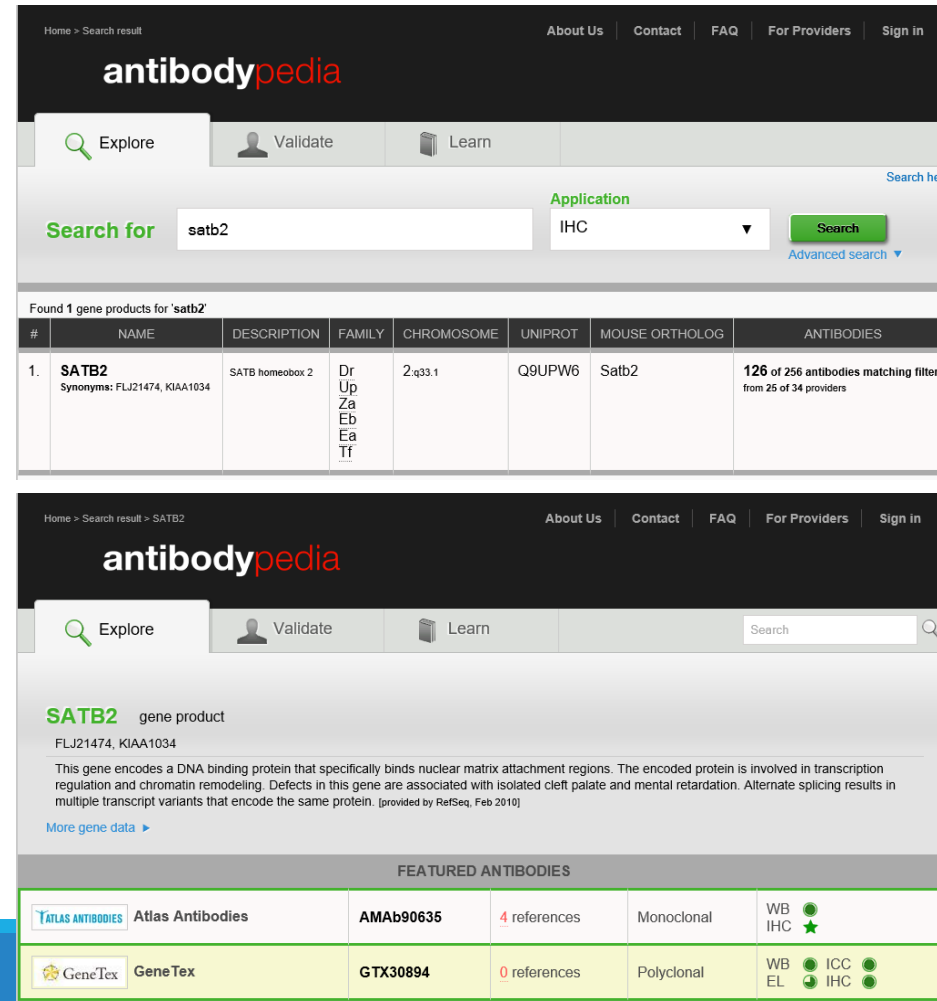
Figure 2. Illustration of a series of immunohistochemistry calibrators after staining. The numbers refer to calibrator levels, from low (1) to high (10) analyte concentrations. A, The illustration shows that rim staining is stronger than central staining because the analyte is attached to the microbead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

Online ressources – “www.antibodypedia.com”

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



The screenshot shows the antibodypedia homepage. At the top, there is a navigation bar with links: About Us, Contact, FAQ, For Providers, and Sign in. Below this is a search bar with the text "Search for" and a dropdown menu for "Application" set to "Any". A green "Search" button is next to the search bar. Below the search bar, there is a link to "Advanced search". The main content area is divided into two columns. The left column is titled "A portal for validated antibodies" and contains text about the resource's scope and a "Search for" section. The right column is titled "Featured Validations" and features a micrograph of a tissue section with a caption: "H00004216-M02 Immunohistochemistry-Paraffin: MEKK4 Antibody (6C6) [H00004216-M02] - Analysis of monoclonal antibody to MAP3K4 on formalin-fixed paraffin-embedded human testis. Antibody concentration 3 ug/ml. More info". At the bottom left, there is a box with content update information: "Content updated 2019-08-08 4068379 reviewed antibodies from 85 providers, covering gene-products encoded by 19165 genes (approximately 94% of all human genes). Primary data available for 1972133 experiments."



The screenshot shows the search results for "SATB2" on the antibodypedia website. The search bar contains "satb2" and the "Application" dropdown is set to "IHC". The results section is titled "Found 1 gene products for 'satb2'" and contains a table with the following data:

#	NAME	DESCRIPTION	FAMILY	CHROMOSOME	UNIPROT	MOUSE ORTHOLOG	ANTIBODIES
1.	SATB2 Synonyms: FLJ21474, KIAA1034	SATB homeobox 2	Dr Up Za Eb Ea Tf	2:q33.1	Q9UPW6	Satb2	126 of 256 antibodies matching filter from 25 of 34 providers

Below the table, there is a section for "SATB2 gene product" with a description: "This gene encodes a DNA binding protein that specifically binds nuclear matrix attachment regions. The encoded protein is involved in transcription regulation and chromatin remodeling. Defects in this gene are associated with isolated cleft palate and mental retardation. Alternate splicing results in multiple transcript variants that encode the same protein. [provided by RefSeq, Feb 2010]". A link to "More gene data" is provided. At the bottom, there is a section titled "FEATURED ANTIBODIES" with a table of recommended antibodies:

ANTIBODIES	Atlas Antibodies	GeneTex
ATLAS ANTIBODIES	AMAb90635	GTX30894
REFERENCES	4 references	0 references
MONOCLONAL	Monoclonal	Polyclonal
WB	WB	WB
IHC	IHC	ICC
EL	EL	IHC

Online resources – “www.antibodypedia.com”

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

antibodypedia

Explore Validate Learn Search

AMAb90635 SATB2 antibody from Atlas Antibodies
FLJ21474, KIAA1034
Eligible for validation within the Antibodypedia Validation Initiative

Western blot Supportive data in Antibodypedia
Immunohistochemistry Enhanced validation

Provider product page for AMAb90635

SELECT THIS ANTIBODY PERFORM KNOCKDOWN OR KNOCKOUT SUBMIT POSITIVE RESULTS GET REWARDED

ANTIBODY DATA

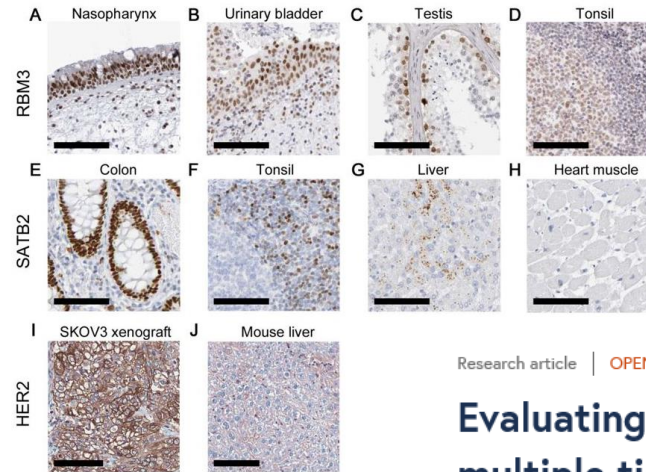
Antibody Data	Product number	AMAb90635
Knockdown Reagents [0]	Provider	Atlas Antibodies
References [4]	Product name	Anti-SATB2
Comments [0]	Provider product page	Atlas Antibodies - AMAb90635
Validations	Antibody type	Monoclonal
Western blot [1]	Description	Protein A purified
Immunohistochemistry [5]	Reactivity	Human
Submit	Host	Mouse
Validation data	Conjugate	Unconjugated
Reference	Antigen sequence	VSGVYFARVAFNRTQGLLSEILSKEDPTASQSL LVNLRAMQNFNLPEVSKDZYQEPESRPSRYS MYSSASSPSSSRIPQAKTSTPTLPIKVGANI NITAAIYDEIQEDMGKAK
Comment	Epitope	Binds to an epitope located within the peptide sequence LVNLRAMQNFNLPE as determined by overlapping synthetic peptides.
Report error	Isotype	IgG
	Antibody clone number	CL0276
	Vial size	100 µl
	Storage	Store at +4°C for short term storage. Long time storage is recommended at -20°C.

BMC Research Notes

Home About Articles Submission Guidelines

Figure 1

From: [Evaluating real-time immunohistochemistry on multiple tissue samples, multiple targets and multiple antibody labeling methods](#)



Research article | OPEN ACCESS | Published: 18 December 2013

Evaluating real-time immunohistochemistry on multiple tissue samples, multiple targets and multiple antibody labeling methods

[Louise Dubois](#), [Karl Andersson](#), [Anna Asplund](#) & [Hanna Björkelund](#) ✉

BMC Research Notes 6, Article number: 542 (2013) | [Download Citation](#)

Starting help to guide test performance characteristics – Validation still required

Online resources – “www.proteinatlas.com”

THE HUMAN PROTEIN ATLAS

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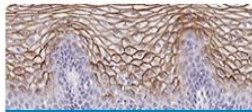
SEARCH¹

SATB2

Search

Fields »

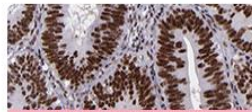
e.g. ACE2, GFAP, EGFR



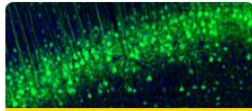
TISSUE ATLAS



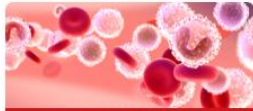
SINGLE CELL TYPE ATLAS



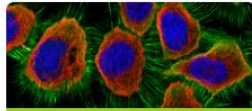
PATHOLOGY ATLAS



BRAIN ATLAS



BLOOD ATLAS



CELL ATLAS

Digital collection History of the Human Protein Atlas



PRESS ROOM



contact@proteinatlas.org

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LICENCE & CITATION

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Thu, 22 Jul 2021
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Tue, 15 Jun 2021
Movie of the month: The Spleen

[all news articles](#)

Version: 20.1
Atlas updated: 2021-02-24
release history

Proteome analysis based on
26941 antibodies targeting
17165 unique proteins

THE HUMAN PROTEIN ATLAS

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SATB2

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1 GENE FOUND¹

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Page 1 of 1

Gene ¹	Gene description ¹	Evidence ¹	Tissue ¹	Cell type ¹	Pathology ¹	Brain ¹	Blood ¹	Cell ¹
SATB2	SATB homeobox 2			RNA			RNA	

Page 1 of 1

TISSUE ATLAS

PRIMARY DATA

TISSUES

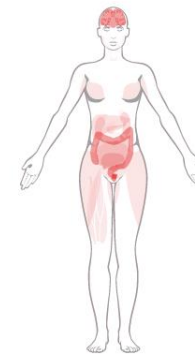
GENE/PROTEIN

ANTIBODIES AND VALIDATION

Dictionary

Tissue proteome

RNA AND PROTEIN EXPRESSION SUMMARY¹

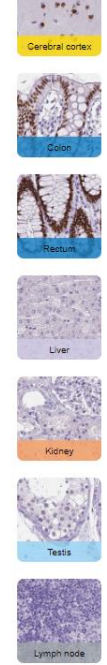
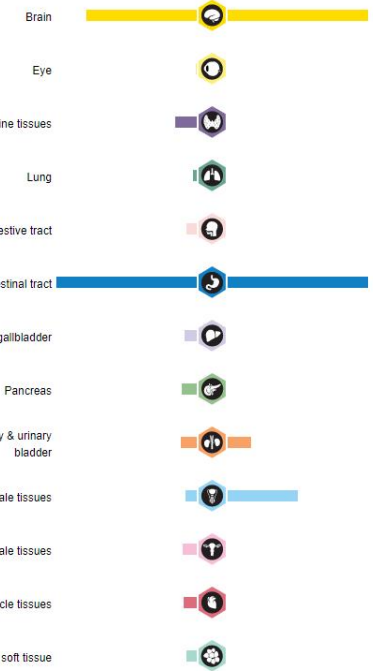


Expression Detection All organs



RNA expression (NX)¹

Protein expression (score)¹



Online resources – “www.proteinatlas.com”

THE HUMAN PROTEIN ATLAS

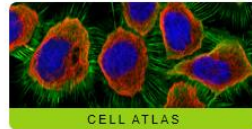
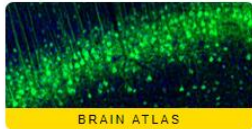
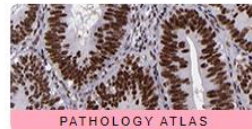
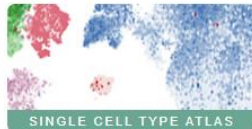
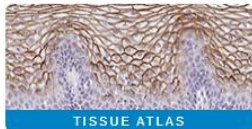
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SEARCH¹

SATB2

e.g. ACE2, GFAP, EGFR

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1 GENE FOUND¹

Show / hide columns ▾ Download: XML | RDF | TSV | JSON | Custom TSV/JSON ▾ Page 1 of 1

Gene ¹	Gene description ¹	Evidence ¹	Tissue ¹	Cell type ¹	Pathology ¹	Brain ¹	Blood ¹	Cell ¹
SATB2	SATB homeobox 2			RNA			RNA	

Page 1 of 1

PATHOLOGY ATLAS

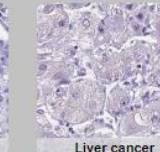
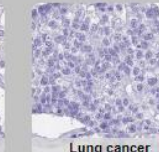
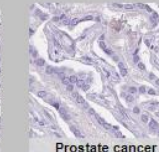
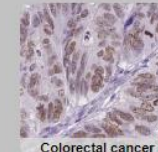
CANCER

GENE/PROTEIN

ANTIBODIES
AND
VALIDATION

Dictionary

PROTEIN EXPRESSION¹



PROTEIN EXPRESSION SUMMARY¹

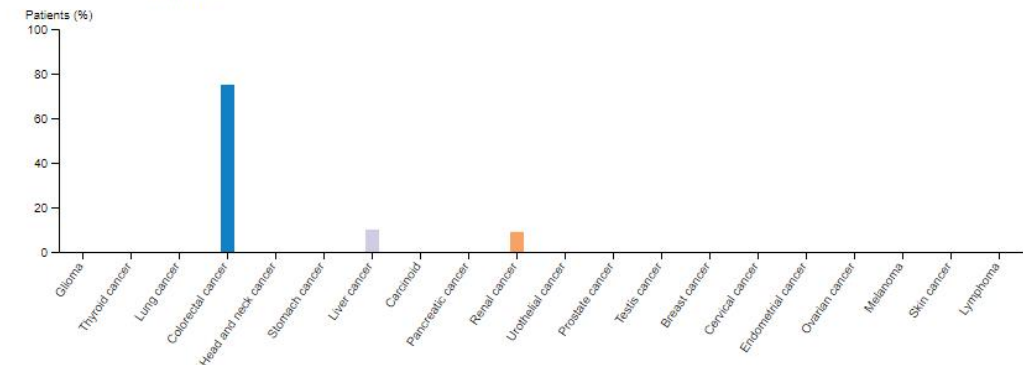
HPA001042 HPA029543 CAB023669 CAB062562 CAB067751 CAB068229 CAB068230 CAB068231

Organ

Expression

Alphabetical

Most colorectal cancers showed moderate to strong nuclear positivity. Other cancer tissues were negative.



Main elements to develop & validate IHC assays



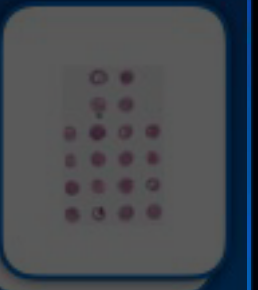
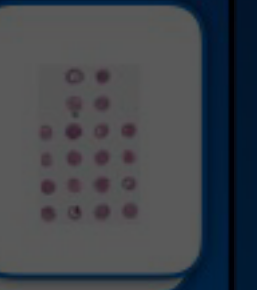


The journey from an antibody to a diagnostic IHC assay with a specific purpose

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

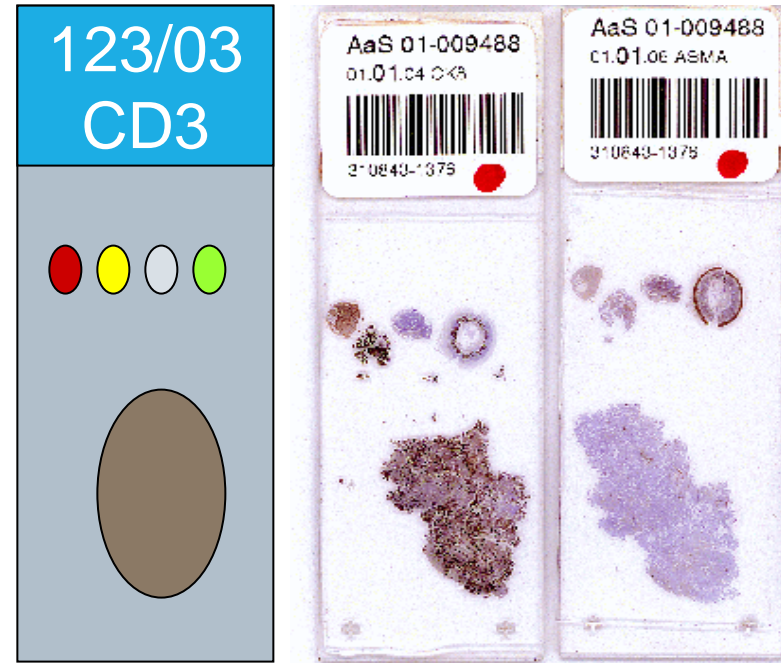


*Immunohistochemical critical assay performance controls

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,*† 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

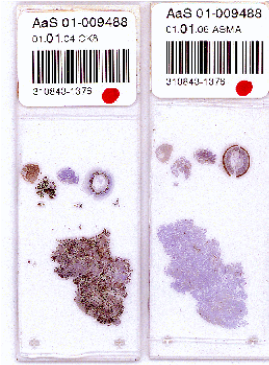
Carol C. Cheung, MD, PhD, JD,*† Clive R. Taylor, MD, DPhil,‡ and Emina E. Torlakovic, MD, PhD†

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, ScD¹²; David L. Rimm, MD, PhD¹³; W. Fraser Symmans, MD¹⁰; Emina E. Torlakovic, MD, PhD^{14,15}; Leticia Varela, MD¹⁶; Giuseppe Viale, MD^{17,18}; Tracey F. Weisberg, MD¹⁹; Lisa M. McShane, PhD²⁰; and Antonio C. Wolff, MD²¹

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

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



Target	2006	2020
Estrogen receptor	12%	46%
ALK	9%	60%

Participants using onslide controls for slide submitted to NordiQC

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†

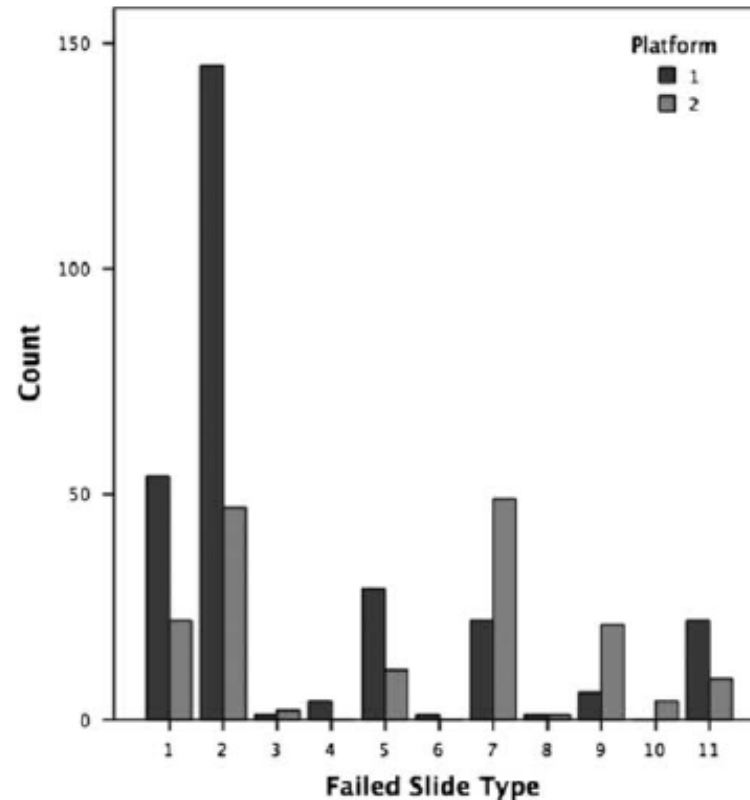


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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;

Class I 0,8%

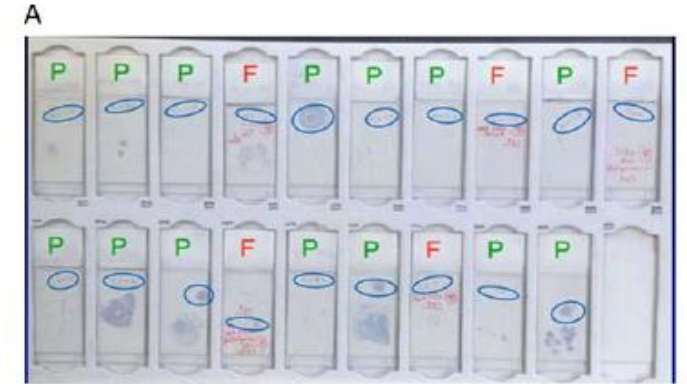
Class II 9,0%

(452/22.234 slides)

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
- Use of critical tissue controls / ICAPCs with relevant range of target analyte is crucial







Conclusions

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

- On-slide TMA controls are preferable to 1 batch 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

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*Immunohistochemical critical assay performance controls

Questions and/or comments



Thank You for the attention and.....