



**Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, October 2nd-4th 2019**

The Tissue Tool Box

-

IHC Critical Assay Performance Controls

*Søren Nielsen,
Director
NordiQC*

Agenda and focus areas

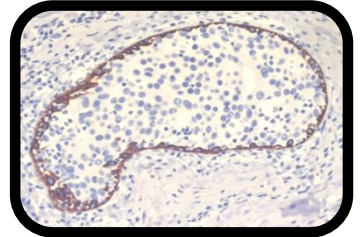
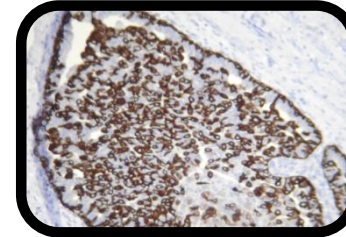
- What is recommended and best practice for IHC controls in diagnostic IHC?
- What are the potentials and limitations for the use of IHC controls ?
- How can IHC controls be used by laboratories and IHC stakeholders?
 - How to use IHC controls to implement new markers.
 - How to use IHC controls to monitor 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

Power of IHC

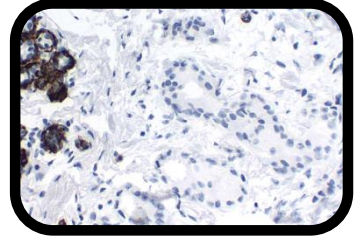
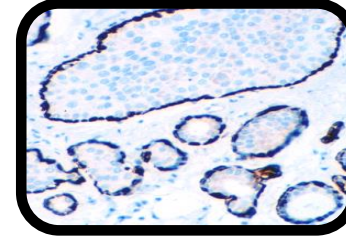
Hyperplasia or In-situ

CK5, CK14, Heavy chain myosin, p63



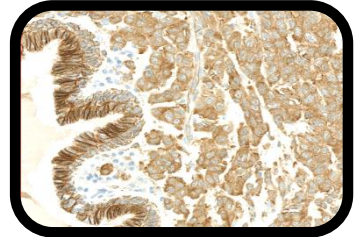
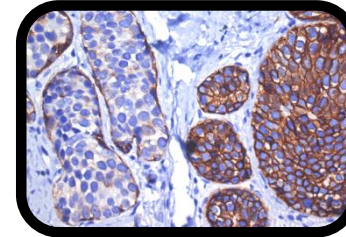
In-situ or invasive

CK5, CK14, Heavy chain myosin, p63



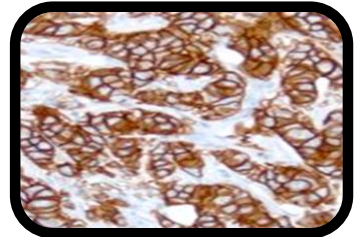
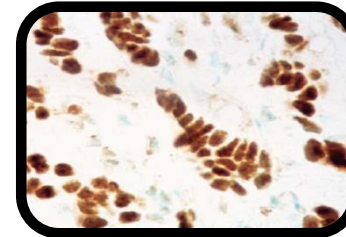
Lobular or ductal lesion

E-cadherin, p120



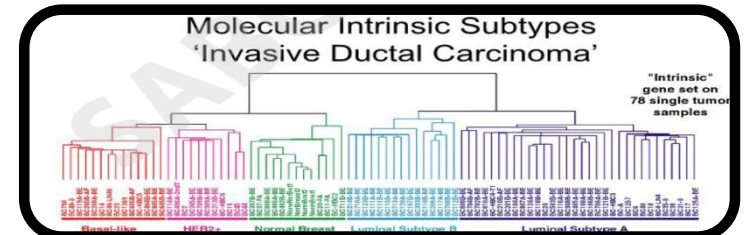
Predictive - Prognostic

ER, PR, HER2, Ki67

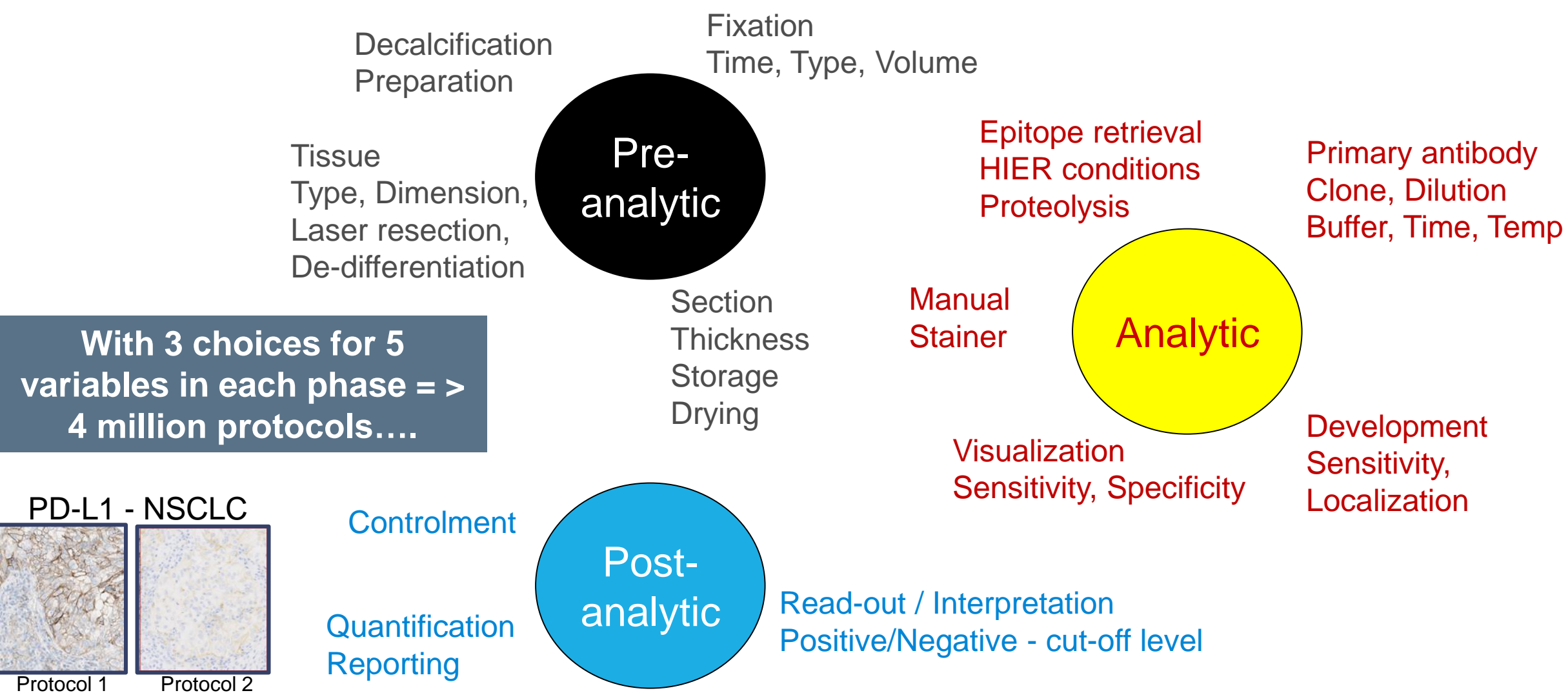


"Molecular risk signature"

IHC4 – ER, PR, HER2, Ki67 (CK5)



... 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

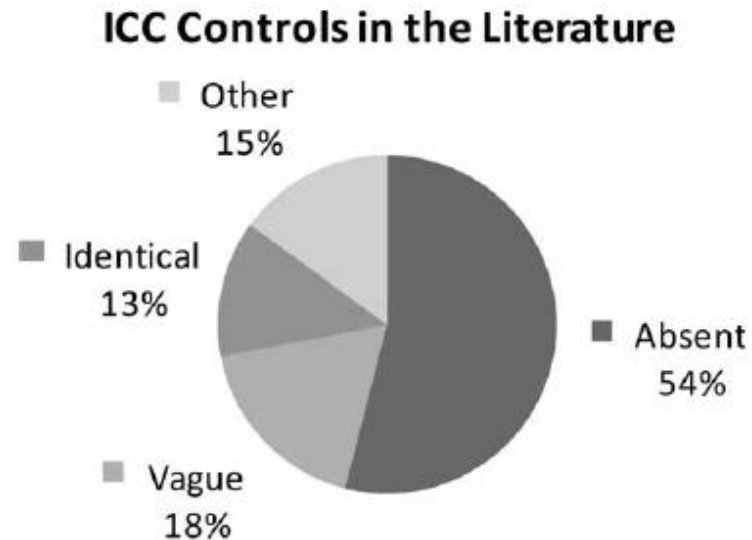


Fig. 1. Description of immunocytochemistry controls in articles reviewed.

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...?

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

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

Metastatic Carcinoma of Unknown Primary:
Diagnostic Approach Using Immunohistochemistry

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

< 5%

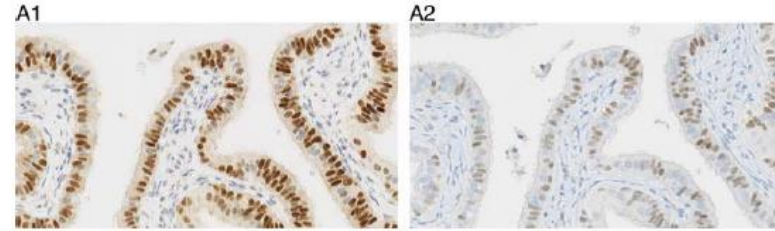
Right choice, use and results reported in positive and negative
IHC control tissues needed to verify data

IHC controls to guide reliability of data...

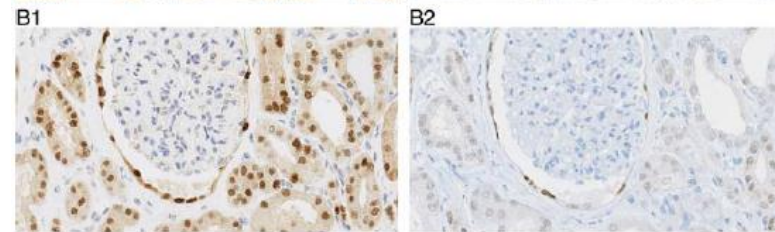
NordiQC Assessments of PAX8 Immunoassays

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

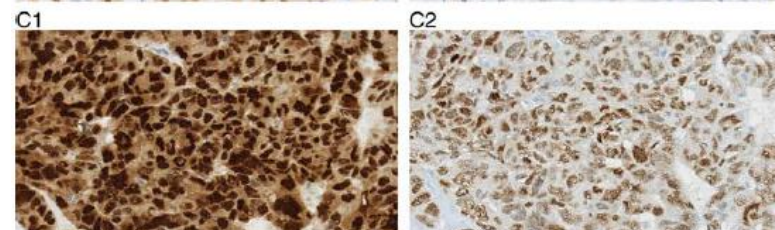
Positive tissue control 1



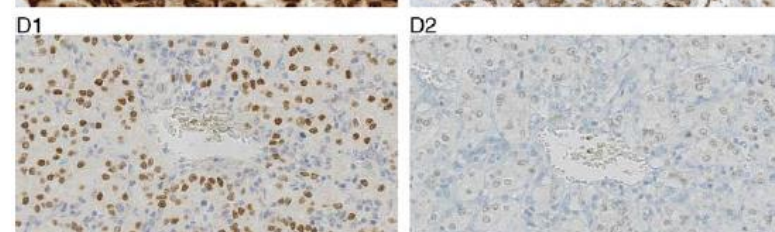
Positive tissue control 2



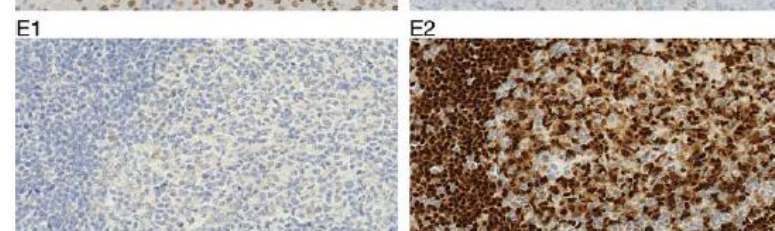
Tumour type 1



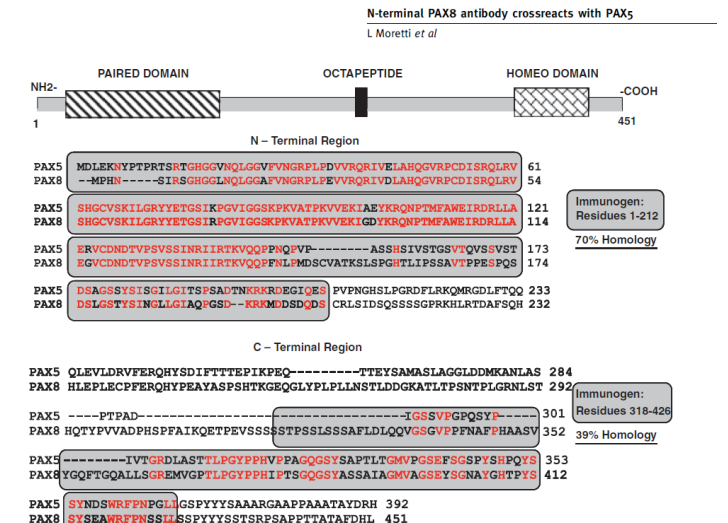
Tumour type 2



Negative tissue control 1



Level of analytical sensitivity



Level of analytical specificity

Protocol 1

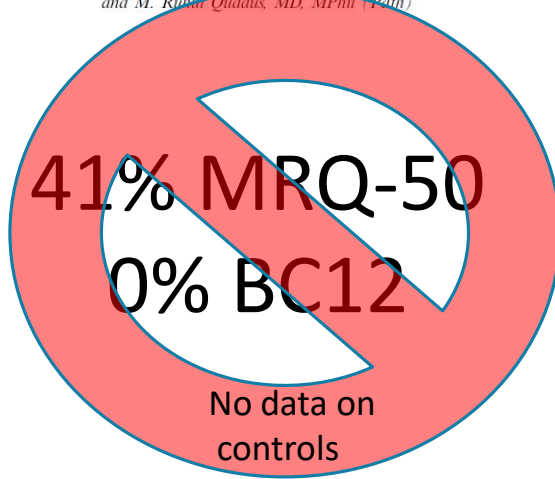
Protocol 2

IHC controls to guide reliability of data...

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

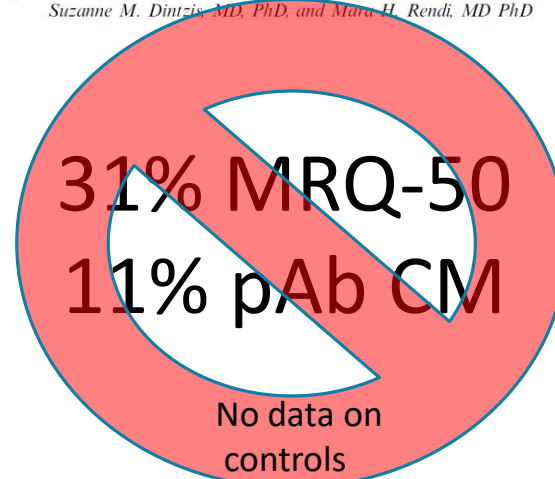
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Unexpected PAX8 Immunoreactivity in Metastatic High-grade Breast Cancer

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Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

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

< 5%

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

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

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

Emina E. Torlakovic, MD, PhD,*†† Carol C. Cheung, MD, PhD, JD,*§
Corrado D'Arrigo, MB, ChB, PhD, FRCPath,§§§ Manfred Dietel, MD, PhD,***
Glenn D. Francis, MBBS, FRCPA, MBA, FFSc (RCPA),†††§§§ C. Blake Gilks, MD,|||
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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),§§§***††
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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.

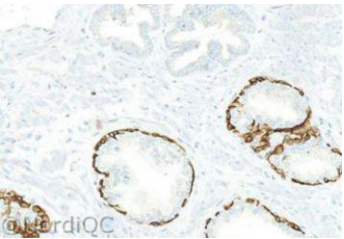
Tissue controls

- 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

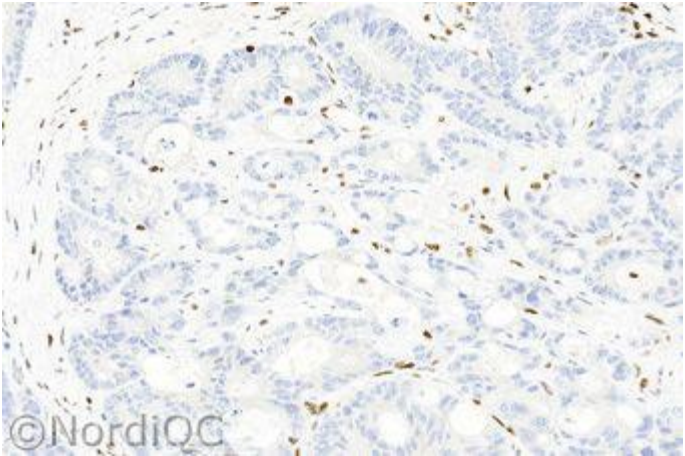
How to use internal tissue controls

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

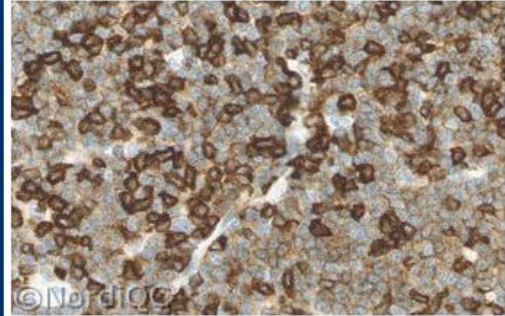


Fig. 4a. Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

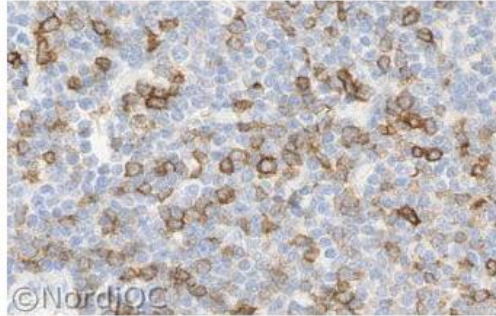


Fig. 4b. Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

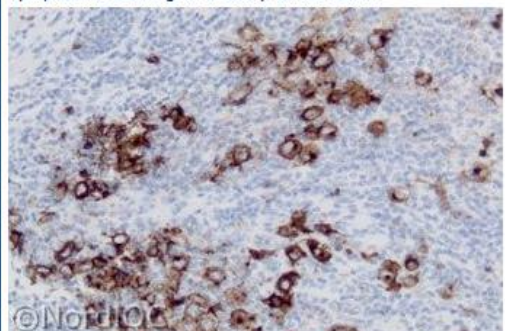


Fig. 2a. Optimal CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

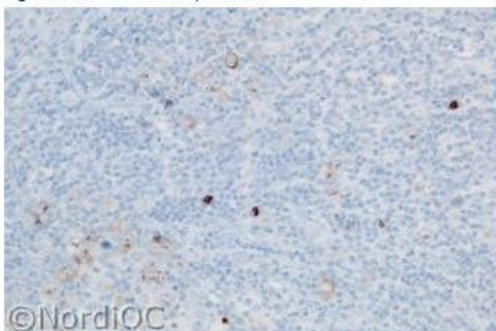


Fig. 2b. CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a.

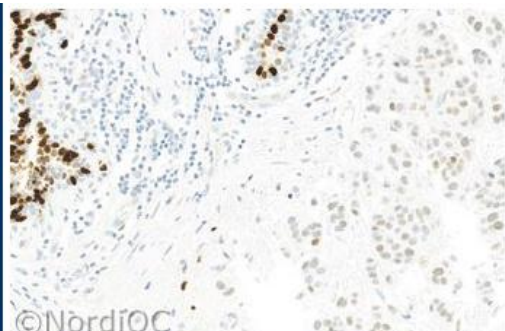


Fig. 3a. Optimal ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the neoplastic cells. Same protocol as in Figs. 1a and 2a.

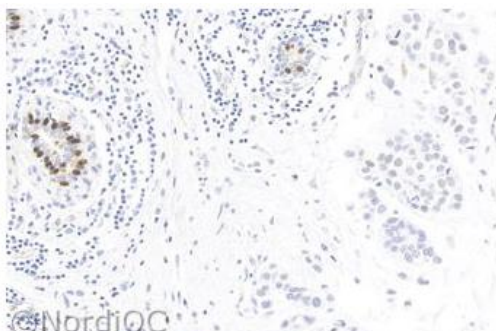
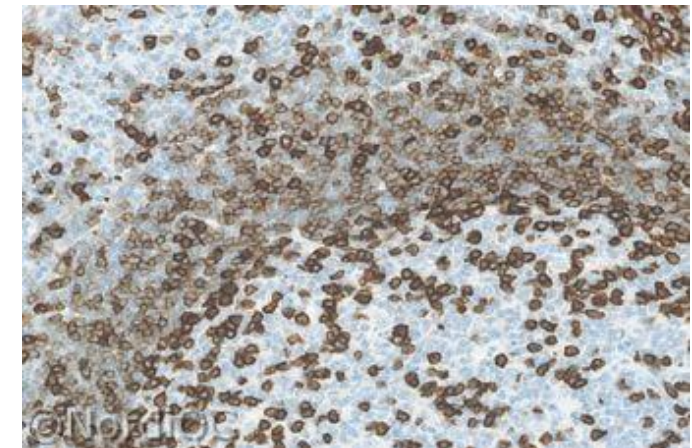


Fig. 3b. Insufficient ER staining of the breast ductal carcinoma no. 3 with 60 - 80 % cells positive using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. Only dispersed neoplastic cells show an equivocal staining.

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**, PR etc



Critical tissue controls = ICAPCs

IHC Critical Assay Performance Controls (ICAPCs)

are basically human positive control tissues with

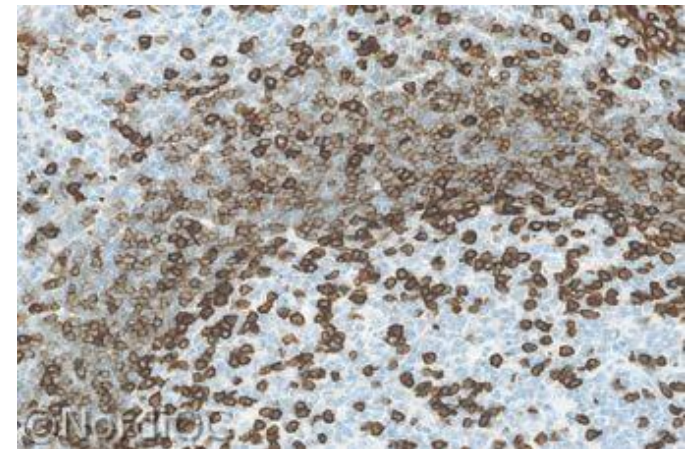
- clinical relevant range of target analyte (antigen) – especially with low limit detection
- well characterized expression pattern – preferable normal tissues
- predictable levels and specified cellular and architectural localization

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






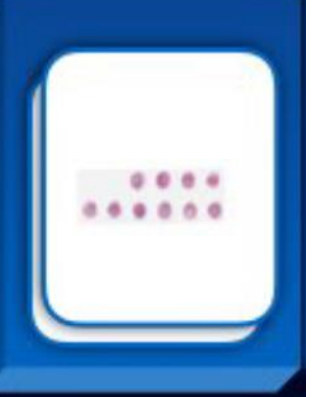
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4. Identification of IHC performance controls providing information that the established level of detection is obtained in each test performed in daily practice.

Based on selection and use of appropriate external tissue controls

External tissue control tool box


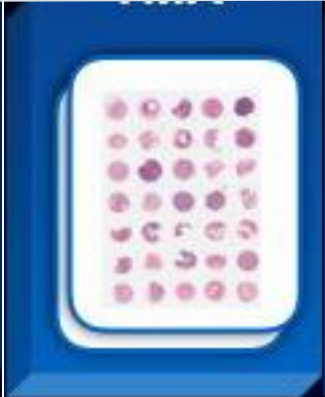




E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 4

Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC* TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical assay performance controls	Maps Ab reaction pattern Test performance characteristics	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility
High expression Low expression No expression	With expression No expression		With expression No expression	High expression Low expression No expression	Method of transfer proof
			20/40 of each Type I/II IHC	+ relevant cut-off	



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

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*Immunohistochemical critical assay performance controls

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

Calibration TMA's

iCAPC*
TMA

Specificity
TMA

Pre-analyt.
TMA



"Gold standard"
tissue controls

"Normal" tissues

iCAPCs processed
as lab procedures

IHC critical assay
performance
controls

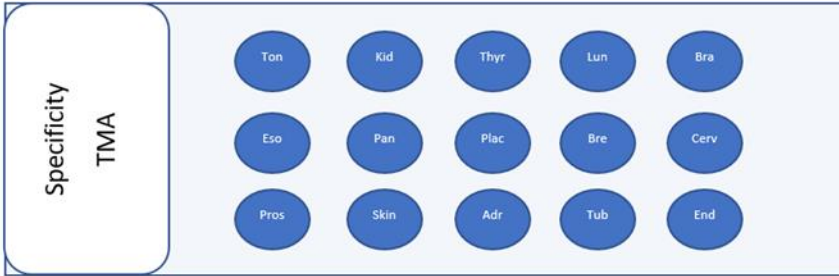
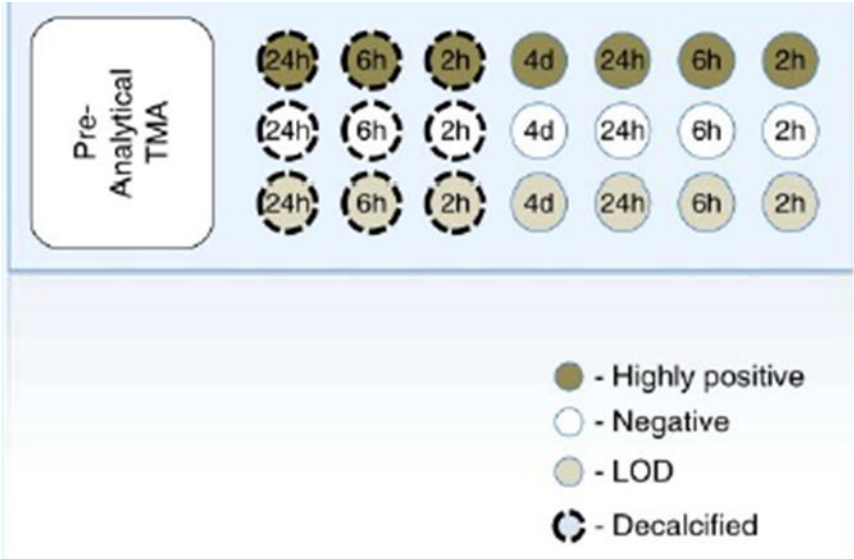
Maps Ab reaction
pattern
Test performance
characteristics

Fixation time
Fixative(s)
Decalcification

High expression
Low expression
No expression

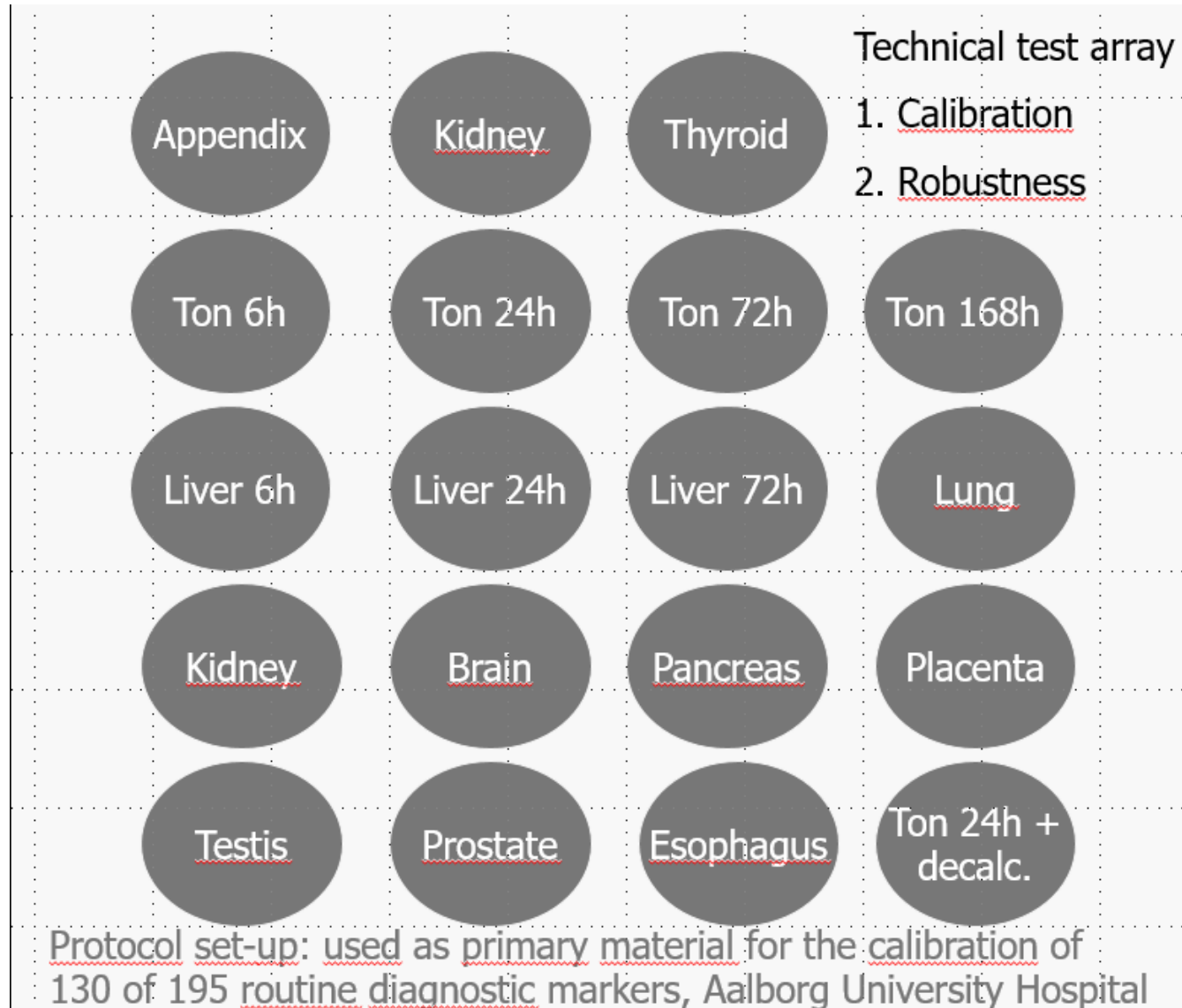
With expression

No expression



*Immunohistochemical critical assay performance controls

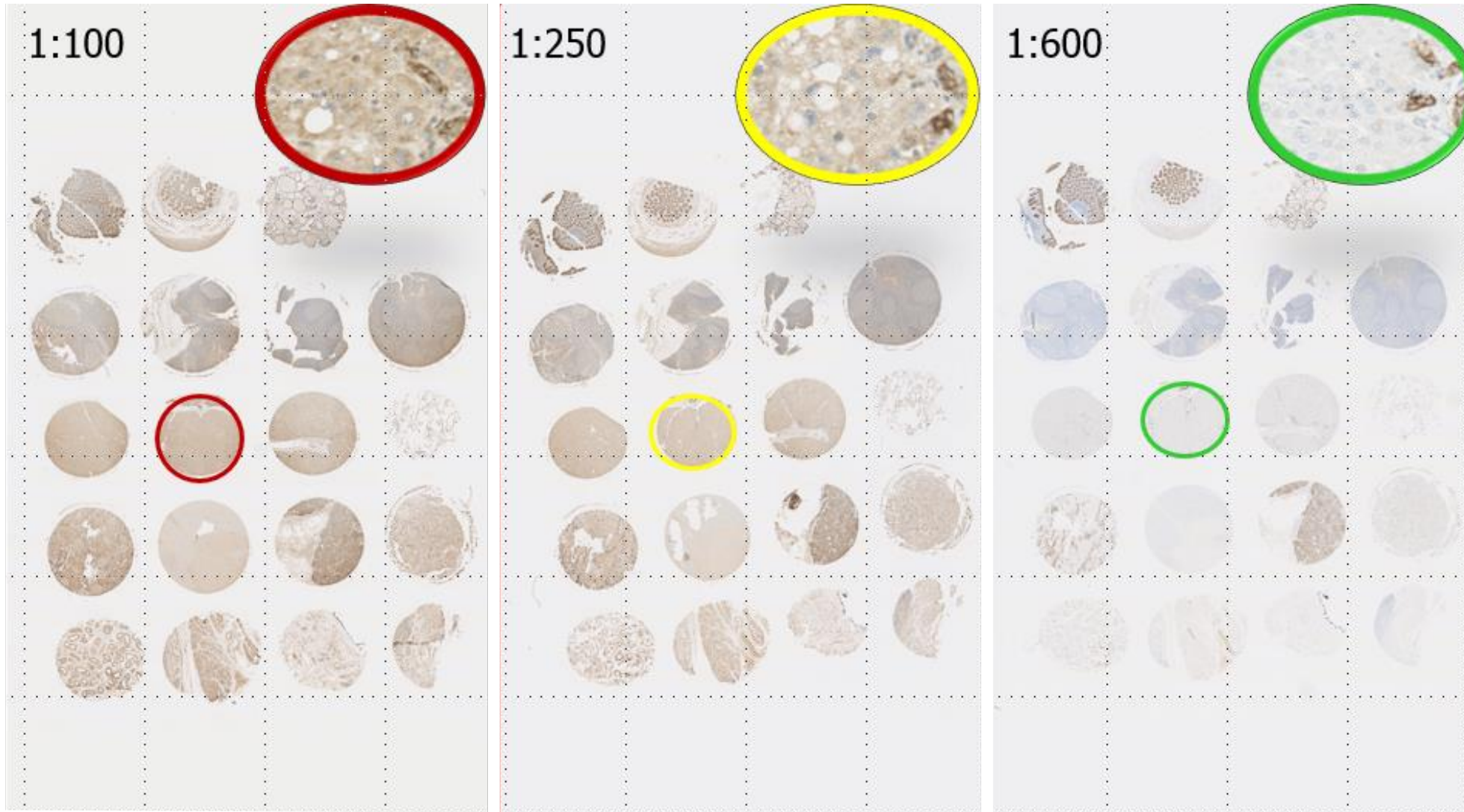
"Poor mans" specificity and pre-analytical TMAs



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

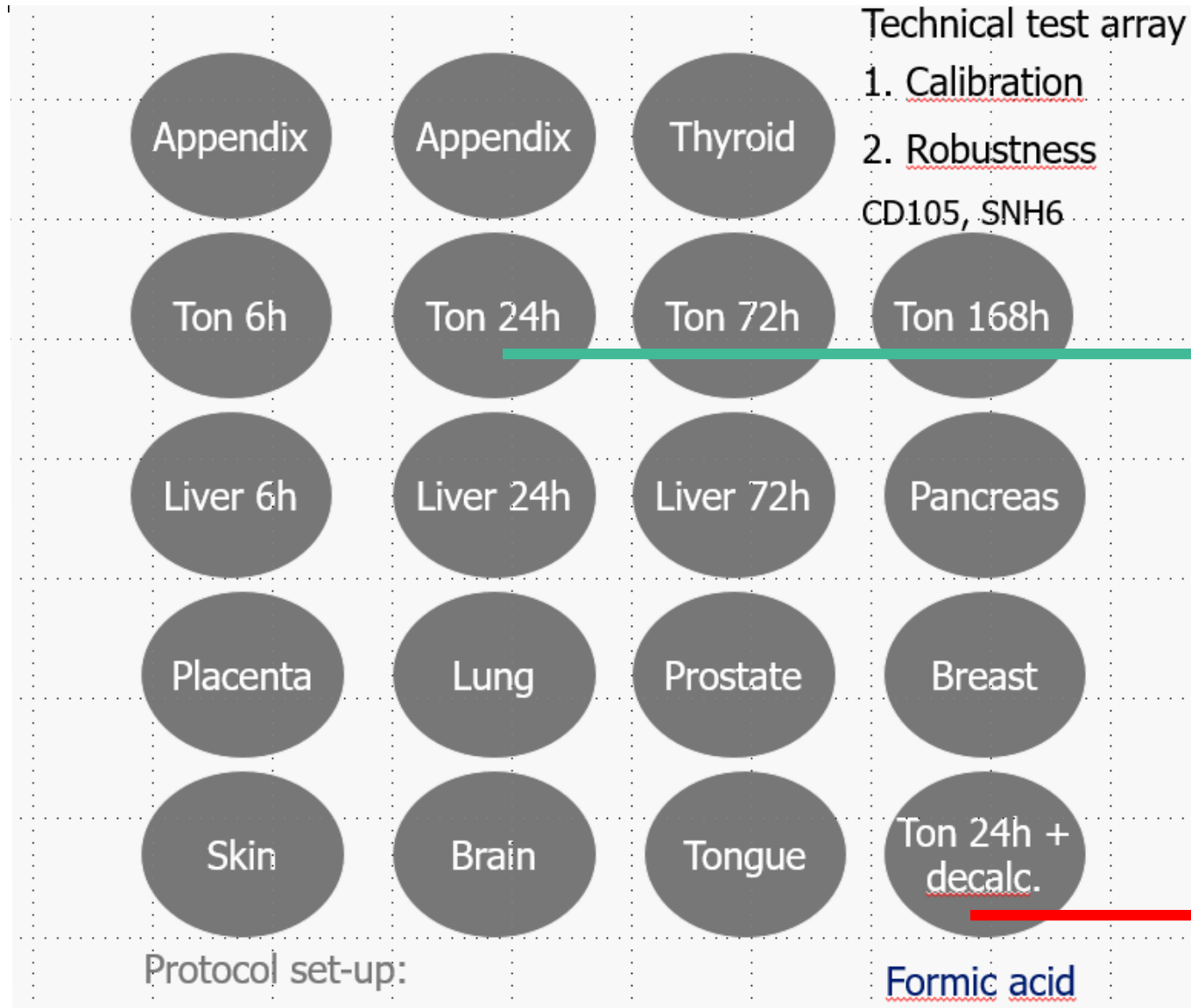


EPCAM calibration

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

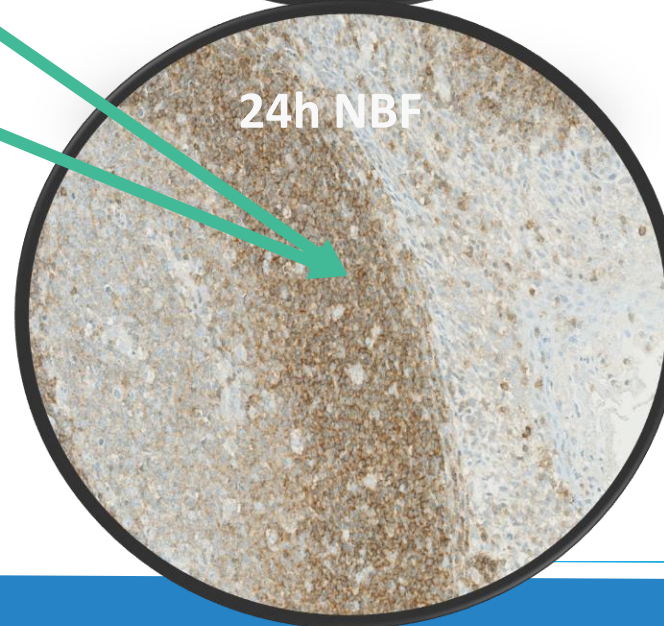
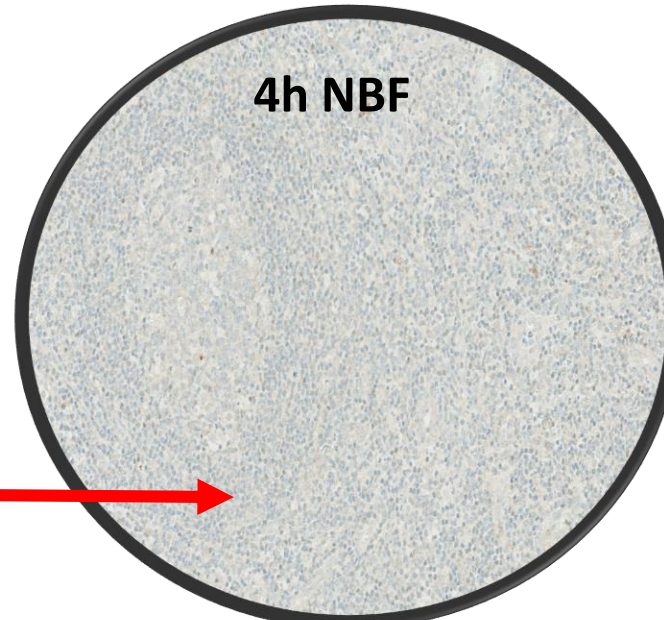
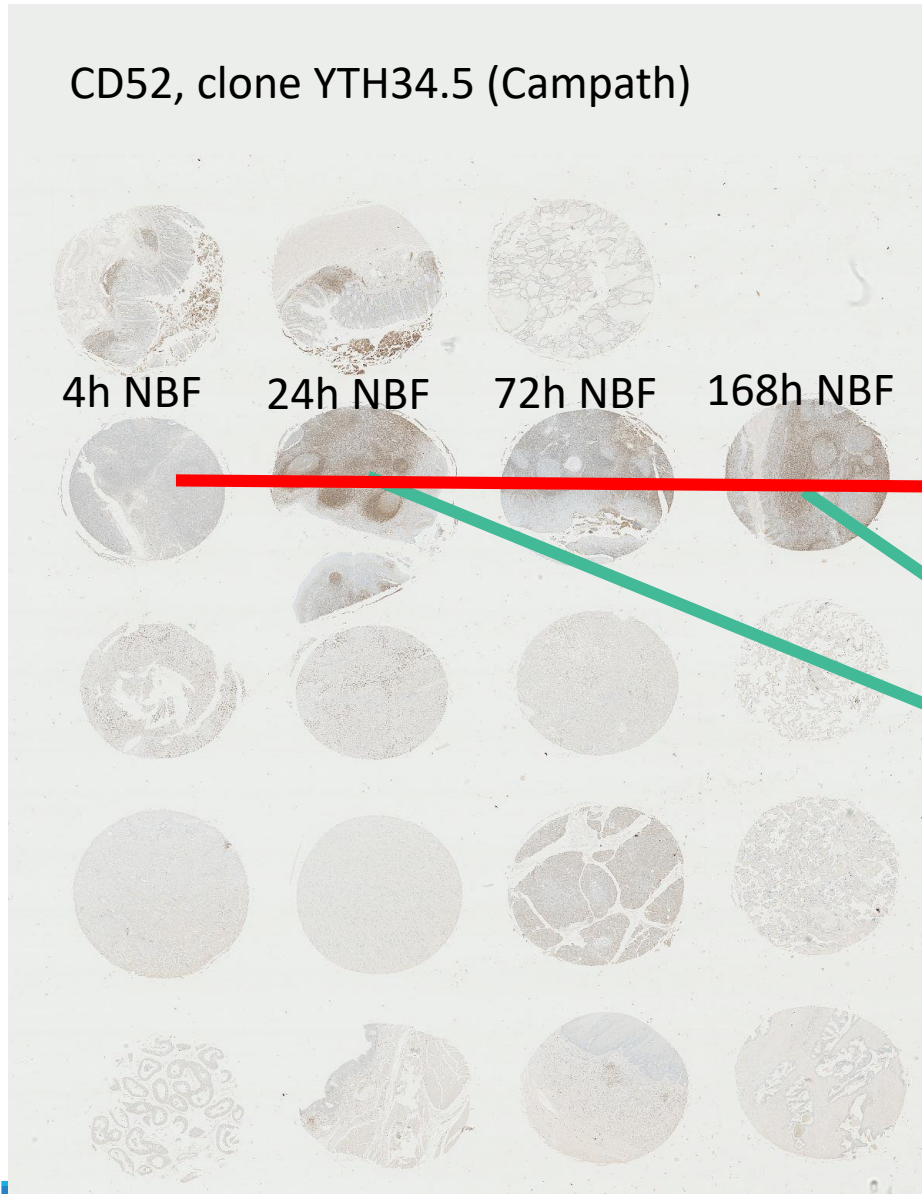


CD105 calibration

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

Source:
NordiQC and Aalborg
University Hospital

"Poor mans" specificity and pre-analytical TMAs



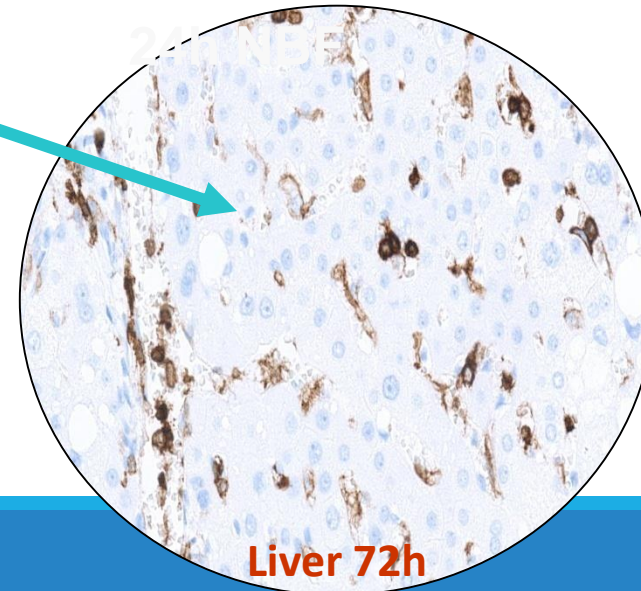
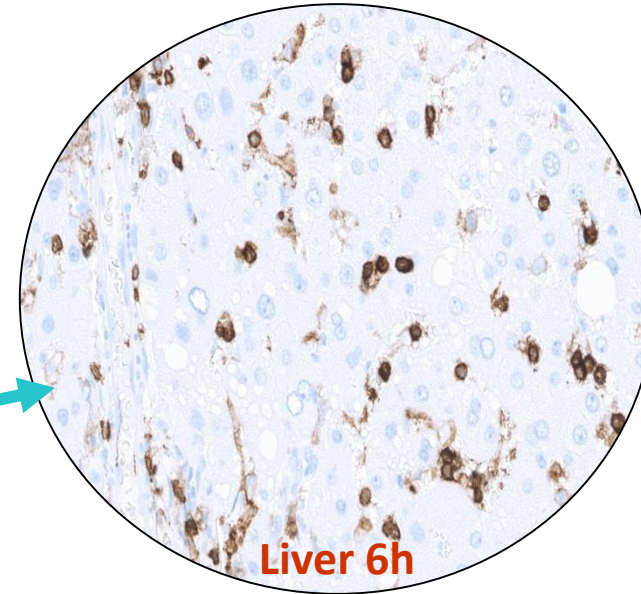
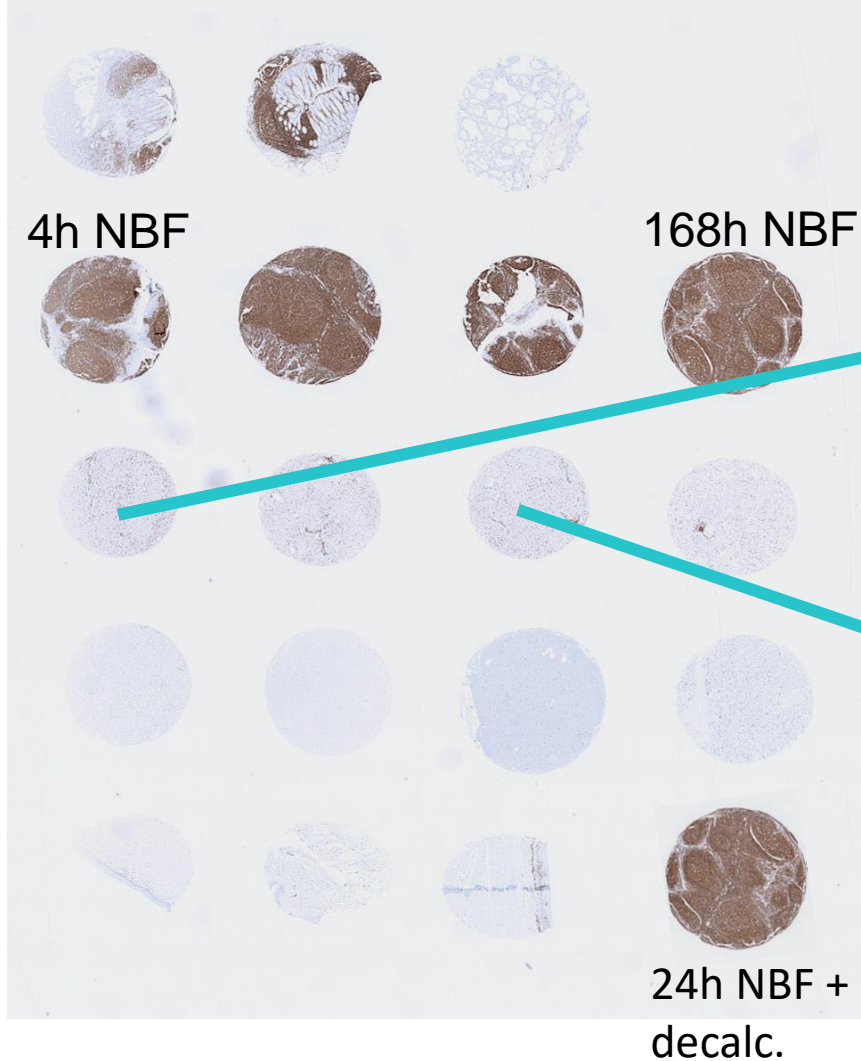
CD52 calibration

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?

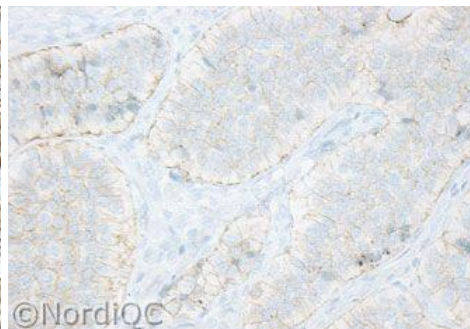
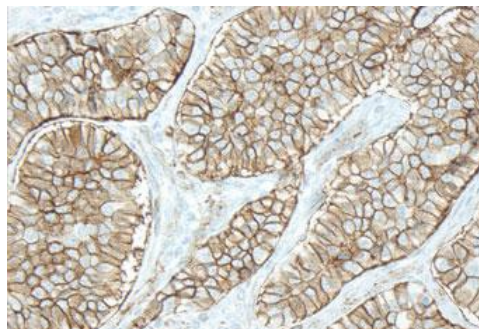
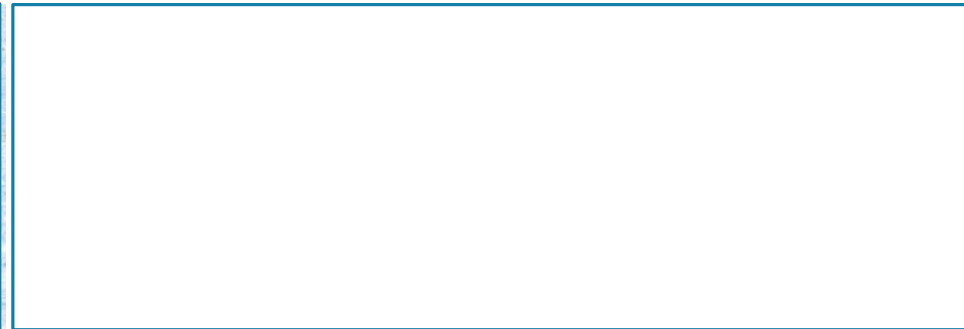
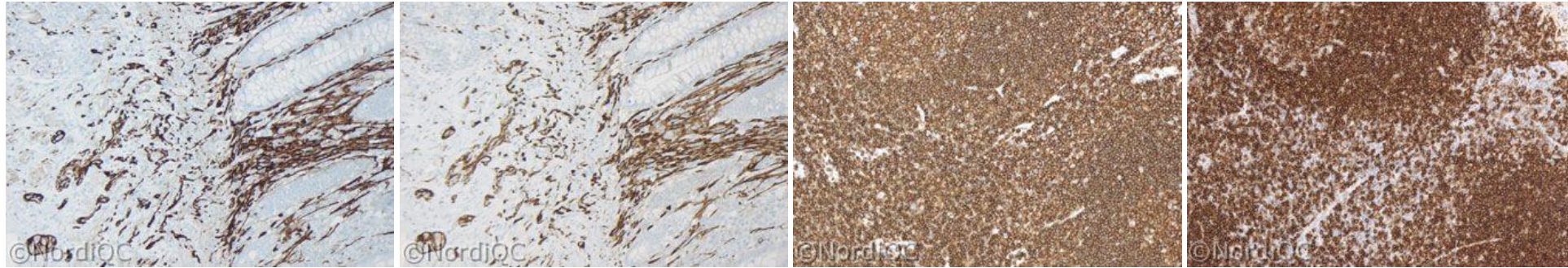
- Analytical sensitivity

- 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; relevant range of target analyte

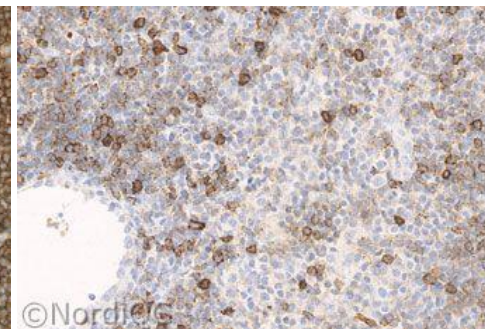
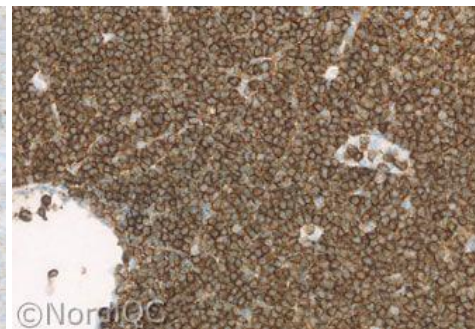


CD56

App – Tonsil – Neuroendocrine carc.

Protocol A

Protocol B



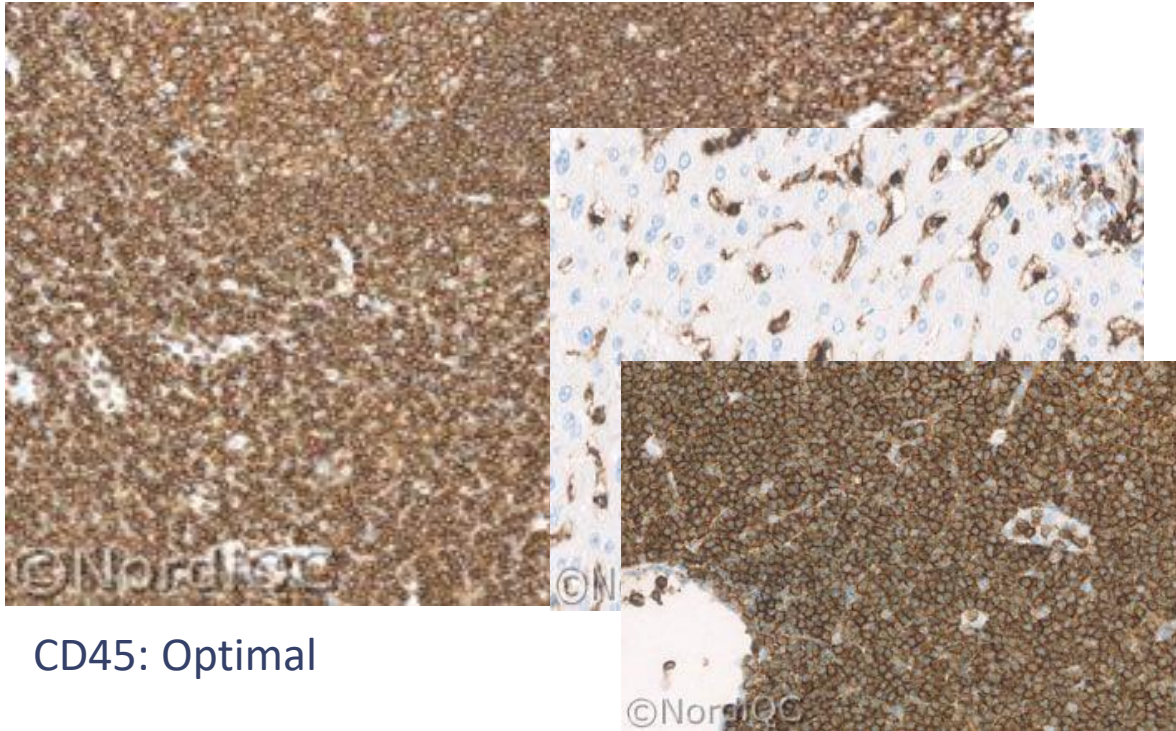
CD45

Tonsil – Liver – B-CLL.

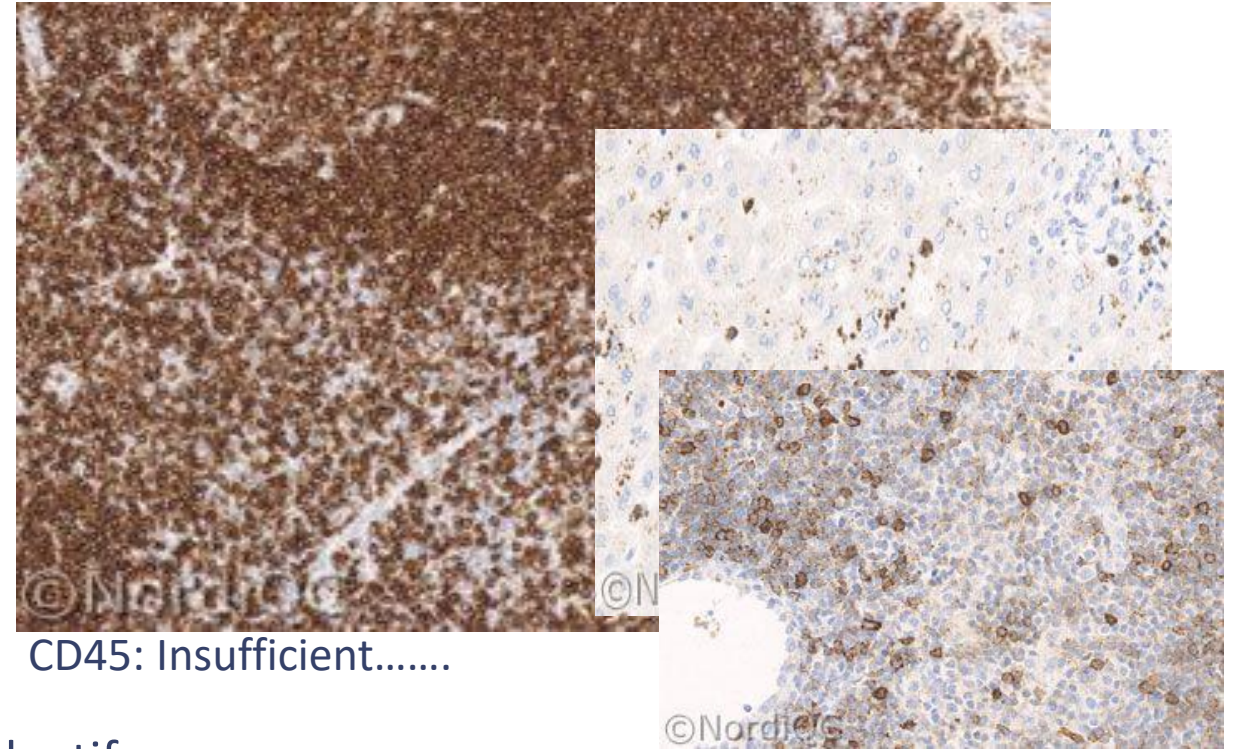
Protocol A

Protocol B

Fit For Purpose; relevant range of target analyte



CD45: Optimal



CD45: Insufficient.....

Tissues/cells with only high level 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 demonstrate the target antigen being expressed at different levels, the 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



iCAPCs – potential and use

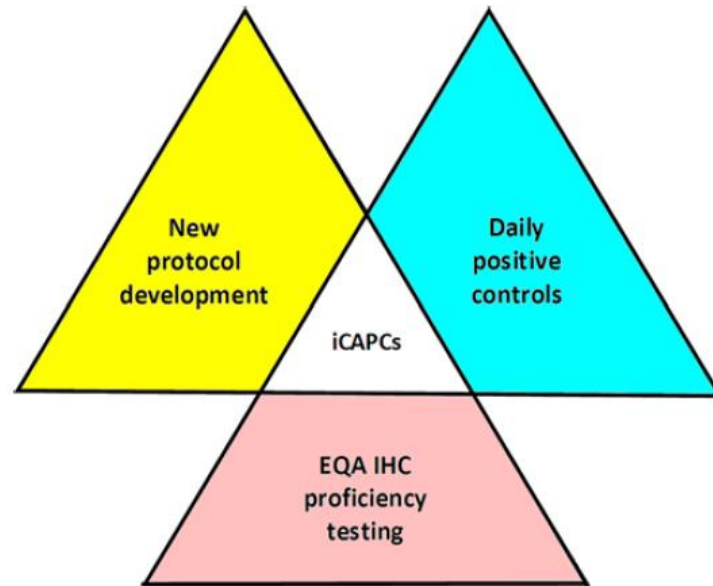


FIGURE 19. The roles of iCAPCs in clinical immunohistochemistry (IHC) laboratories. iCAPCs are an essential part of new protocol development, daily quality controls, and proficiency testing. EQA indicates External Quality Assurance; iCAPC, immunohistochemistry critical assay performance controls.

iCAPS to be used as central element for evaluation of quality;

Expected level – calibration

Analytical sensitivity and specificity

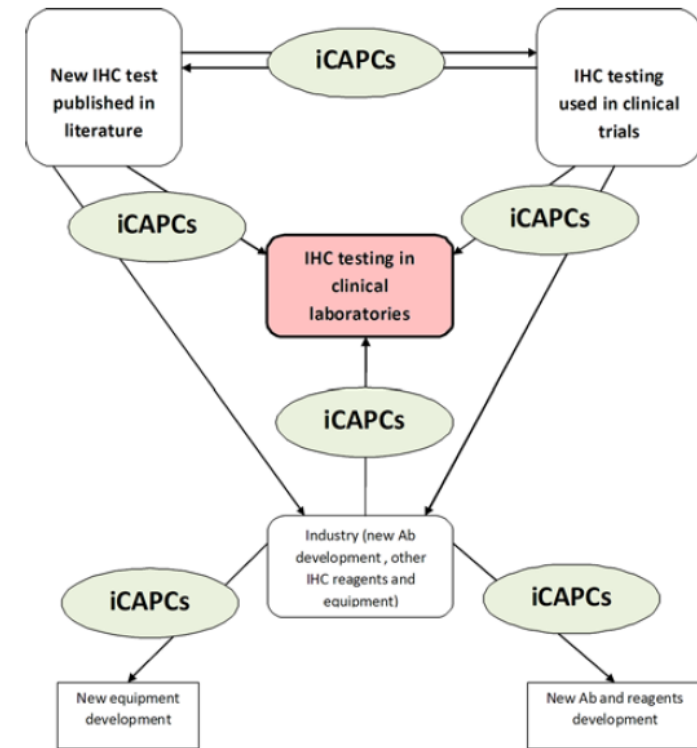


FIGURE 20. iCAPCs and Methodology Transfer. iCAPCs are proposed as important elements for harmonization of immunohistochemistry (IHC) testing between clinical research, product development, and clinical IHC testing. iCAPCs enable IHC harmonization of protocol transfer between research, industry, and clinical laboratories. iCAPC indicates immunohistochemistry critical assay performance controls.

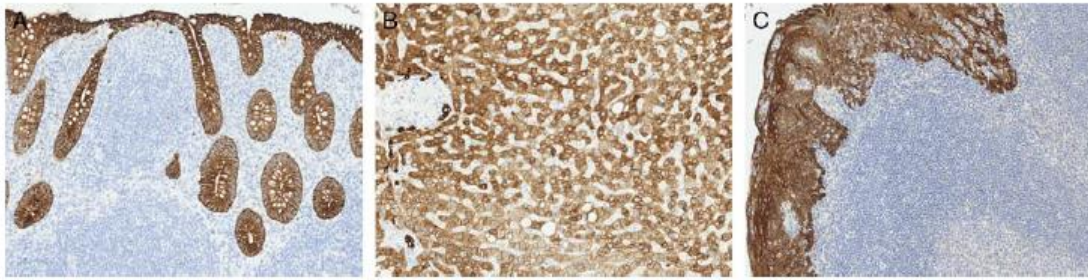


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining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

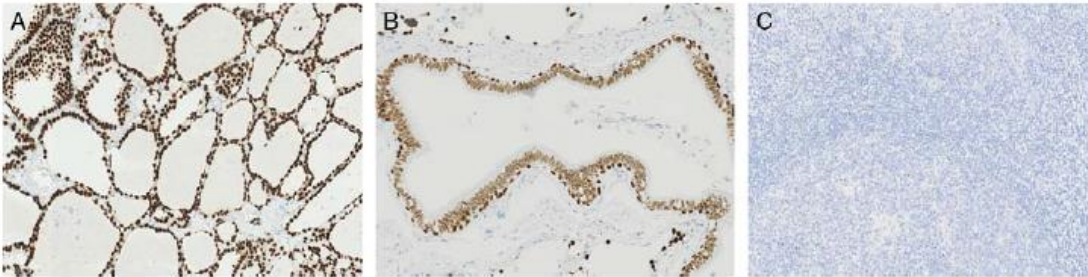


FIGURE 7. TTF-1 iCAPC. A, Thyroid: virtually all epithelial cells must show a strong nuclear staining reaction. B, Lung: virtually all pneumocytes and basal cells of terminal bronchi must show a moderate to strong nuclear staining reaction. Columnar epithelial cells of terminal bronchi must show an at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

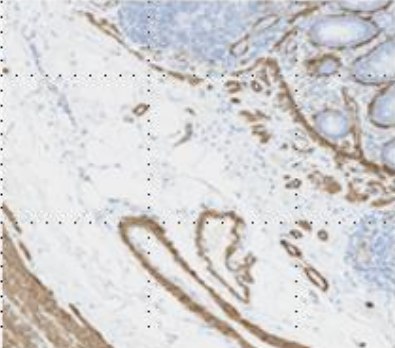
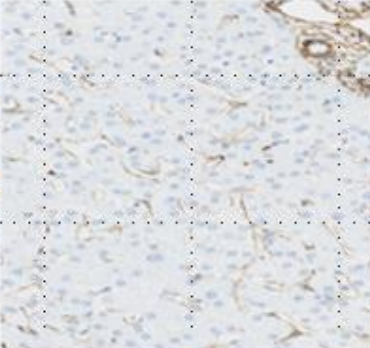
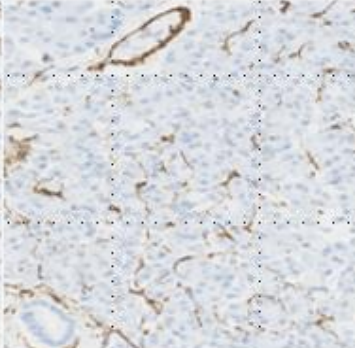
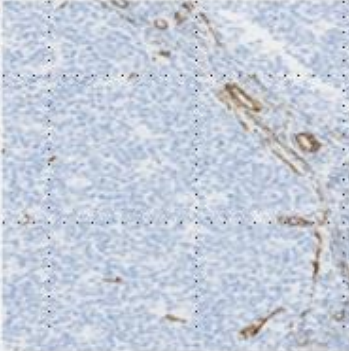
High expression
(Right antibody)

Low expression
(Appropriate sensitivity)

No expression
(Appropriate specificity)

Which tissue
Which cells
Which extension
Which intensity

	High express.	Low ex. (iCAPCs)	Non express.	Comment
CK-PAN	Appendix	Liver	Tonsil	
CK-LMW	Appendix	Liver	Tonsil	
CK-HMW	Tonsil	Pancreas	Liver	
CK7	Liver	Pancreas	Tonsil	
CK20	Appendix	Appendix	Tonsil	Different comp.
CD3	Tonsil	Appendix	Tonsil	
CD20	Tonsil	Appendix	Appendix	Different comp.
CD31	Tonsil	Liver	Appendix	
Vimentin	Appendix	Liver	Liver	Different comp.
Desmin	Appendix	Tonsil	Appendix	Different comp.
ASMA	Appendix	Liver	Appendix	Different comp.
SYP	Appendix	Appendix	Tonsil	Different comp.
CGA	Appendix	Appendix	Tonsil	Different comp.
TTF1	Thyroid	Lung	Tonsil	
CDX2	Appendix	Pancreas	Tonsil	
S100	Appendix	Tonsil	Appendix	Different comp.
Ki67	Tonsi ¹	Tonsil	Tonsil	Different comp.

ASMA (C)	Appendix	Liver	Pancreas	Tonsil
High expression (right ab)	A moderate to strong staining reaction in virtually all smooth muscle cells in muscularis mucosae	A moderate to strong staining reaction in the smooth muscle cells in vessels	A moderate to strong staining reaction in the smooth muscle cells in vessels	A moderate to strong staining reaction in the smooth muscle cells in vessels
Low expression iCAPCs (right sens.)	-	An at least weak to moderate, staining reaction of the majority of the perisinusoidal cells	-	-
Non expression (right spec.)	No staining reaction in the epithelial cells	No staining in the hepatocytes (except lipofuscin)	No staining reaction in the epithelial cells	No staining reaction in lymphocytes
				

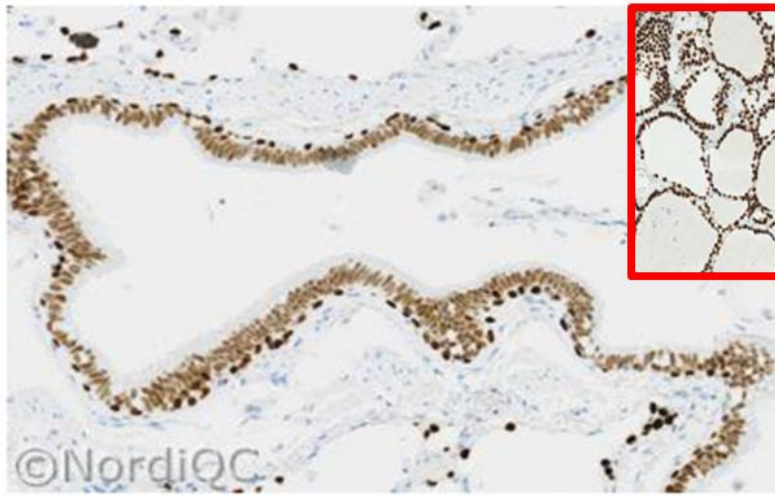


Fig. 2a
Optimal TTF1 staining of the lung using same protocol as in Fig. 1a. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a moderate nuclear staining reaction. No background staining is

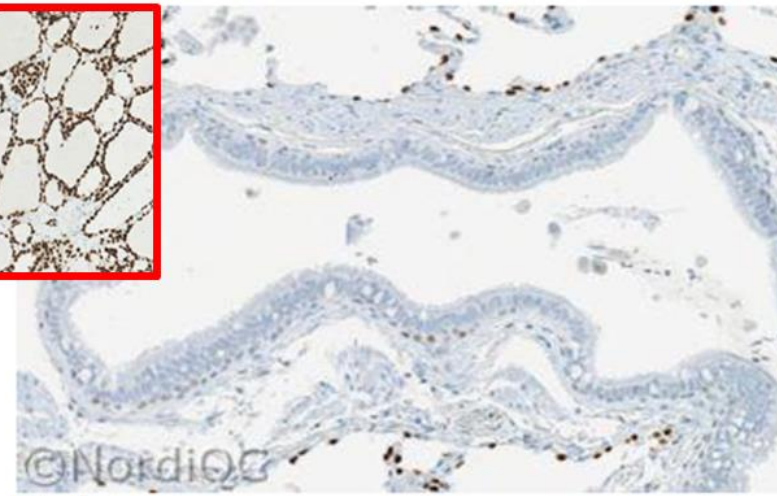


Fig. 2b.
Insufficient TTF1 staining of the lung using same protocol as in Fig. 1b. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show only a weak to moderate positive nuclear staining reaction and no reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

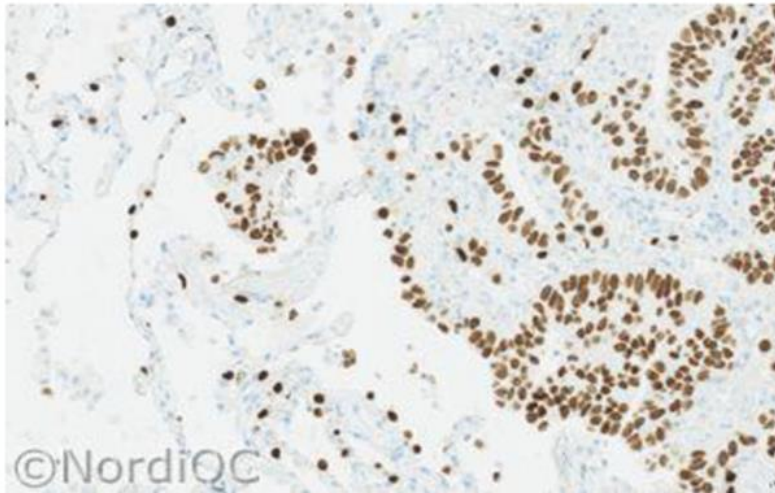


Fig. 4a
Optimal TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1a, 2a & 3a. Tumour (right side) with adjacent normal lung tissue. Virtually all the neoplastic cells

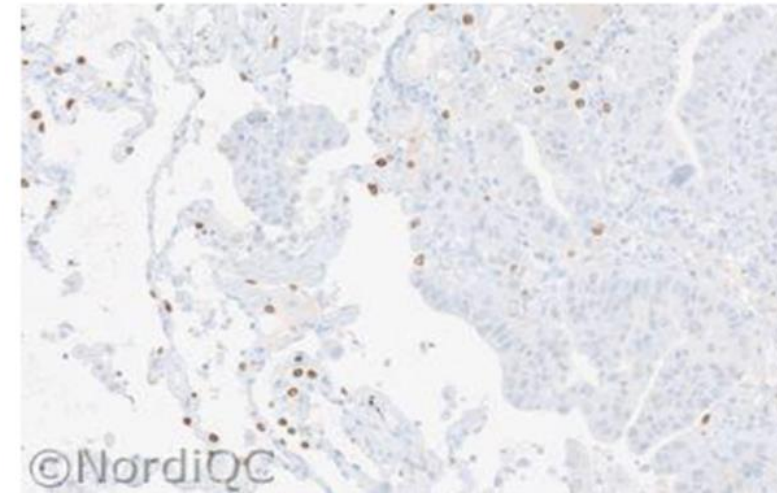


Fig. 4b
Insufficient TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1b, 2b & 3b. Despite a moderate positive staining reaction in the majority of type II

TTF1

iCAPCs:

Thyroid + lung

Epithelial cells
lining terminal
bronchi

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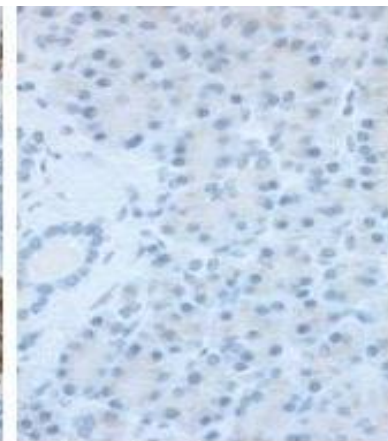
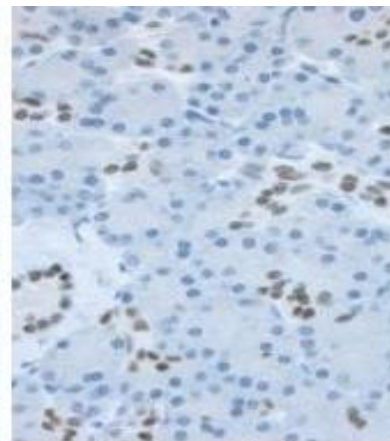
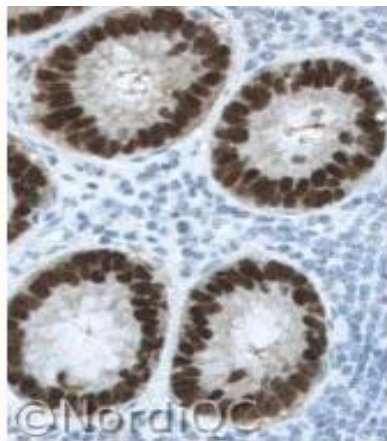


Fig. 1a. Optimal staining for CDX2 using the mAb clone CDX2-88.

Left, colon: A strong nuclear staining is seen in all the enterocytes with a minimal cytoplasmic reaction.

Right, pancreas: A weak to moderate staining is seen in the majority of the ductal epithelial cells.

Fig. 1b. Staining for CDX2 using the mAb clone CDX2-88 with an insufficient protocol.

Left, colon: A moderate to strong nuclear staining is seen in all the enterocytes.

Right, pancreas: No nuclear staining is seen in the ductal epithelial cells. Also compare with Fig 2b – same protocol.

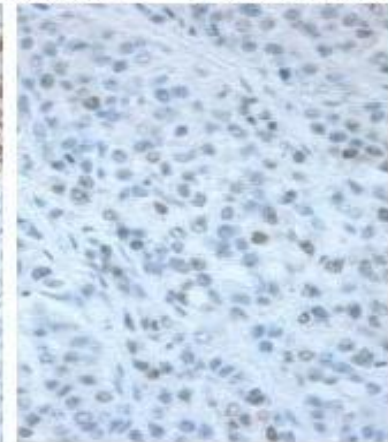
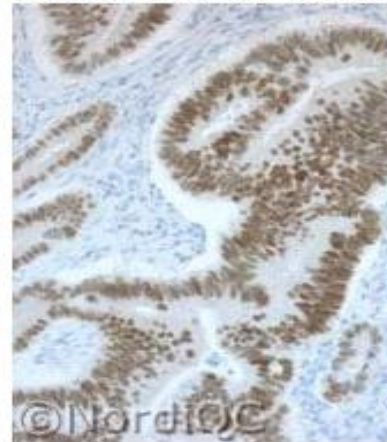
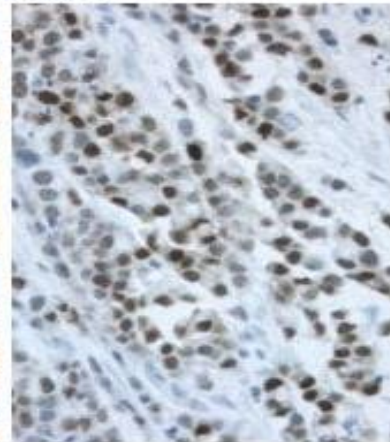
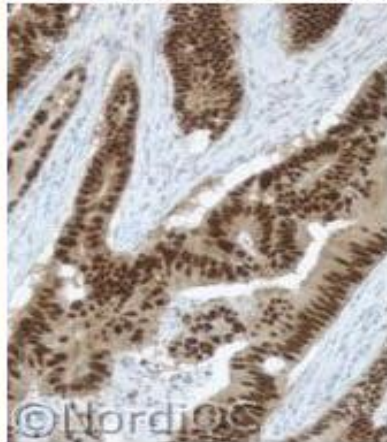


Fig. 2a. Optimal staining for CDX2 using same protocol as in Fig. 1a.

Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show an intense staining while the cytoplasmic compartment is weakly stained.

Right: Colon adenocarcinoma with low expression of CDX2: The majority of the neoplastic cells show a moderate to strong nuclear reaction.

Fig. 2b. Insufficient staining for CDX-2 using same protocol as in Fig. 1b.

Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show a moderate staining, while the cytoplasmic compartment is almost negative.

Right: Colon adenocarcinoma with low expression of CDX2: Only scattered neoplastic cells show a weak nuclear reaction.

CDX2

iCAPCs:

Colon + pancreas

Pancreatic duct
ep. cells

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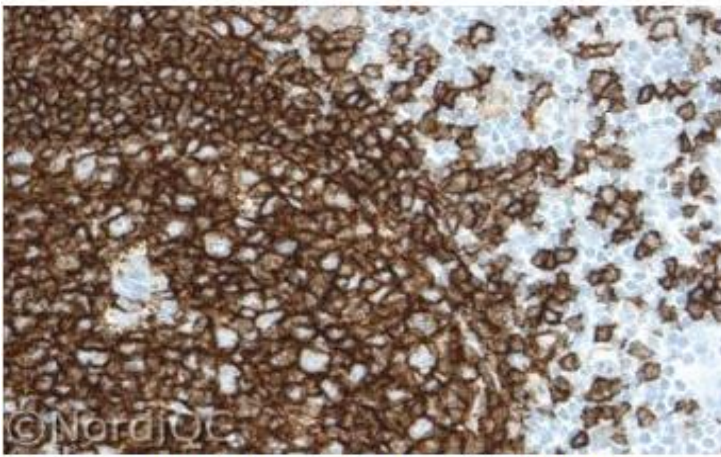


Fig. 1a. Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. HIER was performed using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the B-cells.

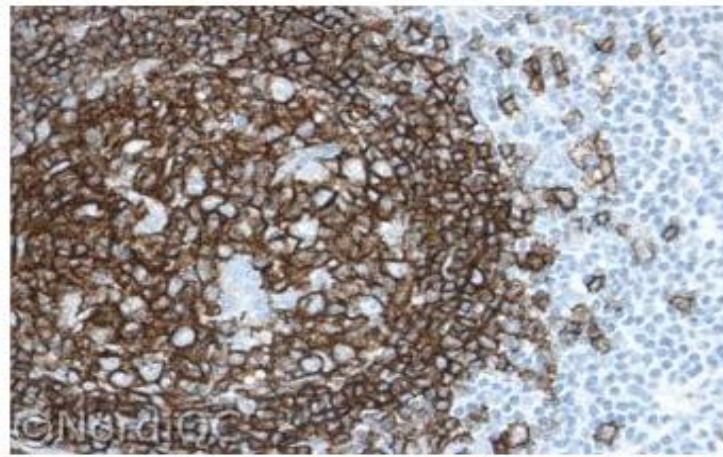


Fig. 1b. Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. No HIER was performed. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors of CD20, hence the relatively strong reaction. Even so, the staining intensity should be improved in order to detect low expressors of CD20 (e.g. B-CLL in Fig. 2a and 2b).

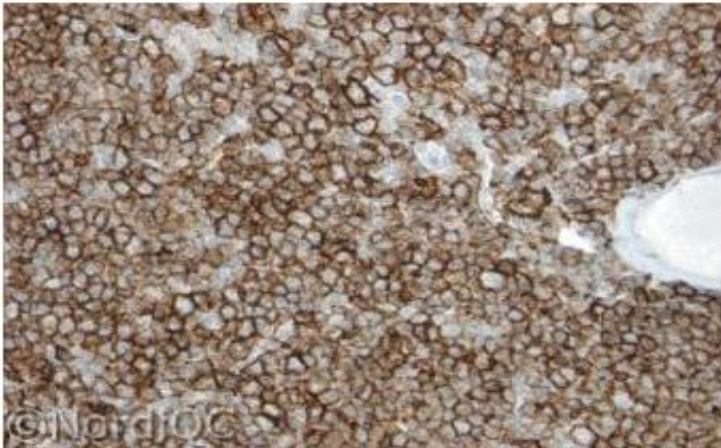


Fig. 2a. B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

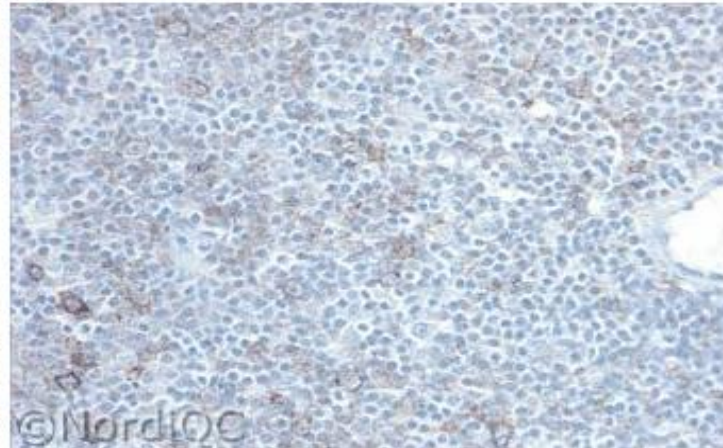


Fig. 2b. B-CLL. Insufficient staining for CD20 using the same protocol as in Fig. 1b. Omitting HIER, only scattered cells are positive. The majority of the neoplastic cells are negative. Compare with the optimal result in Fig. 2a, same field.

CD20:

iCAPCs: ????

Tonsil;

B-cells to be
ASAP....

As strong as
possible...

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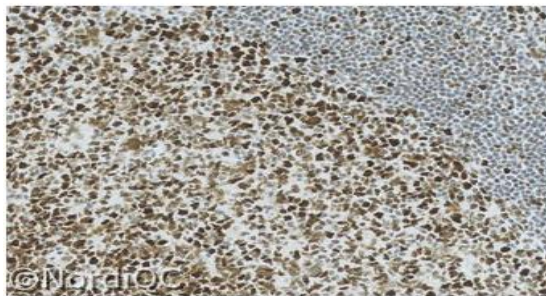


Fig. 1a. Optimal staining for MSH6 of the tonsil using the mAb clone EP49 optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all the mantle zone B-cells show a distinct, moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining.

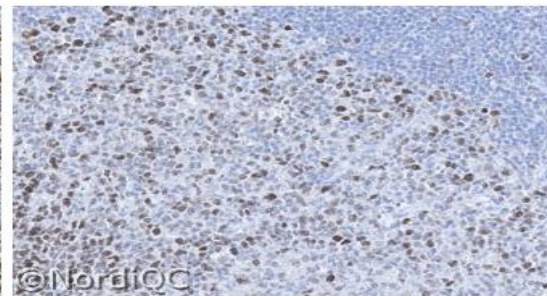


Fig. 1b. Insufficient staining for MSH6 of the tonsil using the mAb clone 44. by a protocol with a too low sensitivity (2-step polymer and too low. conc. of the primary Ab), same field as in Fig. 1a. Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing limited MSH6 are virtually unstained. Also compare with Figs. 2b. & 3b., same protocol.

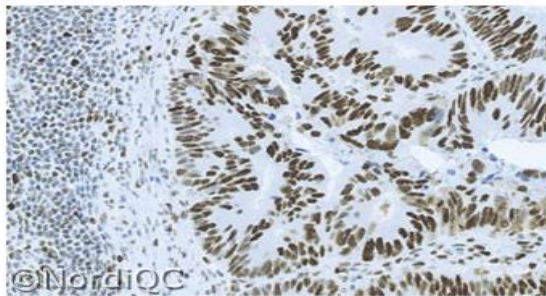


Fig. 2a. Optimal staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1a. The majority of the epithelial and the stromal cells show a moderate to strong nuclear staining. No background staining is seen.

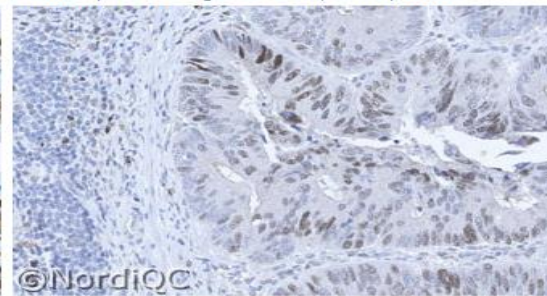


Fig. 2b. Insufficient staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1b., same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction is significantly reduced compared to the result in Fig. 2a. Also compare with Fig. 3b., same protocol.

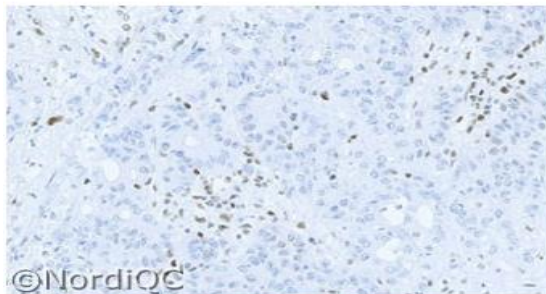


Fig. 3a. Optimal staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1a. & 2a. The neoplastic cells are negative, while the remnants of entrapped lymphocytes and stromal cells show a distinct nuclear staining, serving as internal positive control.

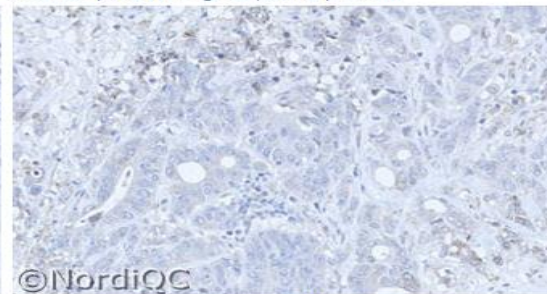


Fig. 3b. Insufficient staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1b. & 2b., same field as in Fig. 3a. No nuclear staining reaction is seen in the neoplastic cells, but as virtually no nuclear staining reaction is seen in the normal cells as stromal cells, the staining pattern can not reliably be interpreted. Also note the weak cytoplasmic staining complicating the interpretation.

MMR:

iCAPCs:

External tissue control

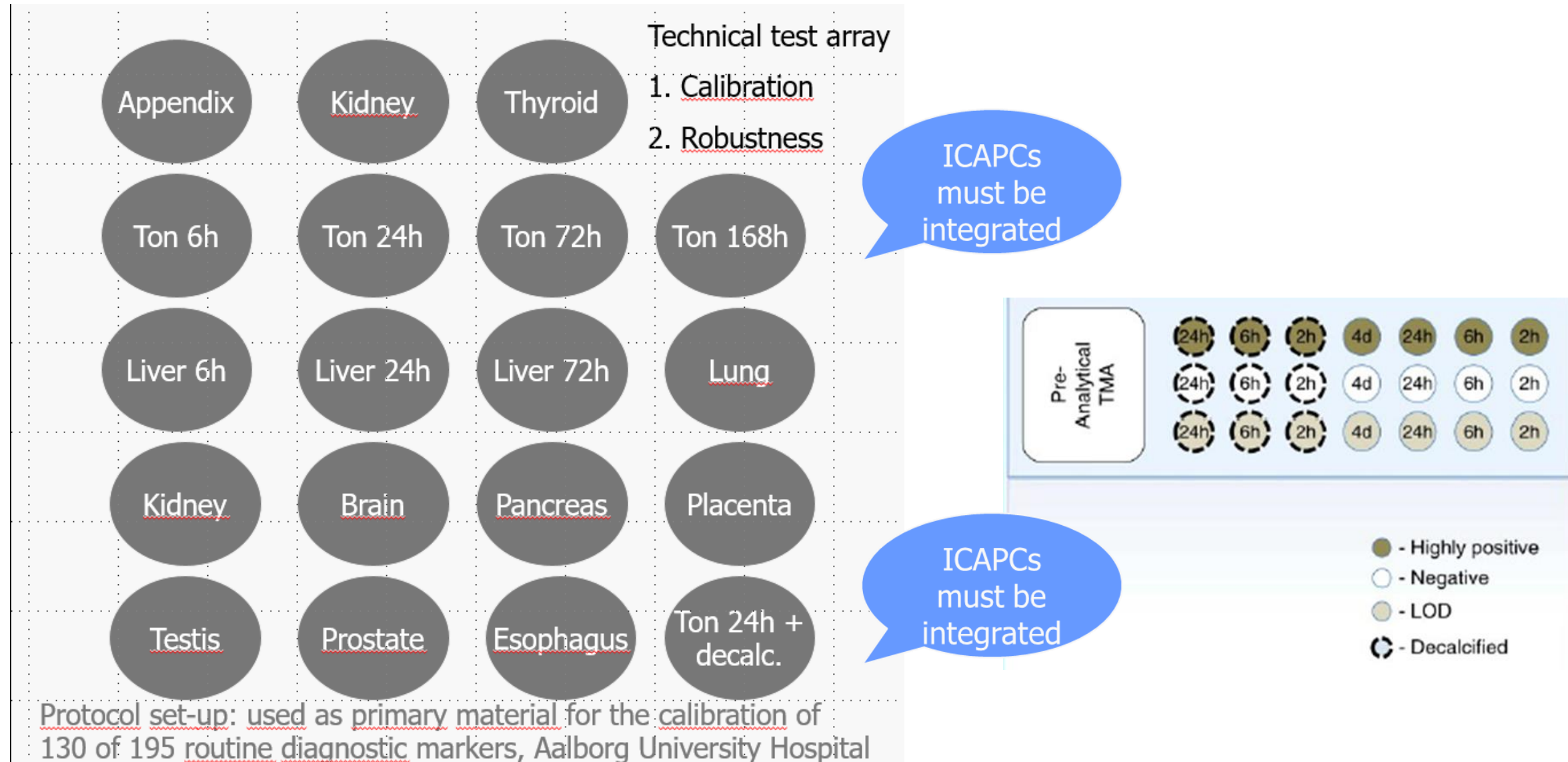
Mantle zone B-cells in tonsil

Assay run-to-run consistency

Internal tissue control

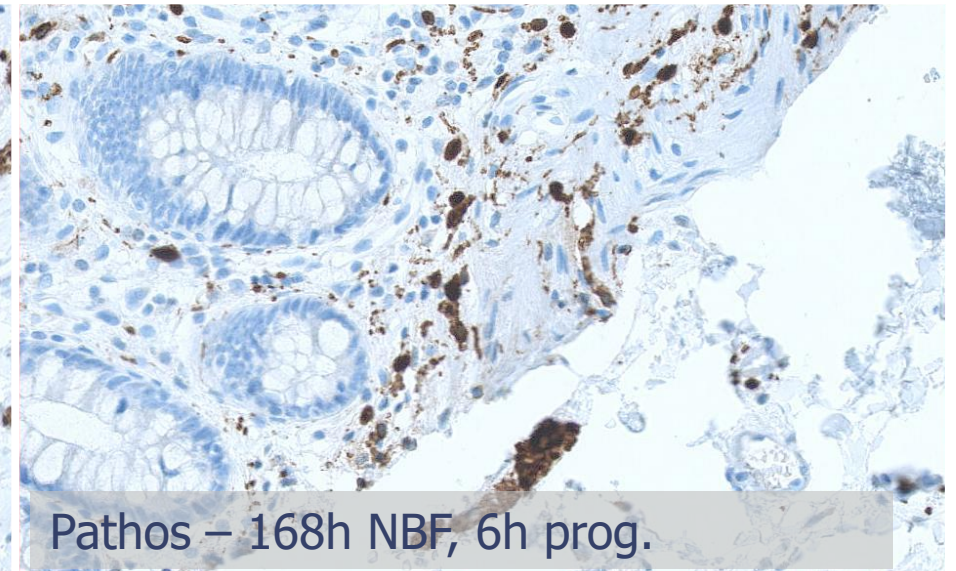
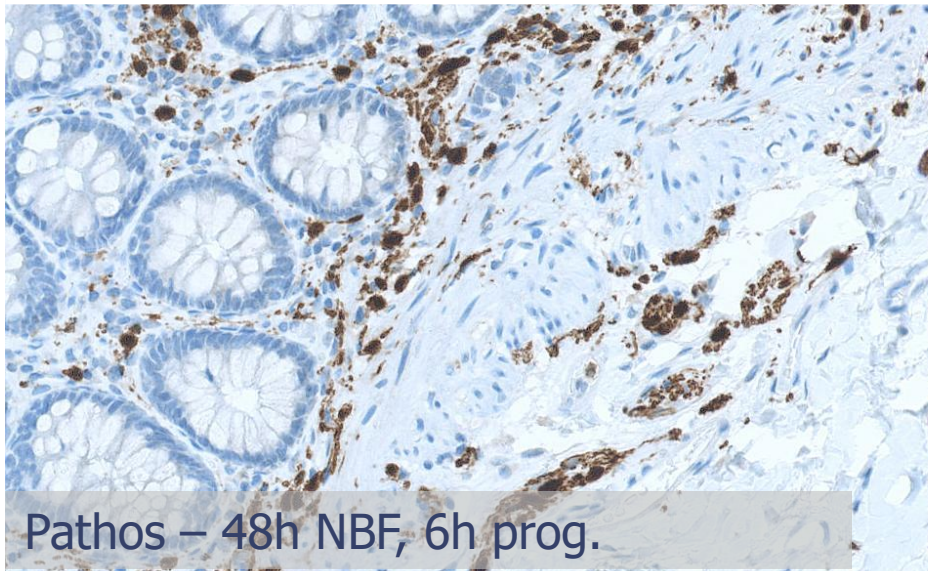
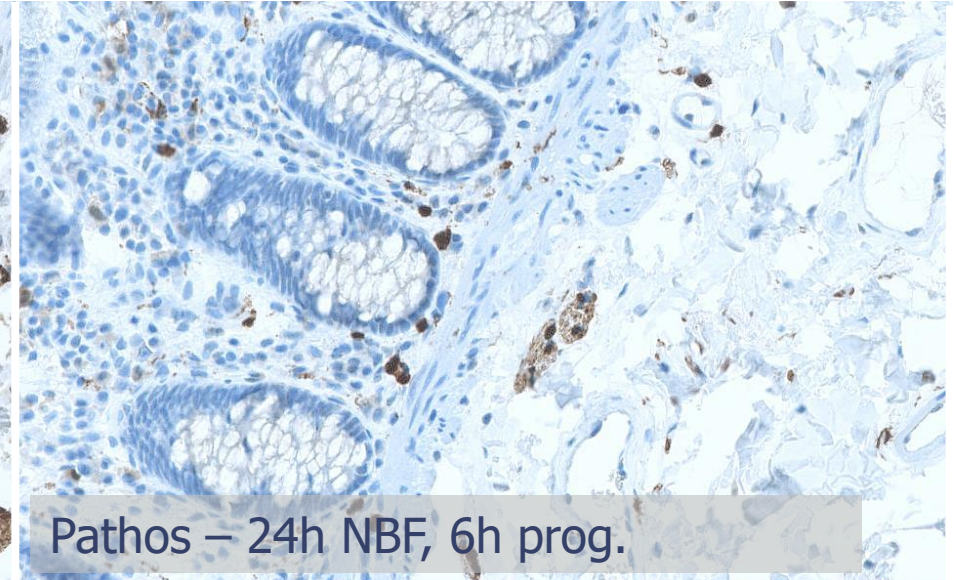
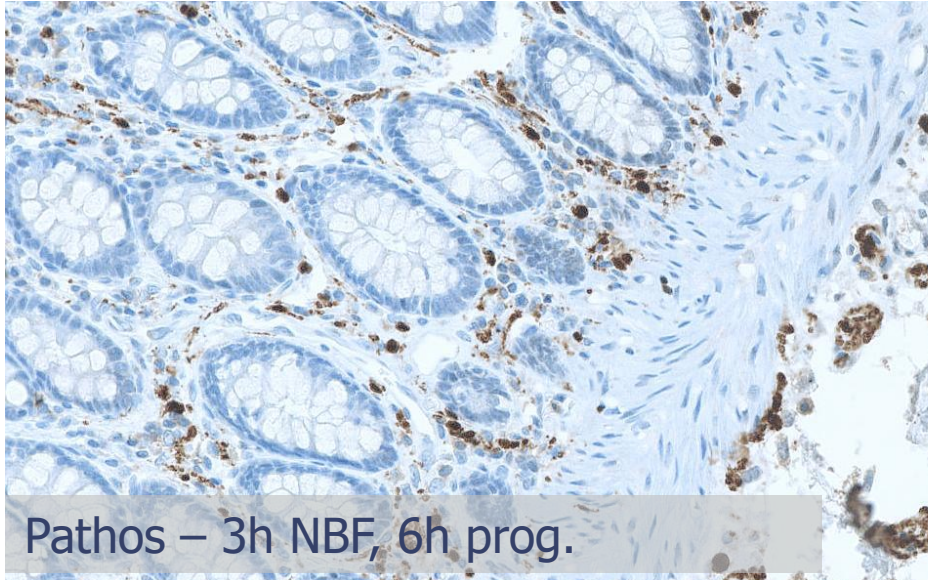
Stromal cells!!

"Poor mans" specificity and pre-analytical TMAs



“Poor mans” specificity and pre-analytical TMAs

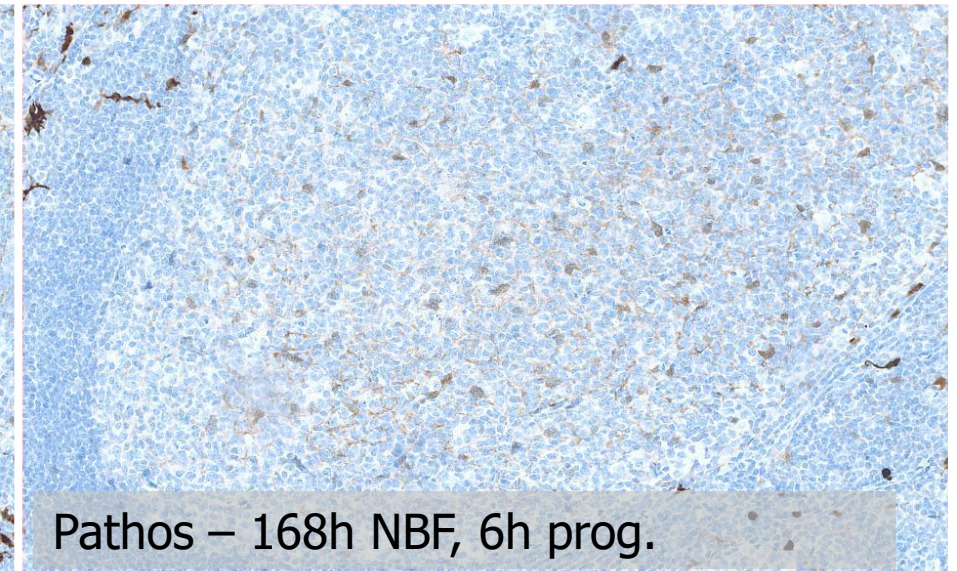
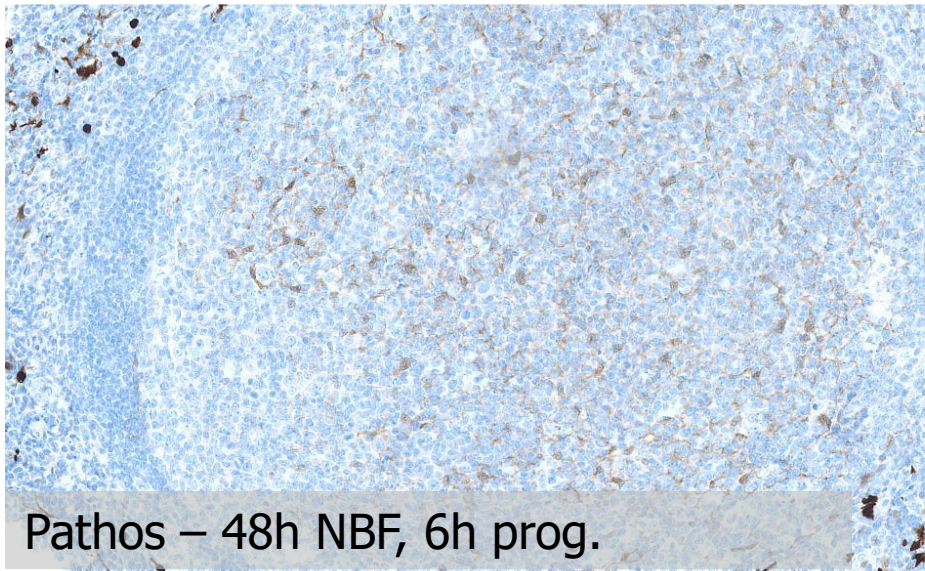
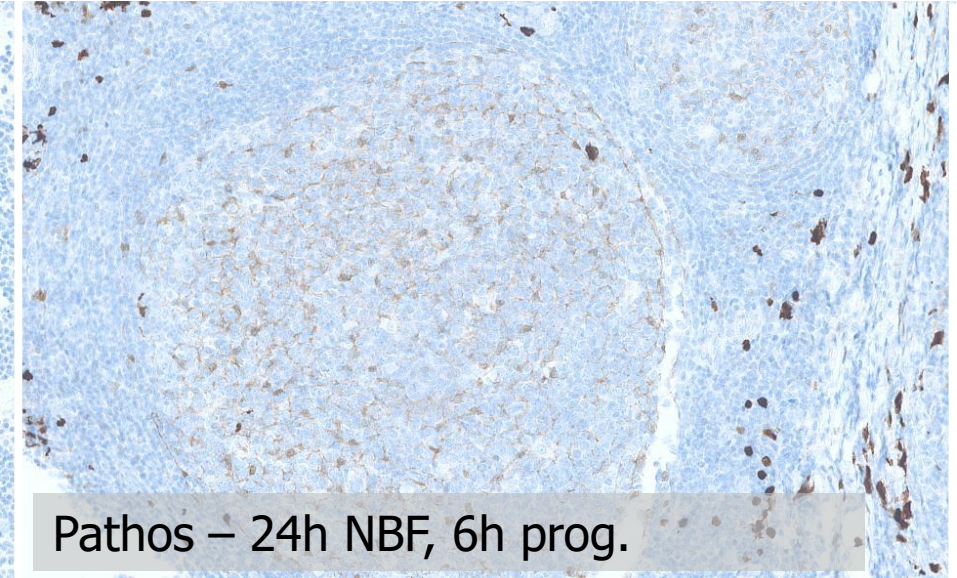
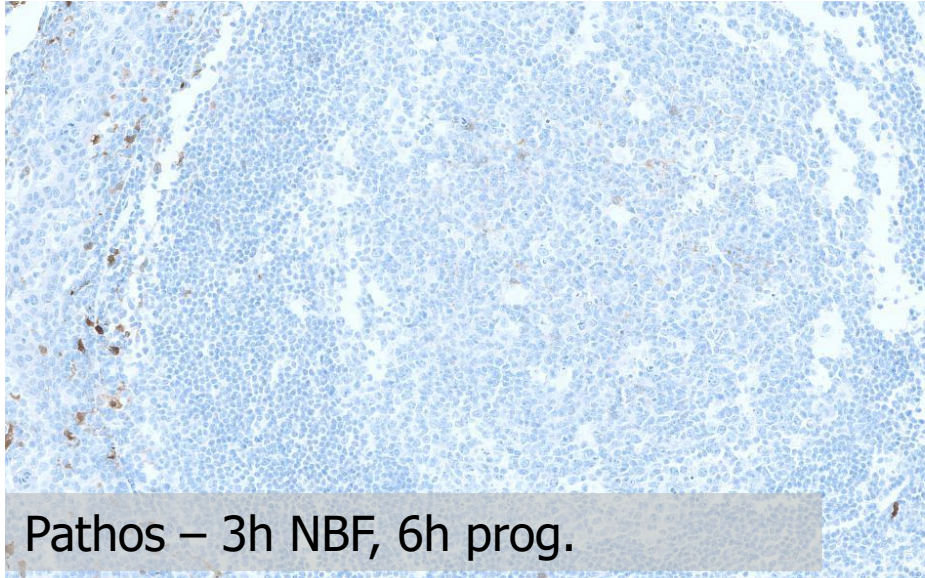
Colon:
S100, polyclonal



“Poor mans” specificity and pre-analytical TMAs

ICAPCs

Tonsil:
S100, polyclonal



Main elements to develop & validate IHC assays



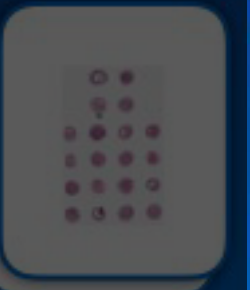



The journey from an antibody to a diagnostic IHC assay with a specific purpose

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
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

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.

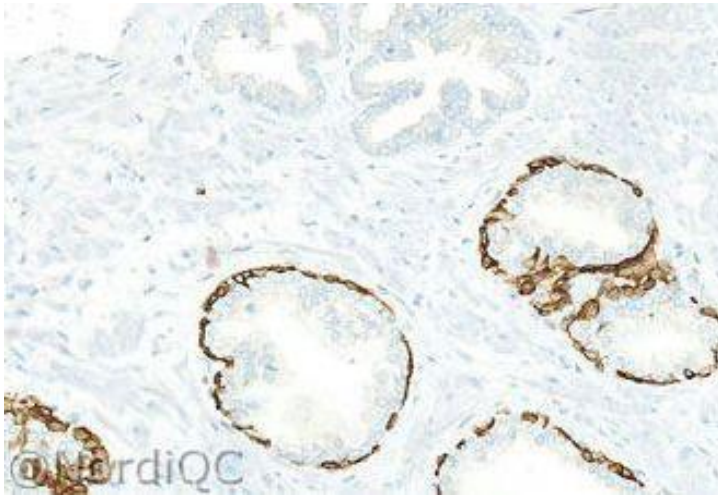
Number perhaps less important compared to use of tissue with full range of expression patterns reflecting the diagnostic use and purpose of test

Identification of purpose of the test

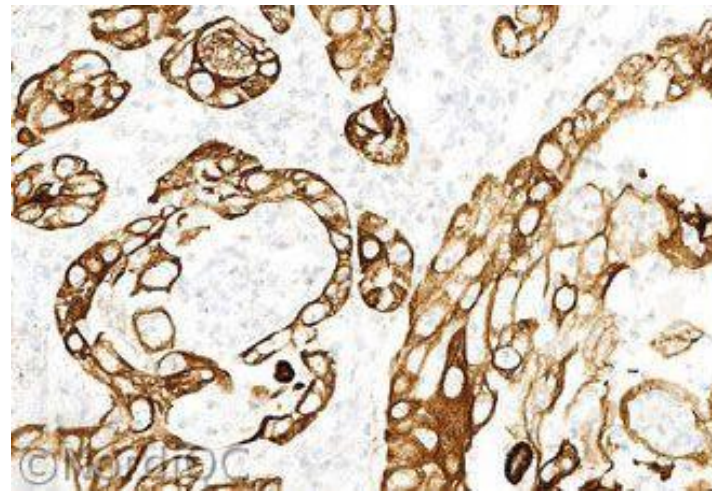
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

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

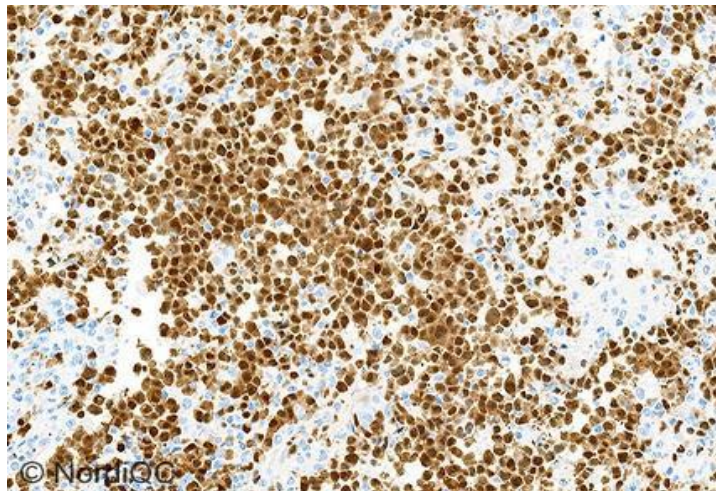
(source; www.nordiqc.org)

Identification of purpose of the test

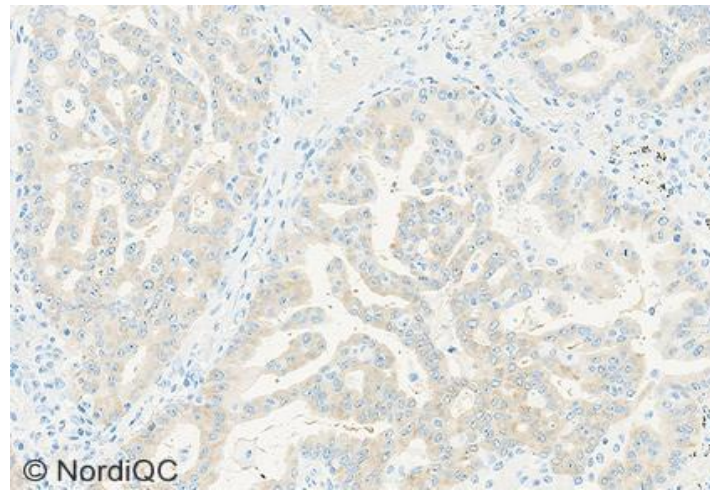
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

IHC for ALK

1. To identify anaplastic large cell lymphoma
2. To identify lung adenocarcinoma with ALK mutation
3.



ALCL



Lung ad. carc + EML-ALK mutation

Same protocol applied for different purposes **not** 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

CK5 for prostate Dx

Purpose 1 / Test 1

Protocol 1

CK5 for carcinoma subtyping

Purpose 2 / Test 2

Protocol 1



Match of
1 Protocol for 2 Purposes

ALK for ALCL

Purpose 1 / Test 1

Protocol 1

ALK for Critonib/RX in lung DX

Purpose 2 / Test 2

Protocol 1



Mis-match of
1 Protocol for 2 Purposes

Purpose 1 / Test 1

Protocol 1

Purpose 2 / Test 2

Protocol 2



Match of
2 Protocols for 2 Purposes

Identification of purpose of the test

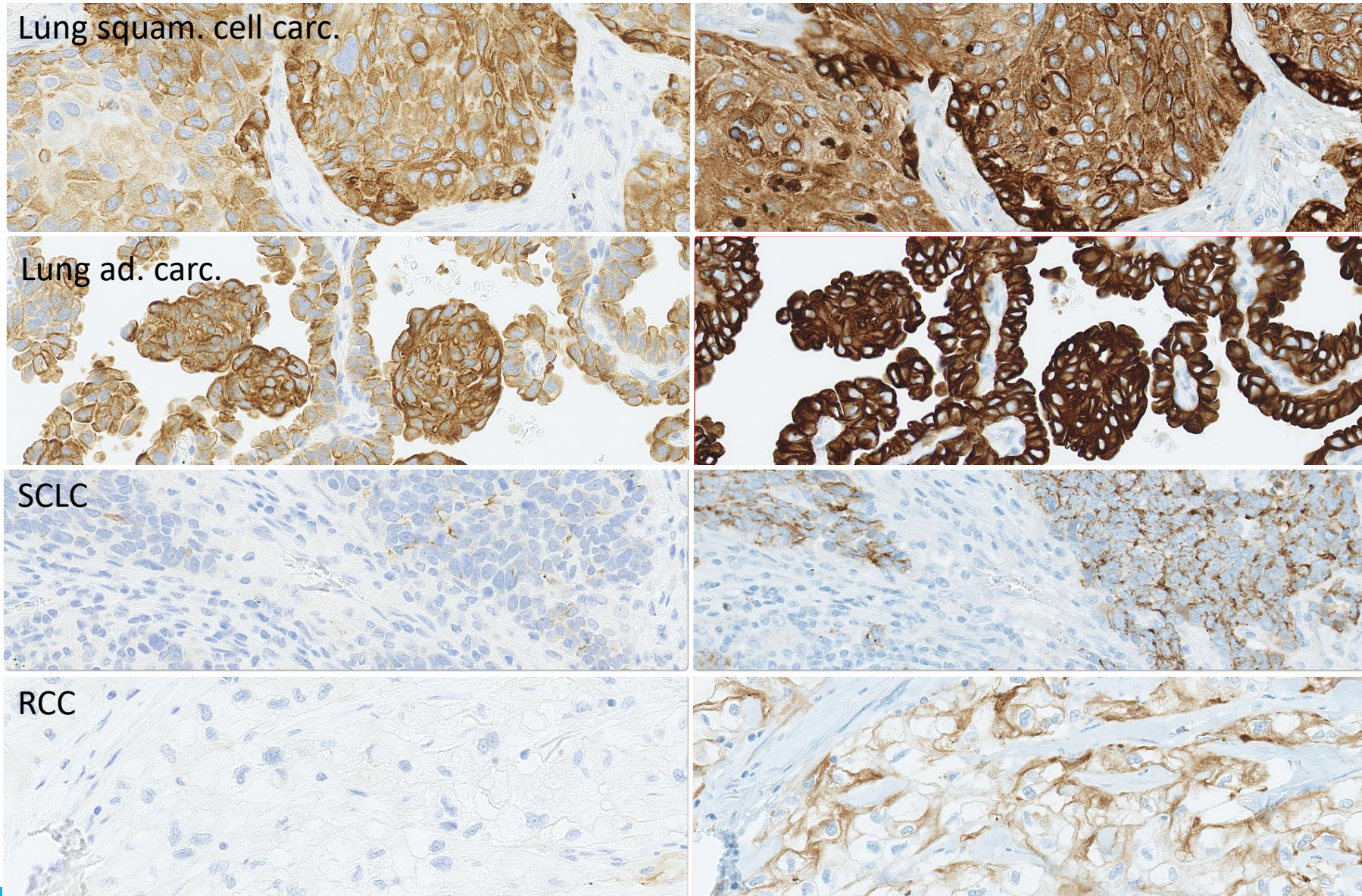
An IHC assay can have one or more purposes and it is crucial to secure the need is fulfilled

	Purpose I (HE)	Purpose II (LE)	Comments
CD34	Dermatofibrosarcoma protuberans	Stem cells / leukemia	Different pre-anal
CD56	Neuroendocrine differentiation	Lymphoma classification	
CD117	GIST	Stem cells / leukemia	Different pre-anal
GATA3	Breast carcinoma – CUP*	Urothelial carcinoma - CUP	
IgK / IgL	Clonality myeloma (Cytopl)	Clonality lymphoma (Membrane)	
Melan A	Melanoma	Sex cord tumours	(mAb A103)
PAX5	B-cell lineage marker (Lymphoma)	Hodgkin	
.....			

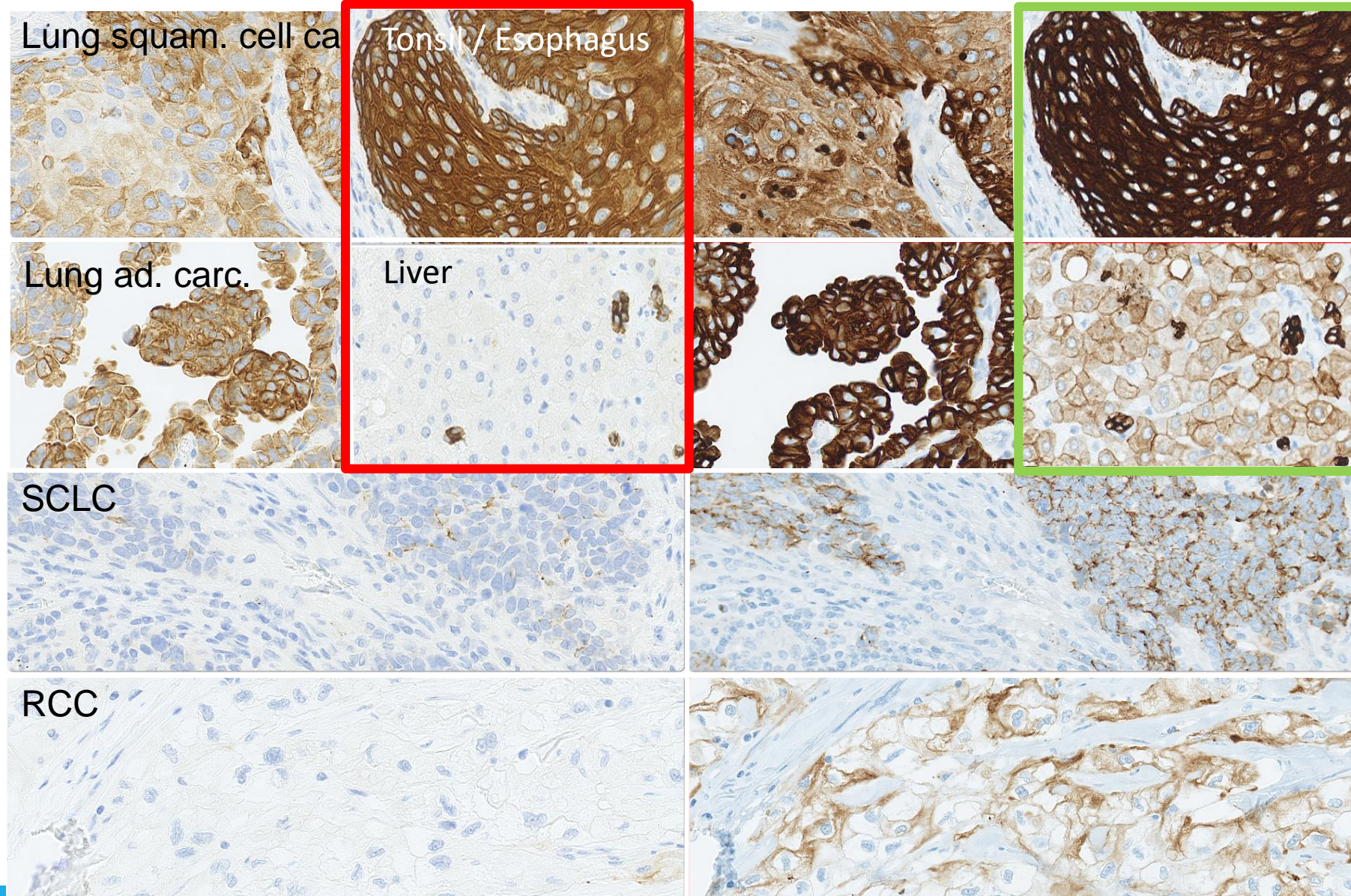
In addition an extensive range within same purpose can be seen....
E.g. Pan-CK for carcinoma identification (primary panel)

* 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



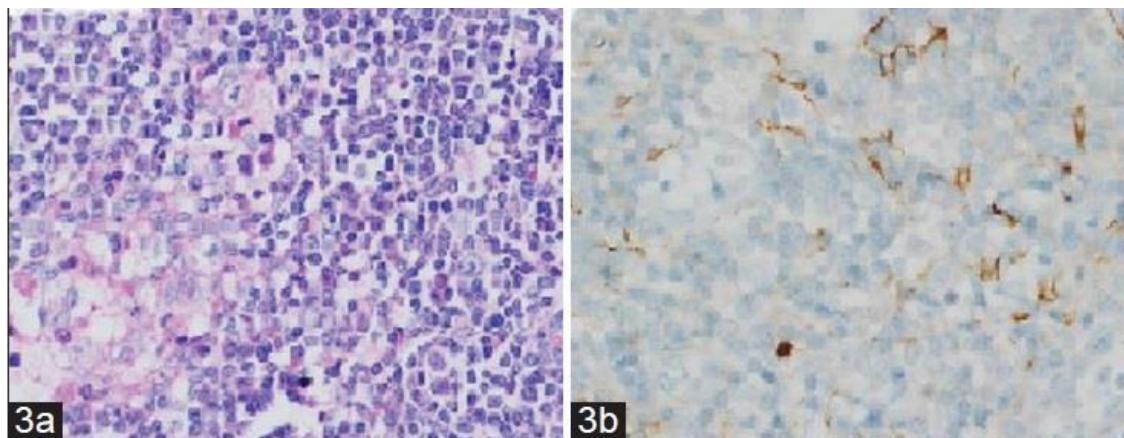
Sensitivity , specificity – what to choose...?

	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 - 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.

Challenges for technical / analytical validation of IHC

1. Limited access to relevant tissues – rare incidences
 - ALK (lung), ROS1
2. New markers not described in details – no data on test performance characteristics
 - SATB2, Claudin-4
3. Limited access to reference material and/or non-IHC method to monitor quality
 - PD-L1 IHC



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

Starting help to guide development – validation still required....

HistoCyte Laboratories
QUALITY IN CONTROL

Products Services About Latest Contact

Control Material from HistoCyte Laboratories Ltd

- Standardised, reliable and cost-effective
- Validated with standardised assays
- Easily determine assay performance

Available now: PD-L1, Her2, ER, PGR, ROST, ALK-Lung (EML4-ALK), ALK-Lymphoma (NPM-ALK), HPV, p16

HistoCyte Laboratories
Quality in Control

Welcome to HistoCyte Laboratories
We manufacture a range of cell line controls for same slide use in immunohistochemistry (IHC) and in situ hybridization (ISH). HistoCyte Laboratories Ltd have developed unique processes that allow the production of high density cell preparations that retain their original morphology. Through careful selection of cell types we can generate a range of positive and negative controls to determine effective performance of reagents used in slide based assessments.

Products
View our range of high quality, reliable control material

Services
We offer a range of contract services to assist in product development

- Biomarker Characterization
- Proof of Concept
- Custom Cell Line Development
- Assay Design
- QMS Auditing

About
Learn more about our company and history

For more information contact: info@histocyte.com

www.histocyte.com

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

StatLab American MasterTech

PRODUCTS SDS CONTACT

PRODUCT DESCRIPTION OR ITEM #

Specimen collection all the way through advanced diagnostics.

Quality products to empower your complete lab workflow are now available for ordering on a single site, delivered with the customer service you've come to know and love.

QUESTION? WE'VE GOT ANSWERS. READ THE FAQ.

Advanced Diagnostics
IHC reagents deliver high quality staining with superior results

Stains
American MasterTech and StatLab stains and kits to meet your needs

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High-quality slides and accessories to meet a wide variety of preferences

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Search all categories of products to support your complete lab workflow

ITEMS IN YOUR CART

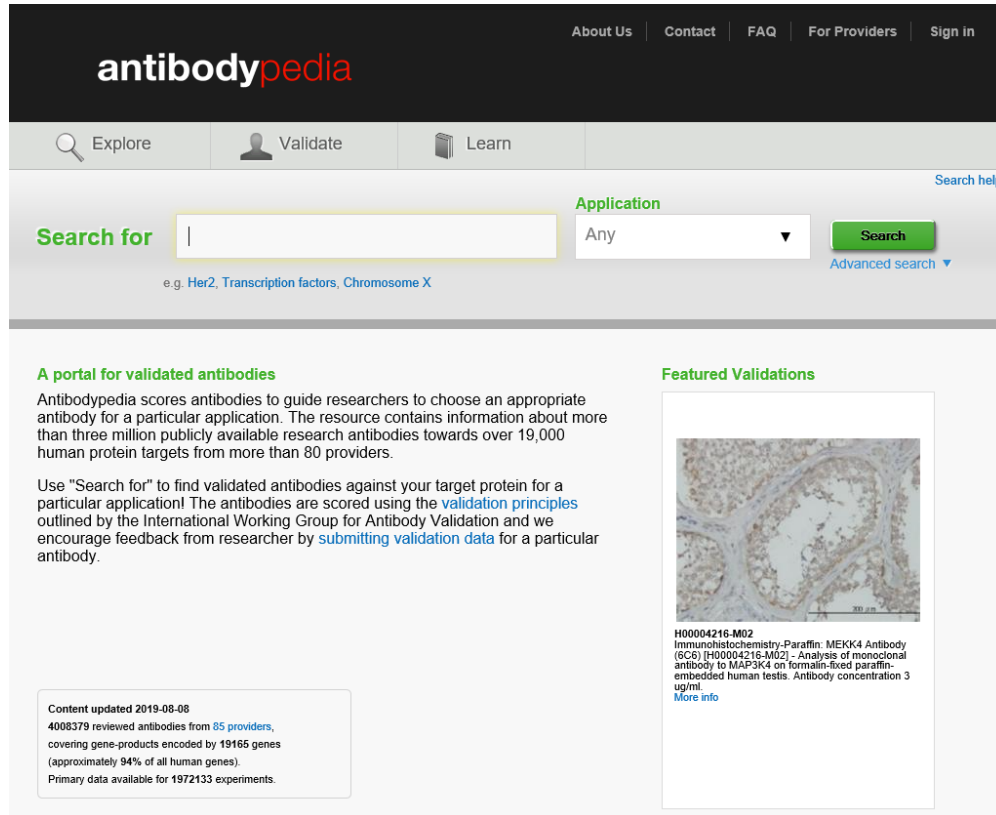
100%

www.statlab.com

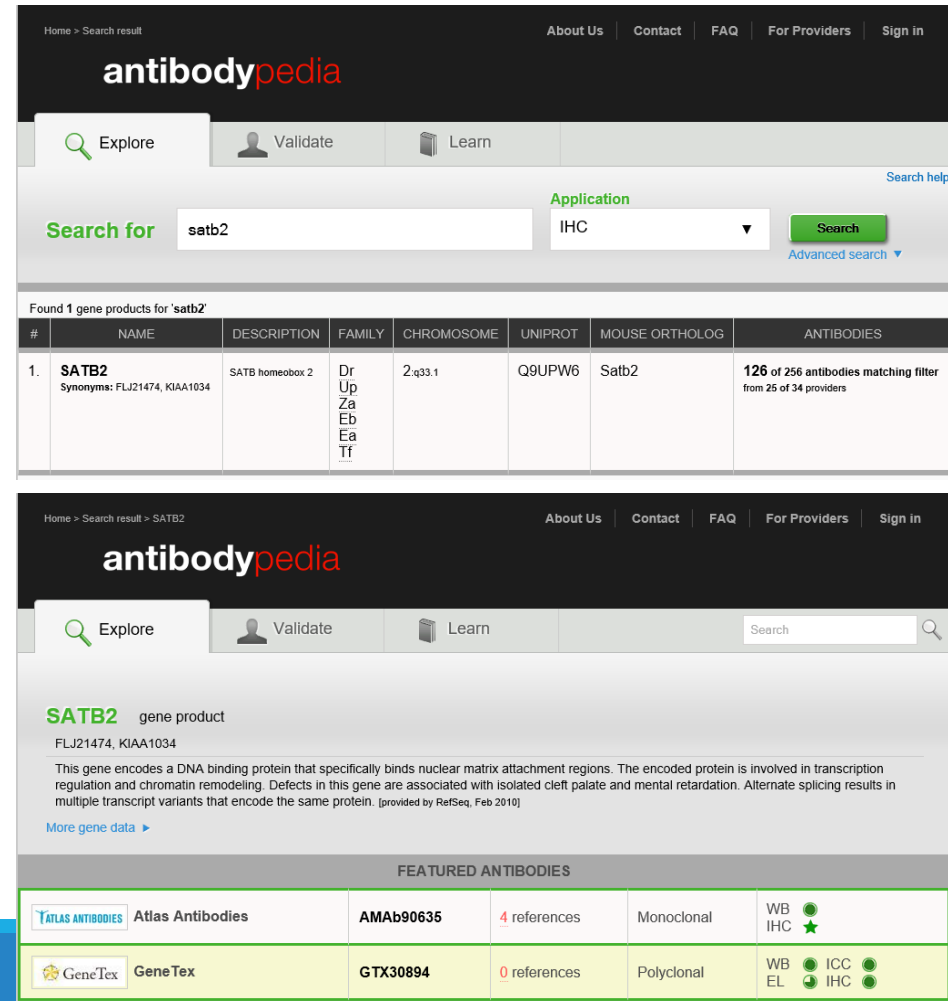
Histoids / Faux tissue
ALK +/-
HER2, PD-L1 with dynamic range

Online resources – “www.antibodypedia.com”

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



The screenshot shows the homepage of antibodypedia. The header includes navigation links: About Us, Contact, FAQ, For Providers, and Sign in. Below the header is a search bar with a magnifying glass icon and the word 'Explore'. To the right of the search bar are icons for 'Validate' and 'Learn'. The main content area features a 'Search for' input field, an 'Application' dropdown menu set to 'Any', and a green 'Search' button. Below the search bar, there is a link to 'Advanced search'. A section titled 'A portal for validated antibodies' provides information about the resource, stating that it scores antibodies to guide researchers. It mentions that the resource contains information about more than three million publicly available research antibodies towards over 19,000 human protein targets from more than 80 providers. A 'Featured Validations' section displays an immunohistochemistry image of a tissue section. Below the image, the text reads: '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'. A footer box indicates that the content was updated on 2019-08-08, that 408,379 reviewed antibodies from 85 providers covering gene-products encoded by 19,165 genes (approximately 94% of all human genes) are available, and that primary data is available for 197,213 experiments.



The screenshot shows the search results for 'SATB2' on the antibodypedia website. The header is identical to the homepage. The search bar contains 'satb2' and the 'Application' dropdown is set to 'IHC'. The 'Search' button is green. Below the search bar, there is a link to 'Advanced search'. The results section is titled 'Found 1 gene products for 'satb2''. It contains a table with the following columns: #, NAME, DESCRIPTION, FAMILY, CHROMOSOME, UNIPROT, MOUSE ORTHOLOG, and ANTIBODIES. The table has one row for SATB2, with the following details: # 1, NAME SATB2 (Synonyms: FLJ21474, KIAA1034), DESCRIPTION SATB homeobox 2, FAMILY Dr Up Za Eb Ea Tf, CHROMOSOME 2:q33.1, UNIPROT Q9UPW6, MOUSE ORTHOLOG Satb2, and ANTIBODIES 126 of 256 antibodies matching filter from 25 of 34 providers. Below the table, there is a section for 'SATB2 gene product' with the gene ID FLJ21474, KIAA1034. The text describes the gene as encoding a DNA binding protein that specifically binds nuclear matrix attachment regions. It mentions that 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. The text is attributed to RefSeq, Feb 2010. A link to 'More gene data' is provided. Below the gene product section is a section titled 'FEATURED ANTIBODIES' which contains a table with the following columns: Provider, Antibody Name, ID, References, Type, and Applications. The table has two rows: Atlas Antibodies (AMAb90635, 4 references, Monoclonal, WB IHC) and GeneTex (GTX30894, 0 references, Polyclonal, WB EL, ICC, IHC).

#	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

Provider	Antibody Name	ID	References	Type	Applications
Atlas Antibodies	AMAb90635	4 references	Monoclonal	WB IHC	
GeneTex	GTX30894	0 references	Polyclonal	WB EL	ICC IHC

Online resources – “www.antibodypedia.com”

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

The screenshot shows the antibodypedia website interface. At the top, there's a navigation bar with 'Home', 'Search result: SATB2', and 'AMAb90635'. Below this, the 'antibodypedia' logo is prominent. A search bar and navigation tabs (Explore, Validate, Learn) are visible. The main content area displays the antibody profile for 'AMAb90635', identified as a 'SATB2 antibody from Atlas Antibodies' with accession numbers 'FLJ21474, KIAA1034'. It notes the antibody is 'Eligible for validation within the Antibodypedia Validation Initiative'. A progress bar shows 'Western blot' as 'Supportive data in Antibodypedia' and 'Immunohistochemistry' as 'Enhanced validation'. A 'Provider product page for AMAb90635' link is provided. Below this, a workflow diagram illustrates the process from 'SELECT THIS ANTIBODY' to 'GET REWARDED'. The 'ANTIBODY DATA' section at the bottom provides detailed information:

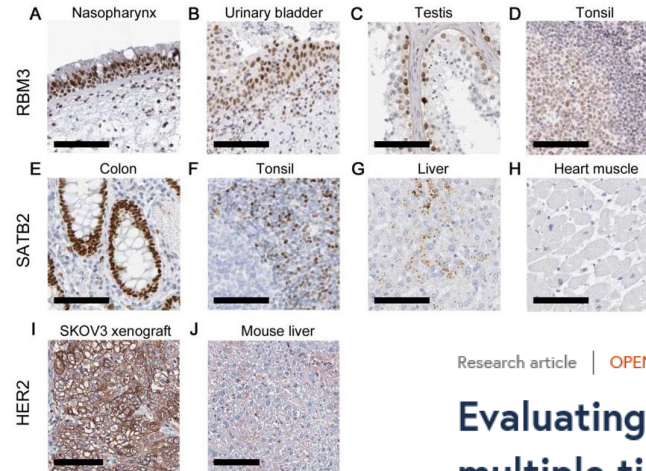
Antibody Data	Product number
Knockdown Reagents [0]	AMAb90635
References [4]	Atlas Antibodies
Comments [0]	Product name
Validations	Anti-SATB2
Western blot [1]	Provider product page
Immunohistochemistry [5]	Atlas Antibodies - AMAb90635
Submit	Antibody type
Validation data	Monoclonal
Reference	Description
Comment	Protein A purified
Report error	Reactivity
	Human
	Host
	Mouse
	Conjugate
	Unconjugated
	Antigen sequence
	VSGAVFARVAFNRTGGLLEILKEEDPTASQSL LNLNRMQNFNLNLPFVSKDQIQEPEPSSQVPS MYSSASSPSSSRIPQAKTSTPTLPIKVGANI NITAAIYDEIQEDMGKAK
	Epitope
	Binds to an epitope located within the peptide sequence LVNLRAMQNFNLNLP as determined by overlapping synthetic peptides.
	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

Role of non-IHC methods to guide quality / accuracy

Target	Treatment	Indication	Test methods	Considerations
HER2	Herceptin	Breast C. GI cancer	IHC ISH	<p>Predictable value - responders</p> <p>Clinical accuracy – confidence</p> <p>Test commercially available</p> <p>Test complexity – to perform</p> <p>Test complexity – to analyze</p> <p>Test turn-around-time</p> <p>Tissue sample size / type</p> <p>Number of relevant targets</p> <p>.....</p>
ALK ROS1	Crizotinib	NSCLC	IHC ISH NGS	
PD-1 CD274	Keytruda	NSCLC Urothelial Head & Neck	IHC; PD-L1	

*Only indicative overview and does not reflect any approved regulatory status or guidelines

The challenge to validate a PD-L1 assay

E Torlakovic et al. AIMM 2017;25:151-159
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 3

44 - 62% of the participants in NordiQC PD-L1 IHC runs C1 – C4 used LDT's

How is correct accuracy identified? – do I identify right proportion of pos / neg tumours?

How is correct index identified? - do I identify tumours with the clinical relevant range from weak to strong?

By access to reference material (e.g. slides / tumours) tested with validated IHC assay

By access to second line non-IHC test as ISH to confirm accuracy of LDT

The central challenges to meet

As outlined in the references:

*E Thunissen et al.
Lung cancer 113 (2017) 102-105
PD-L1 IHC in NSCLC with a global
and methodological perspective

*E Thunissen et al.
Arch Pathol Lab Med. 2017-0106-SA
doi: 10.5858/arpa.

**E Torlakovic et al. AIMM 2017;25:151-159
Evolution of Quality Assurance for Clinical
Immunohistochemistry in the Era of
Precision Medicine: Part 3

1. Identify the purpose of the test – E.g. detection of PD-L1 in NSCLC for KEYTRUDA® decision
2. Develop in-house IHC (LDT) – E.g. using tissue tool-box
3. Perform IHC with reference test – 40* – 100** NSCLCs tested with validated PD-L1 test
4. Perform IHC with developed LDT – Same 40* – 100** NSCLCs
5. Use relevant cut-off's for treatment – 0%, ≥1%, ≥50%
6. Analyze concordance – ≥90% then LDT is validated (if <90% LDT must be recalibrated)
7. Assay reproducibility – Secure stability of reagents, monitor lot-to-lot variations etc etc

Main elements to develop & validate IHC assays



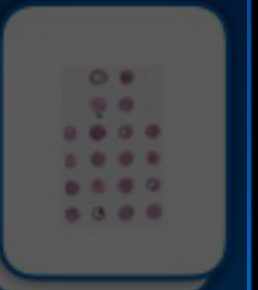
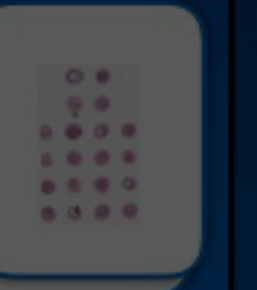


The journey from an antibody to a diagnostic IHC assay with a specific purpose

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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 – [Method transfer](#).

Based on selection and use of appropriate external tissue controls

External tissue control tool box

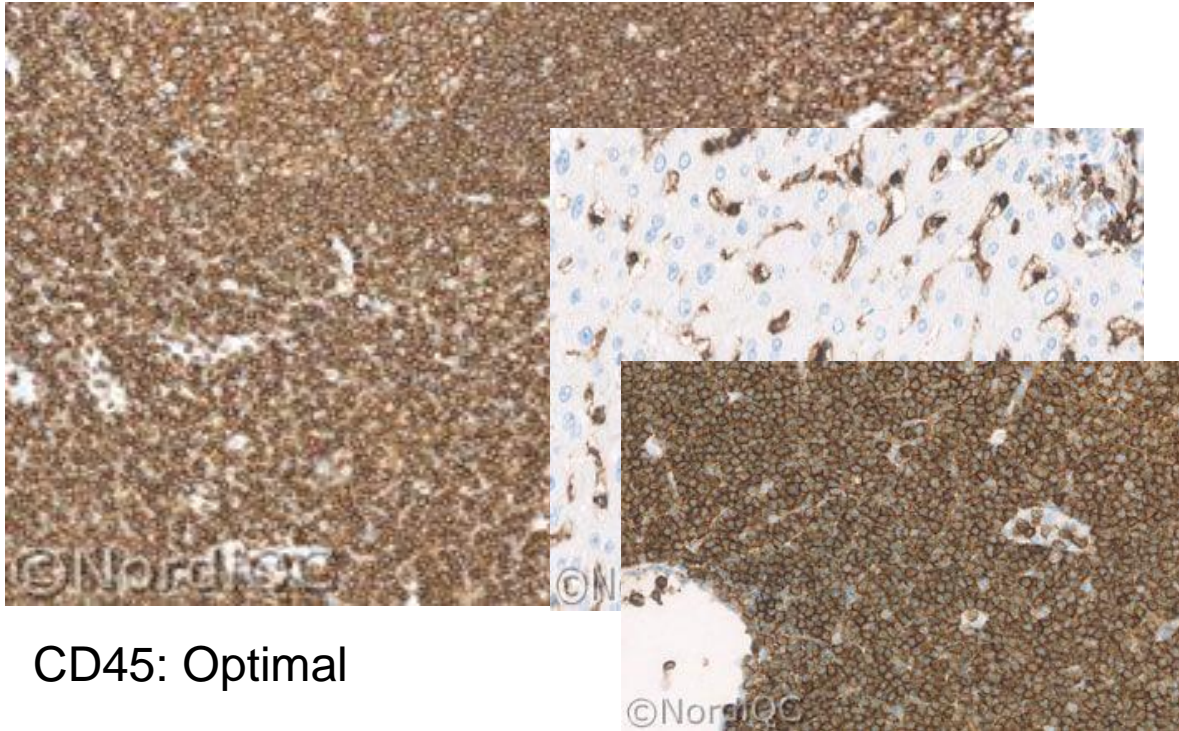
E Torlakovic et al. AIMM, 2017; 25:227-230
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4

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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

Tissue controls; Fit for purpose - relevant range of analyte



CD45: Optimal



Insufficient.....

Tissues/cells with only high expression levels 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 demonstrate the target antigen being expressed at different levels, the controls must reflect this !

Composition of TMA for QC of diagnostic IHC

B1:	Appendix,	Hepar,	Tonsil,	Pancreas	
	CD2	ASMA	BCL2	MMR	CDX2
	CD3	CD4	BCL6	S100	CGA
	CD19	CD31	CD2		SYP
	CD34	CD34	CD3		CK7
	CD117	CD45	CD4		PP
	CEA	CD68	CD5		SMAD4
	CGA	CK Pan	CD8		SYP
	CK20	CK LMW	CD10		
	DOG1	CK8	CD20		
	MMR	CK18	CD21		
	S100	HEPA	CD23		
	SYP	Arginase	CD38		
			CD56		
			CD79a		
			CD138		
			CK Pan		
			CyD1		
			EMA		

ether inclusive:

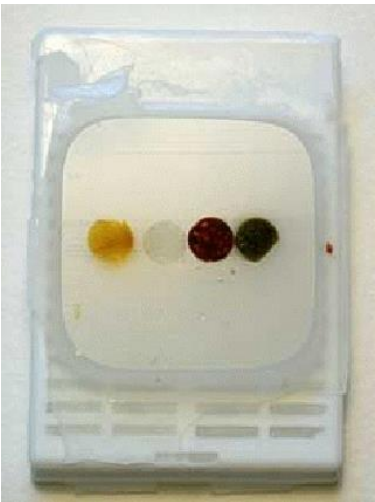


Used together inclusive:

HE

LE

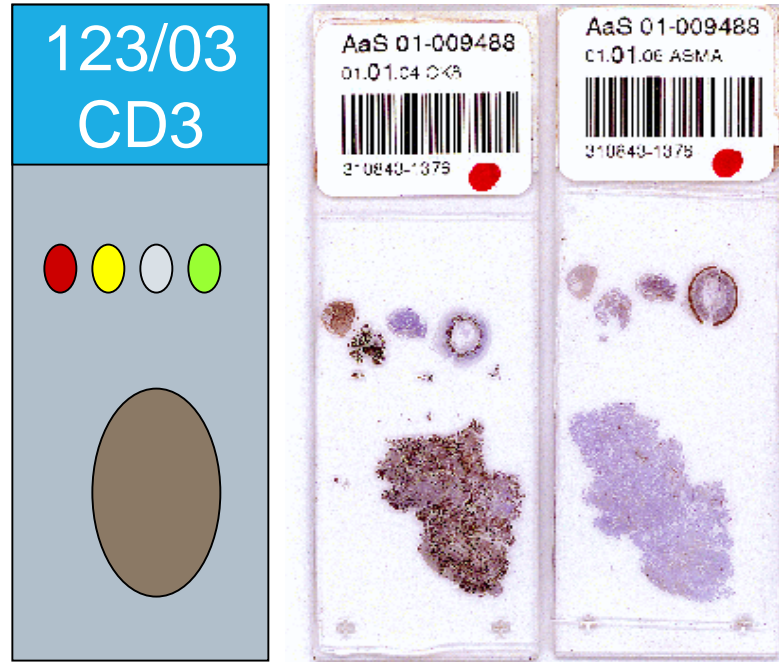
NE



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		

Application of TMA for QC of diagnostic IHC

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,||

TABLE 3. (continued)

	Special Considerations
Cut and submit “own on-slide control” if sending patients’ unstained slides to another laboratory for IHC testing	The positive controls should match patients’ sample tissue processing so far as is possible This is difficult if the sender does not know which IHC assays will be performed or if the sender does not have dIHC laboratory and has no positive controls
Use on-slide positive controls	“Run” or “batch” positive controls are not recommended
Date unstained slides with on-slide controls	Without the date when the slides are prepared, it will be impossible to determine if a unexpected weak result is due to variation in protocol or to an “expired” positive control

dIHC indicates diagnostic immunohistochemistry; iCAPCs, immunohistochemistry critical assay performance controls; SOP, standard operating procedure.



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

Application of TMA for QC of diagnostic IHC

RESEARCH ARTICLE

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

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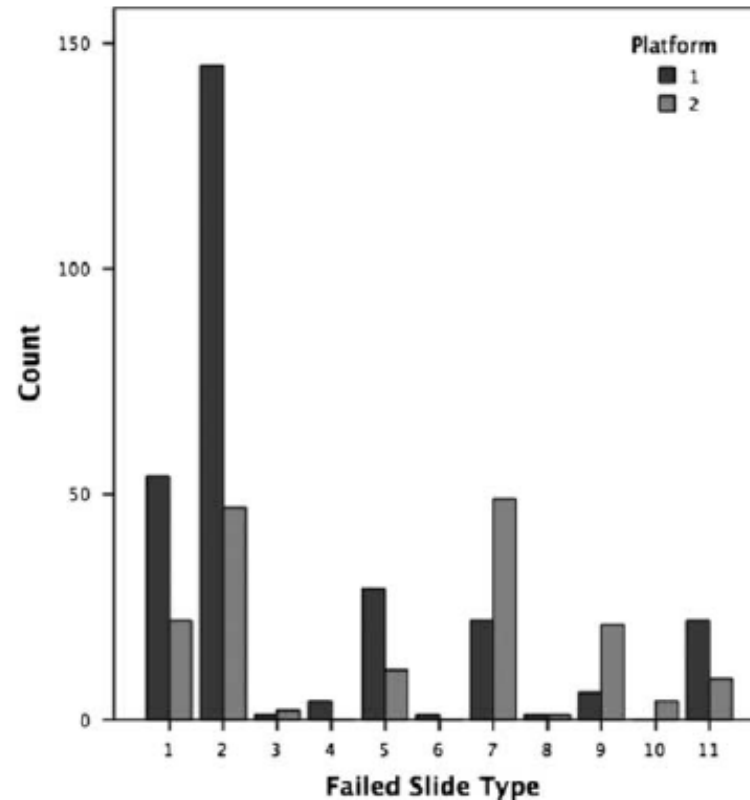


FIGURE 1. Frequency of failed immunohistochemistry slides by category and platform.

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%

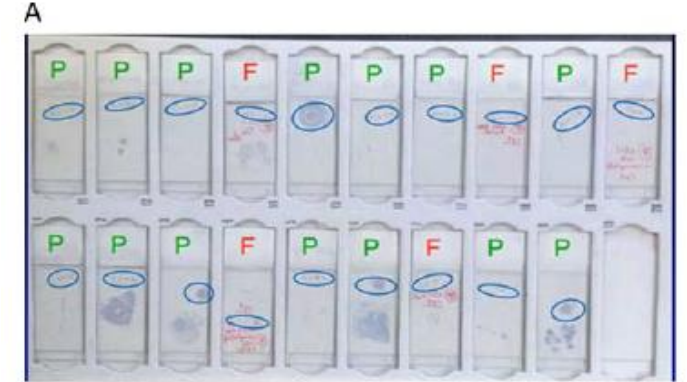
Class II 9,0%

(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


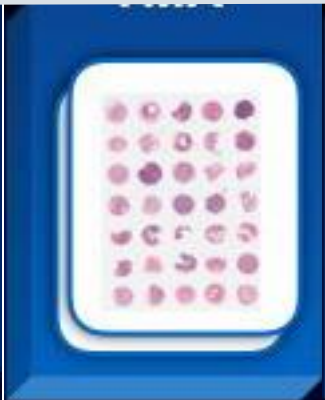




Conclusions

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.

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

Questions and/or comments



Thank You for the attention and.....