

The Tissue Tool Box

IHC Critical Assay Performance Controls

Søren Nielsen, Scheme Director NordiQC



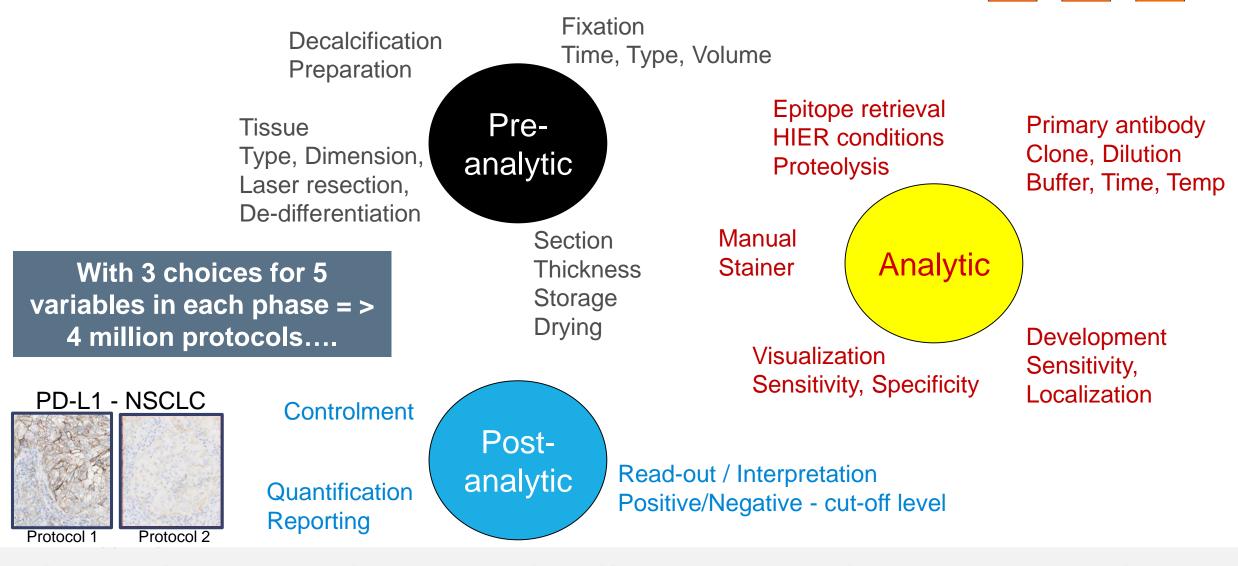
Agenda and focus areas

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

The role and concept behind ICAPCs -

IHC Critical Assay Performance Controls

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



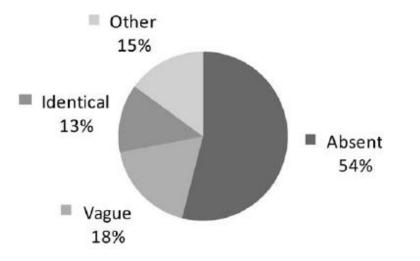
The right control material will expose right or wrong choices

Importance of IHC controls have been neglected....

Documentation of Diagnostic Cytopathology, Vol 39, No 4 2011

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 Glady



ICC Controls in the Literature

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 of false...?

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

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

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 Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

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

41% MRQ-50 0% BC12

31% MRQ-50 11% pAb CM



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

IHC controls to guide reliability of data...

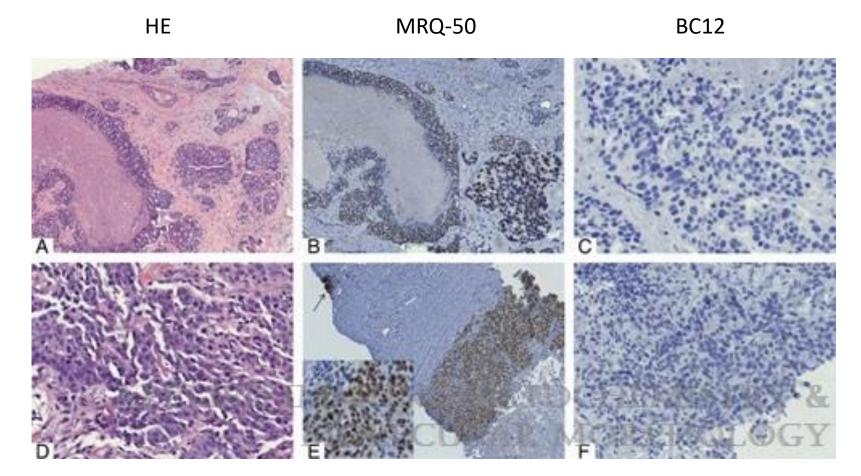


FIGURE 1

Aberrant Immunostaining of Breast Carcinoma by MRQ-50 PAX8 Antibody

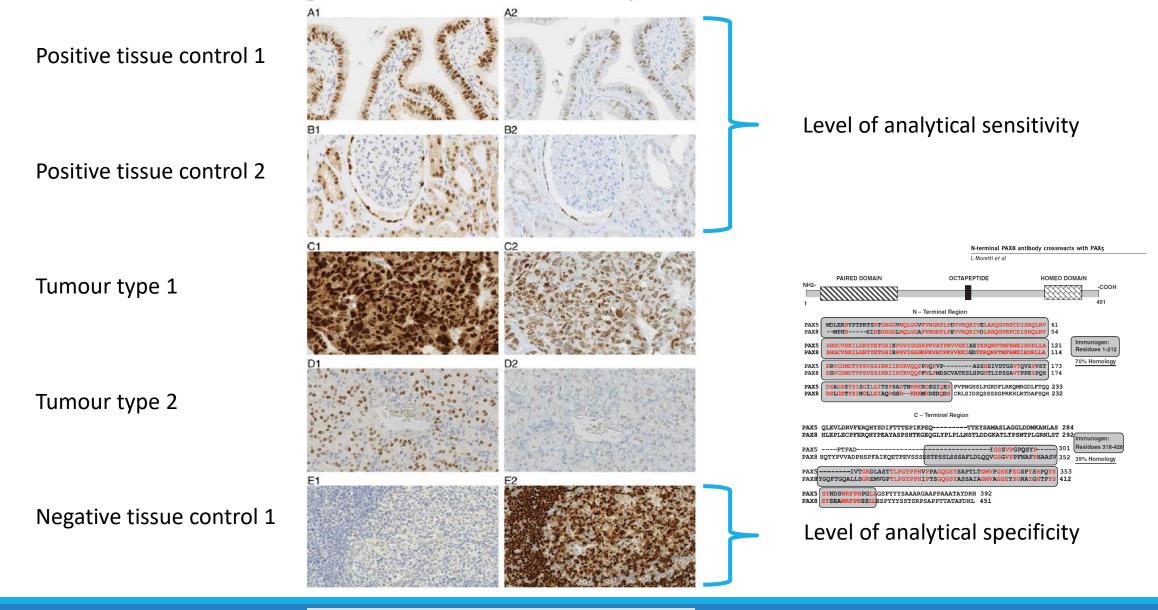
Singh, Kamaljeet; Hansen, Katrine; Quddus, M. Ruhul

Applied Immunohistochemistry & Molecular Morphology28(4):e37-e38, April 2020.

doi: 10.1097/PAI.000000000000682

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.

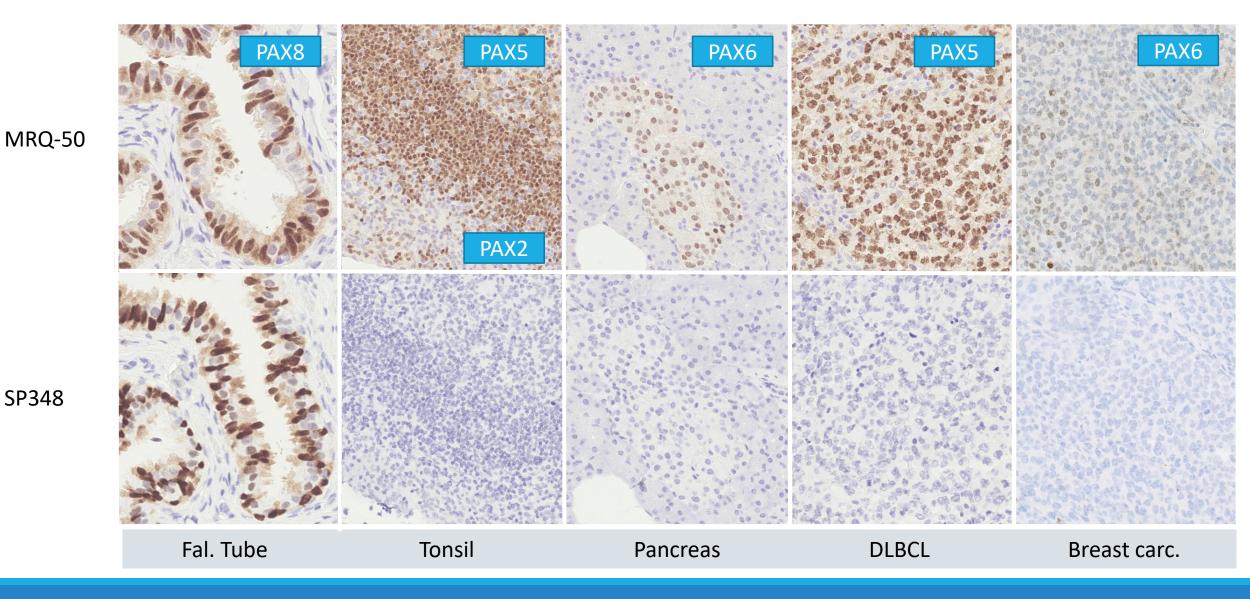
IHC controls to guide reliability of data... NordiQC Assessments of PAX8 Immunoassays and Mogens Vyberg, MD*† Ole Nielsen, HT.‡ Michael Bzorek, HT.\$ Soren Nielsen, HT.\$ and Mogens Vyberg, MD*†



BC12 / SP348

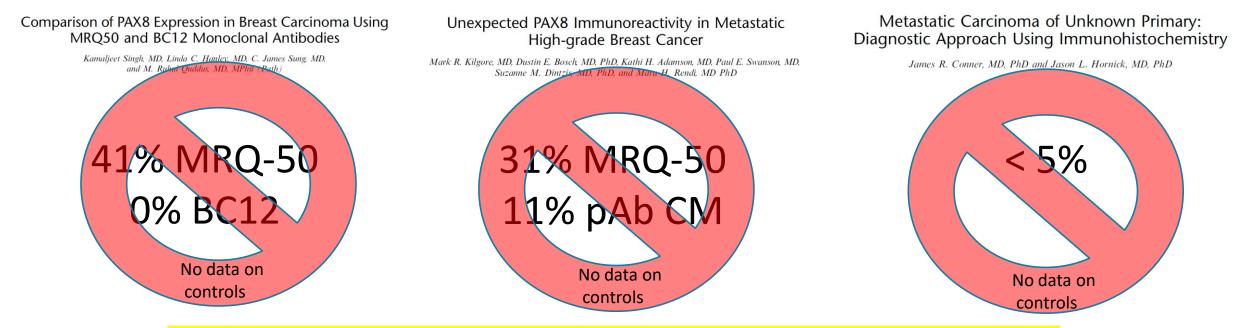
MRQ-50 / pAb

IHC controls to guide reliability of data...



SP348

IHC controls to guide reliability of data... PAX8 expression in breast cancer – true of false...?



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









PAX family Group	Protein structure/domains	Protein family member	Embryonic Expression Domain	Expression/Mutation in human disease
Ĭ		PAX1	Skeleton, thymus 3rd/4th pharyngeal pouch	Klippel-Feil Syndrome, Jarcho-Levin Syndrome
		PAX9	Skeleton, Teeth, Thymus	Jarcho-Levin Syndrome, Oligodentia
		PAX2	Kidney, CNS	Hyperproliferative dysplastic kidney, Renal hyperplasia, Bladder and renal cancer,Coloborna Syndrome
Ш		PAX5	B-Cells, CNS	Lymphomas
		PAX8	Kidney, Thyroid, CNS	Congenital hypothyroidism, Thyroid carcinomas/adenomas
Ш		PAX3	Neural Crest, CNS somites/muscle	Waardenburg Syndrome Types I/III, Melanoma, Rhabdomyosarcoma
12.002		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,‡‡ Søren Nielsen, HT, CT,§§|| || Eugen B. Petcu, MD, PhD,§ Paul E. Swanson, MD,¶¶ Clive R. Taylor, MD, PhD,## and Mogens Vyberg, MD§§|| ||

REVIEW ARTICLE

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

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

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

References central for the area of IHC controls

The 4-paper evolutions series

Recommendations and road-map for IHC QA provided by

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

International Quality Network for Pathology (IQN-PATH)

Published AIMM 2017 (Jan-April)

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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Abstract: Technical progress in immunohistochemistry (IHC) as well as the increased utility of IHC for biomarker testing in reactions medicine avails us of the opportunity to reasons clinical IHC as a laboratory test and its proper characterization as a special type of immunoassay. IHC, as used in curtest clinical applications, is a descriptive, qualitative, cell-based, soundly mordinear, in sits protein immuneasary, for which the readout of the results is principally performed by pathologists rather than by the instruments on which the immunoassay is erformed. This modus operandi is in contrast to other asservwhere the instrument also performs the readout of the test resul izg, 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 BHC test is developed and its subsequent classical uses, as well as the role of pathologists is the analytical and postaunlytical planes of BHC testing. This paper is the first of a 4-part series, under the general title of "Evolution of Quality Awarance for Classical Immunologistchemistry in the Eas of Procision Medicine."

Key Words: biomarkers, quality awarance, quality control, validation, immunohistochemistry (Appl Immunhoschem Mel Merphel 2017;25:4-11)

In the era of precision medicine, biomarker testing using immunobiatochemistry (HIC) has not only become more precise but also more complex.^{1,6} 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 3: Technical Validation of Immunohistochemistry (IHC) Assays in Clinical IHC Laboratories

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

Abstract: Validation of immunohistochemistry (BIC) anarys is a orbitet that is of grant impectance to clinical practice as well as basic ecounds and clinical trials. When applied to clinical practice and forcued on patient safety, validation of BIC anarys crusts onlyactue evidence that IRC anarys used for patient care as e⁻¹ fine-parpose.¹ Validation of BIC assays needs to be properly informed by and modeled to assess the parpose of the BIC assay, which will further determine what sphere of validation. These concepts will be defined in this review, part 3 of the 4-part series "Production of Quality Anneance for Clinical Immunohistocheming in the East of Phasilian Madaline."

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

(Appl Immunohierechem 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 This has also led to increasingly stringent criteria therapy." 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.15 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 accelerat he translation of novel cancer therapeutics into the clinic. 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 2: Immunohistochemistry Test Performance Characteristics

Emina E. Torlakovic, MD, PhD,*1‡ 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,#] Jacqueline A. Hall, PhD,§¶ Jason L. Hornick, MD, PhD,Bill Merdol Dvahim, PhD,*** Antonio Marchetti, MD, PhD,††† Keith Miller, FIBMS,*** J. Han van Krieken, MD, PhD,‡‡‡ Soren Nielsen, BMS,§§§]] Paul E. Swamson, MD,§§§ Mogens Vyberg, MD,§§§]] Xiaoge Zhou, MD,JHB*** Clive R. Taylor, MD,††† and From the International Society for Immunobistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path)

Abstract: All laboratory uses have test performance characteristics (TPCs), whether or sot they are exploitly known to the laboratorism or the puthologist. TPCs are thus also an integral characteristic of immunohistochemistry (BHC) tests and other in sits, cell-haud molecular assays such as DNA or RNA in sits hybridization or aptasere-based testing. Boassane of their descriptive, in sits, cell-based nature, BHC tests have a limited repetotive of appropriate TPCs. Although entry is for TPCs are relevant to IBC, proper selection of informative TPCs is nonchieves soutial south and measures in the IBC laboratory. This paper doesn'the site TPCs that are relevant to BHC testing and emphasizes the role of TPCs in the validation of HIC testing. This is part 2 of the 4-part series "Evolution of Quality Assurance for Clinical Intranobiotochemistry in the Era of Precision Modicine."

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

(Appl Immunohistochew 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 4: Tissue Tools for Quality Assurance in Immunohistochemistry

Carol C. Cheung, MD, PhD, JD, *† Corrado D'Arrigo, MB, ChB, PhD, FRCPah, §§ Manfred Dietel, MD, PhD, *[Glenn D, Francis, MBBS, FRCPA, MBA, FFSe (RCPA), 8**†† Regon Falton, MD, PhD, **, C. Blohe Gilks, MD, §§ Jacqueline A, Hall, PhD, §§*† Jaron L, Hornick, MD, PhD, **, I, Han van Krieken, MD, *** Antonio Marchetti, MD, PhD, ††††† Keith Miller, FIBMS,*** J, Han van Krieken, MD, PhD, §§ Soren Nielsen, BMS, §§575 Paul E, Swanson, MD, ##6 (Tive R, Taylov MD, *** Mogent Vyhore, MD, §§) [§§575 Yaoge Zhou, MD, ††††††† Emina E. Torlakoric, MD, PhD, §§§57] §§ and From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Society for Immunohistochemistry and Molecular Morphology (ISIMM)

Abstract: The rembers of diagnostic, prognostic, and predictive immersibilitodicensisty. (BEQ: tests are incensing: the implementation and videbatis of new BEC tests, revolutions of existing tests, as well as the or-paring and for daily usality assurance mechanical for proper quality looks, specifically tissue tools that will mable absoratories to successfully tarry cest these presenses. This paper dentifies, through the laws of labonatory insign tools, how validation, volification, and result fails of IBC tests on the performed in order to develop and maintain high quality. The for-purpose: IBC tooring in the era of problem malaxies. This is the fatal pour of the 4-pan states of gradient of Quality Assorators for Clinical Instantonionscheristry in the Eras of Precision Medicine."

Key Words: intransbistochemistry, quality tooh, tinne tools, test development, quality ossananon, biomarker, validation Udppl howaeoloosuhem Mol Merphel 2016;00:000-000)

Before the decision to implement a new immunohistorelevant to test development and maintenance need to be contemplated (see parts 1 to 3 of the Evolution series). To introduce a new HIC tota, a series of steps must be followed that require careful planning, from test development through to on-poing quality monitoring. For this process to be successful, proper tissue tools, which are a contension 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 <u>tissue</u> controls are necessary for the validation of immunohistochemical staining results.

 Tissue controls are the most valueable tool to monitor the specificity and sensitivity for IHC

- Internal positive and negative tissue control
 - Cells/structures within the patient material
- External positive and negative tissue control
 - Slide next to patient material

How to use internal tissue controls

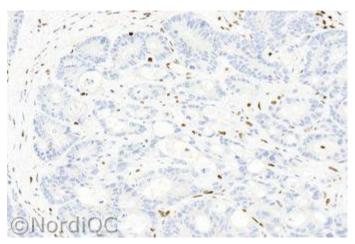
Appl Immunohistochem Mol Morphol • Volume 22, Number 4, April 2014

Standardization of Negative Controls

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

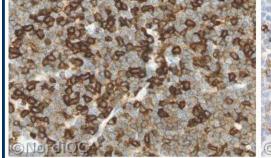
IHC Assay	Use	Comments
Cytokeratin 5	Demonstration of basal cells in glandular structures of prostate to differentiate between benign (positive) and malignant (negative) glands	Interpretation of the results in the tumor directly depends on clear demonstration of internal positive control Tested sample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



The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells normal T-cells are clearly demonstrated. cond staining reaction

nsufficient staining for CD5 of the same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. The neoplastic cells are virtually negative and only the

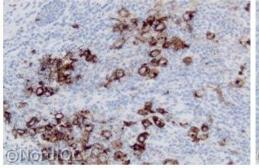


Fig. 2a. Optimal CD15 staining of the Hodgkin lymphoma 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

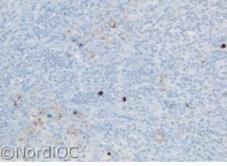
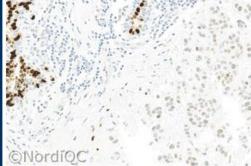


Fig. 2b. CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a

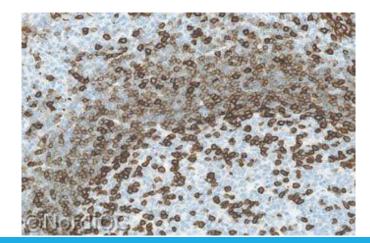


staining of the breast ductal no. 3 with 60 - 80 % cells positive. A weak but distinct nuclear staining is seen in the appropriate proportion of the protocol as in Figs. 1b and 2b - same field as in Fig. 3a. neoplastic cells. Same protocol as in Figs. 1a and 2a.

carcinoma no. 3 with 60 - 80 % cells positive using same 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

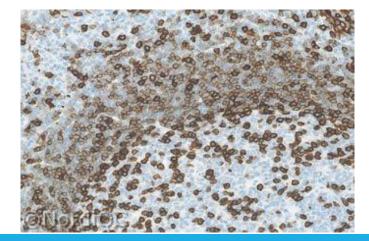
- clinical relevant range of target analyte (antigen) especially with low limit detection
- well characterized expression pattern preferable normal tissues
- predictable levels and specified cellular and architectural localization

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

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Main elements to develop & validate IHC assays

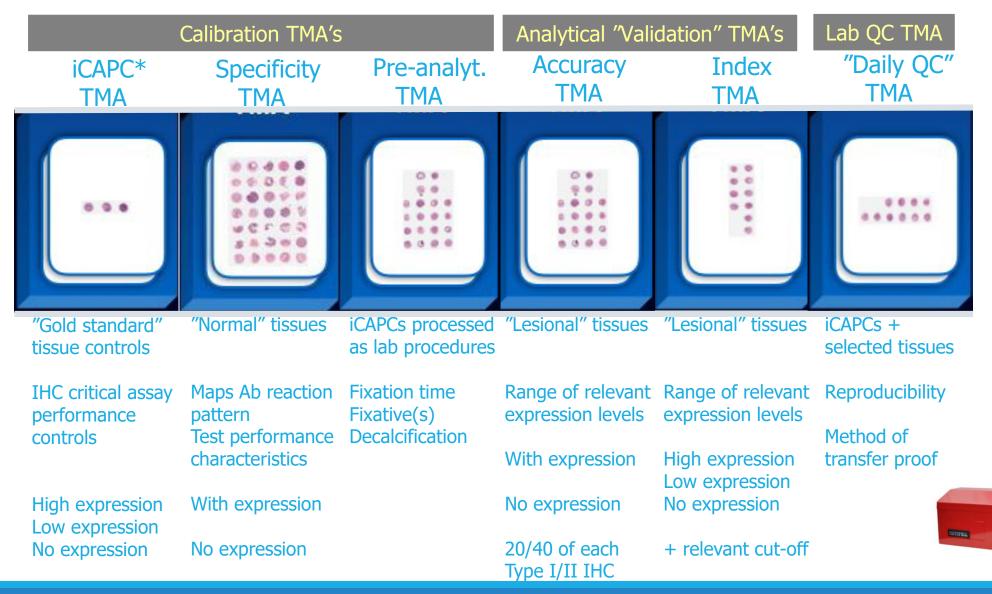
The journey from an antibody to a diagnostic IHC assay with a specific purpose

- 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

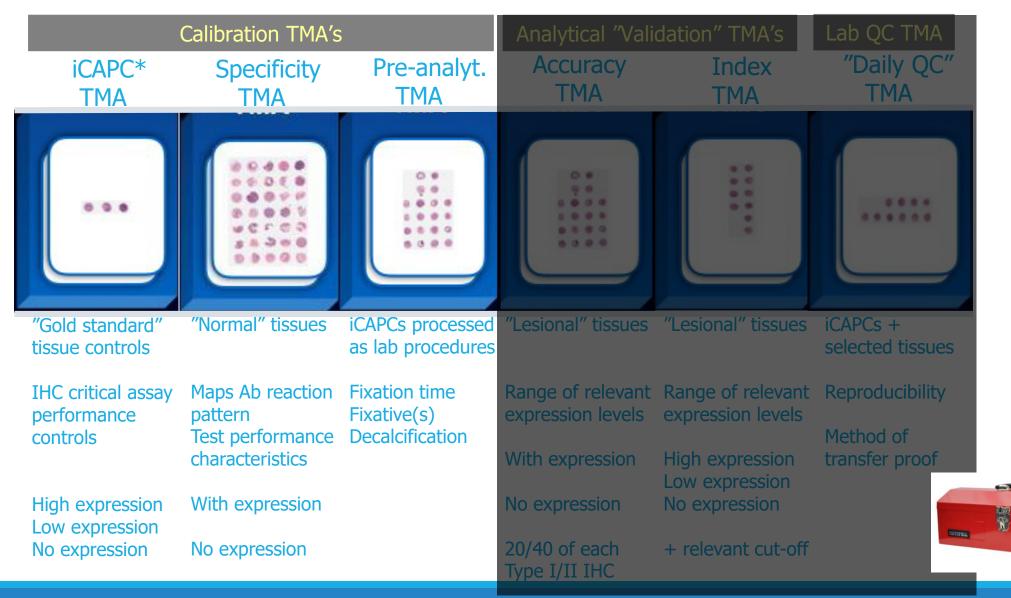
External tissue control tool box

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



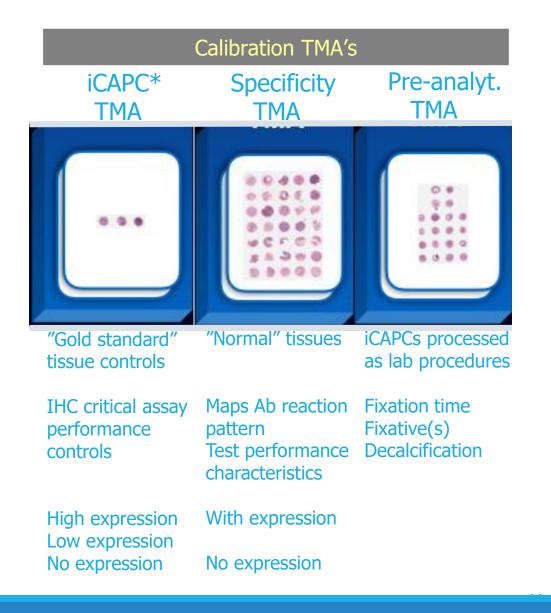
External tissue control tool box

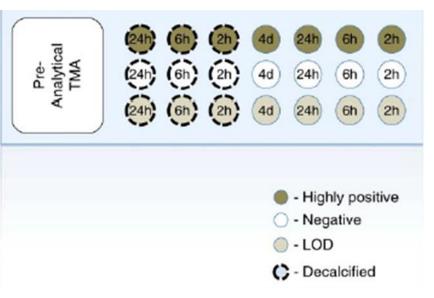
E Torlakovic et al. AIMM, 2017; 25:227-230 Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4



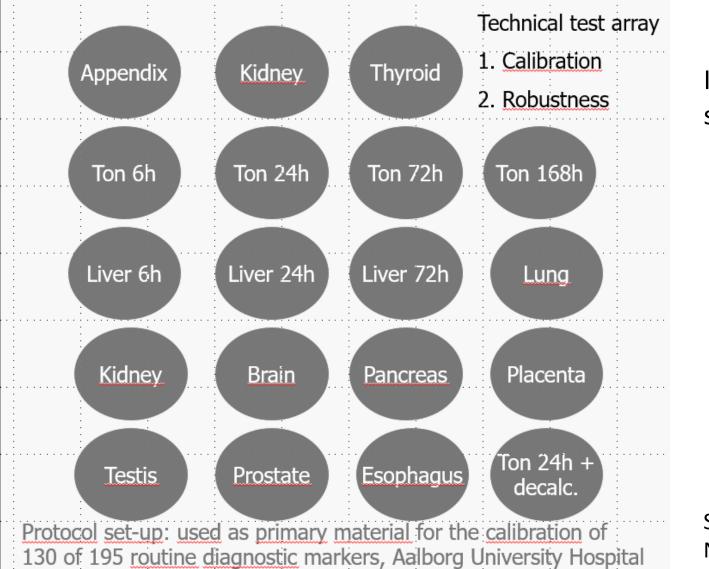
External tissue control tool box

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



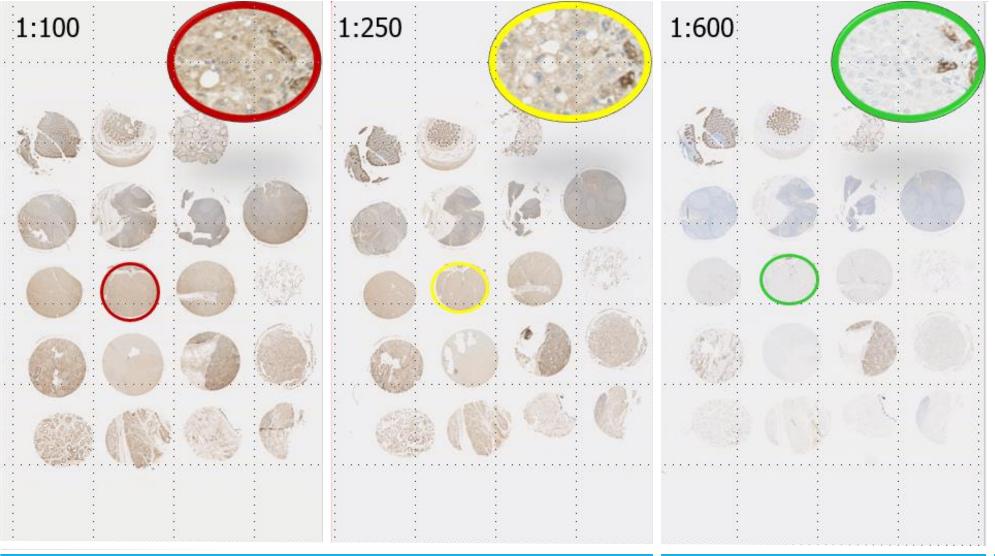






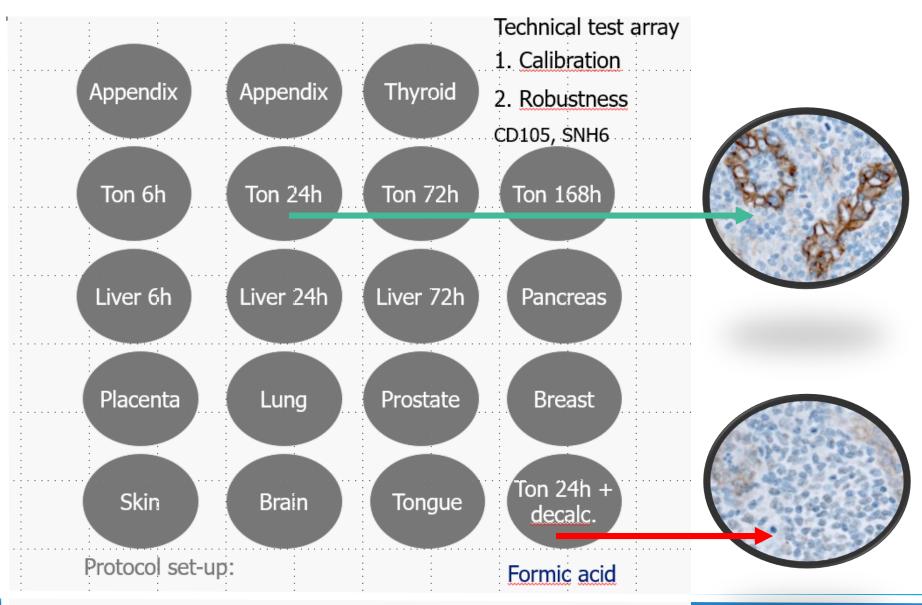
Inspirational set-up to address issue of specificity and impact on pre-analytics





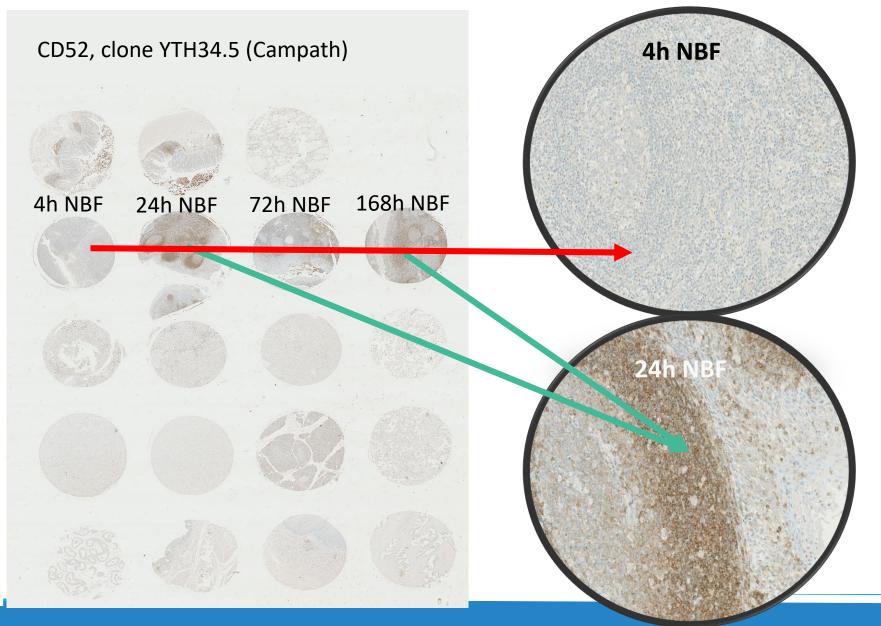
EPCAM calibration

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



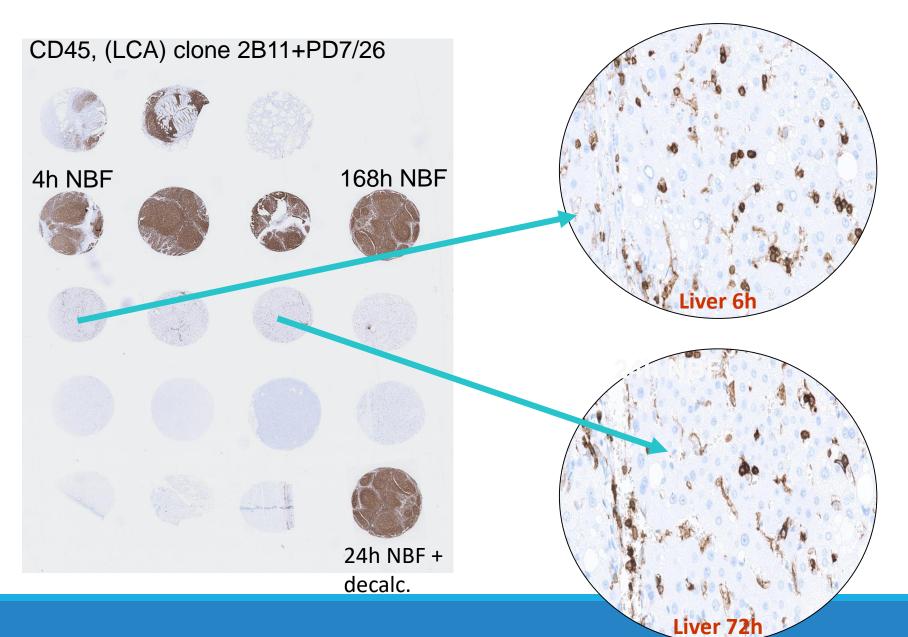
CD105 calibration

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



CD52 calibration

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



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?

Test Performance Characteristics - TPCs

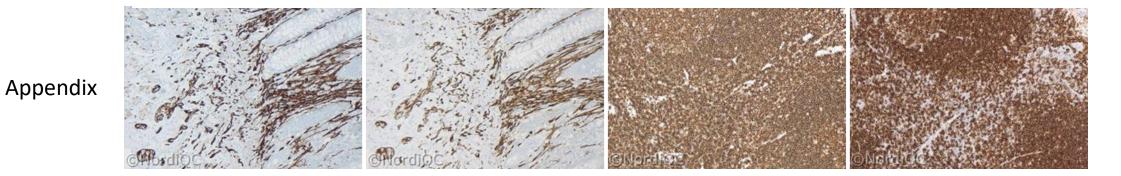
Test performance characteristics;

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 – <u>CRITICAL CONTROLS - ICAPCs</u>?





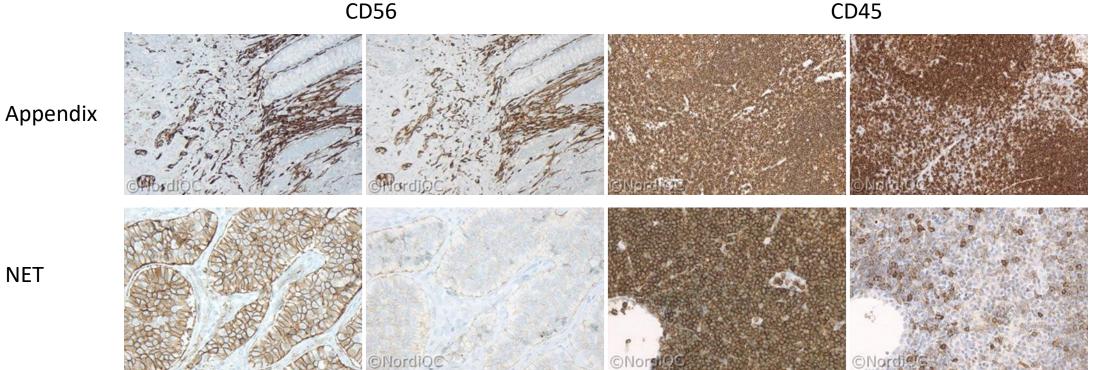
Tonsil



CD45



CD56



Tonsil

B-CLL

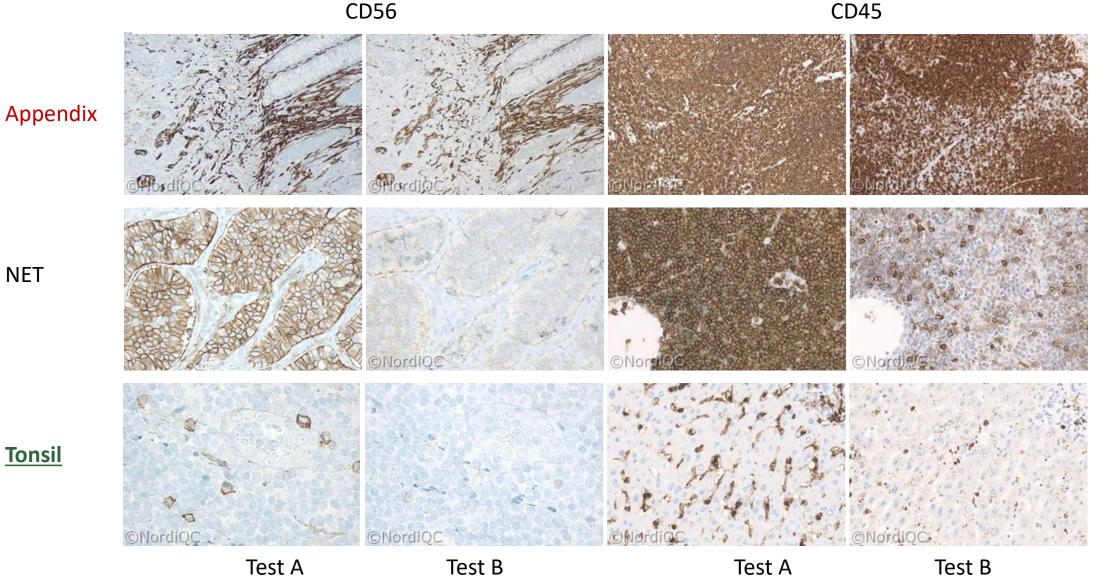
NET

Test A





CD56



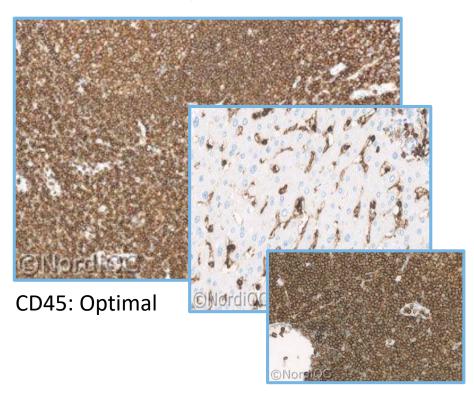
Tonsil

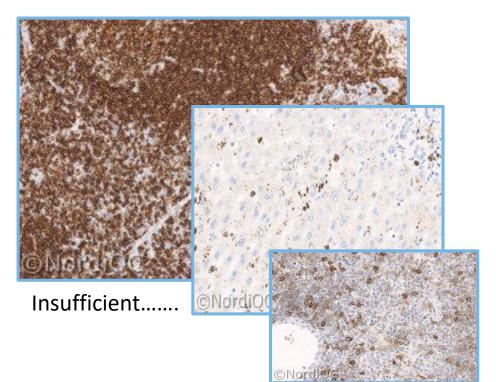
B-CLL

<u>Liver</u>

NET

<u>Tonsil</u>





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 ?

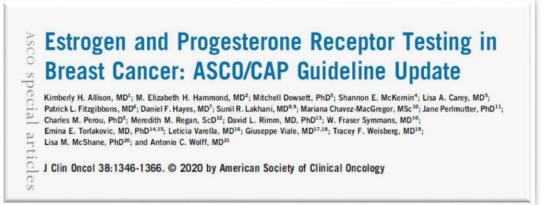
What is the expected staining pattern ?

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

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iCAPCs – potential and use

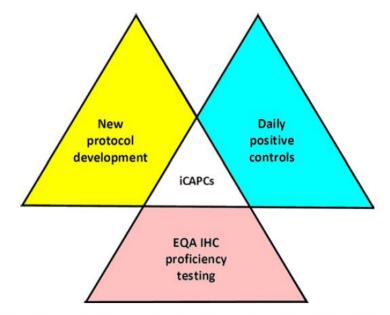


FIGURE 19. The roles of iCAPCs in clinical immunohistochemistry (IHC) laboratories. iCAPCs are an essential part of new protocol development, daily quality controls, and proficiency testing. EQA indicates External Quality Assurance; iCAPC, immunohistochemistry critical assay performance controls.

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

Expected level – calibration Analytical sensitivity and specificity

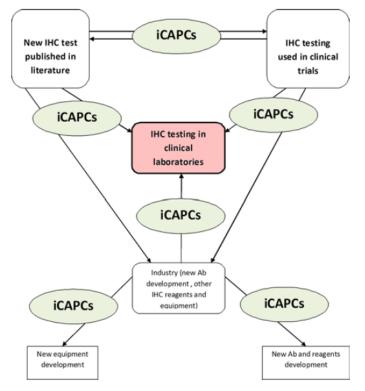


FIGURE 20. iCAPCs and Methodology Transfer. iCAPCs are proposed as important elements for harmonization of immunohistochemistry (IHC) testing between clinical research, product development, and clinical IHC testing. iCAPCs enable IHC harmonization of protocol transfer between research, industry, and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

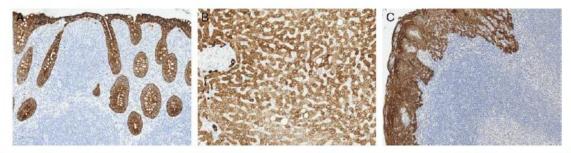


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining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

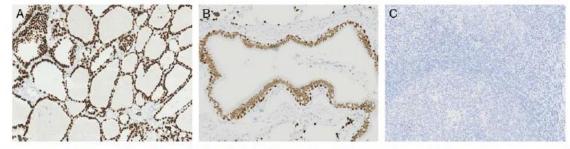


FIGURE 7. TTF-1 iCAPC. A, Thyroid: virtually all epithelial cells must show a strong nuclear staining reaction. B, Lung: virtually all pneumocytes and basal cells of terminal bronchi must show a moderate to strong nuclear staining reaction. Columnar epithelial cells of terminal bronchi must show an at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

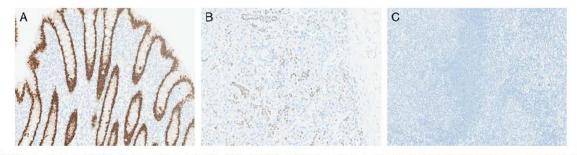


FIGURE 8. CDX-2 iCAPC. A, Appendix: virtually all epithelial cells must show a strong nuclear staining reaction. A weak cytoplasmic staining reaction in addition to strong nuclear staining is often present. B, Pancreas: the majority of epithelial cells of intercalated ducts must show a weak to moderate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

Generel expected patterns

High expression (Right antibody)

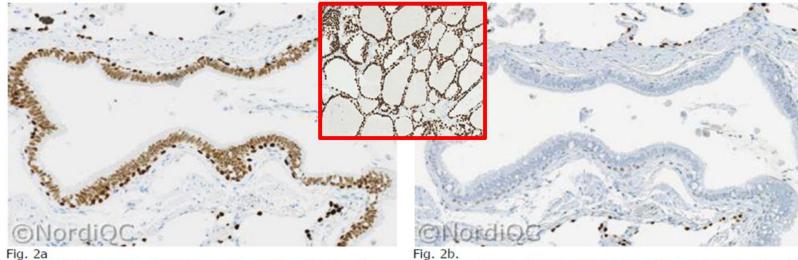
Low expression (Appropriate sensitivity)

No expression (Appropriate specificity)

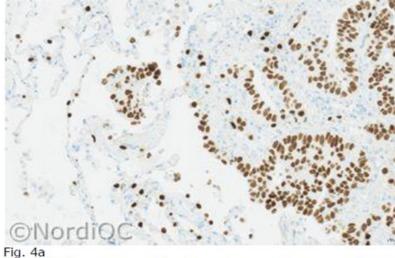
Which tissue Which cells Which extension Which intensity

	High express.	Low ex. (iCAPCs)	Non express.	Comment
CK-PAN	Appendix	Liver	Tonsil	
CK-LMW	Appendix	Liver	Tonsil	
CK-HMW	Tonsil	Pancreas	Liver	
СК7	Liver	Pancreas	Tonsil	
СК20	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 <u>sens</u> .)		An at <u>least weak</u> <u>to moderate</u> , staining reaction of the <u>majority of</u> <u>the perisinusoidal</u> <u>cells</u>		
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

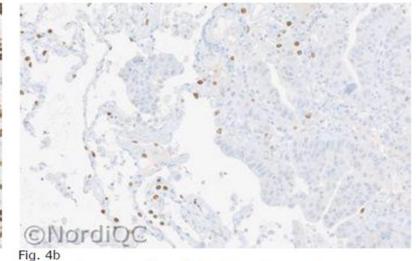


Optimal TTF1 staining of the lung using same protocol as in Fig. 1a. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a moderate nuclear staining reaction. No background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



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

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.



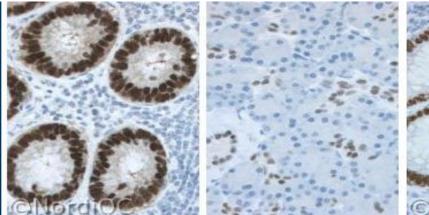
Insufficient TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1b, 2b & 3b. Despite a moderate positive staining reaction in the majority of type II

TTF1

iCAPCs: Thyroid + lung

Epithelial cells lining terminal bronchi

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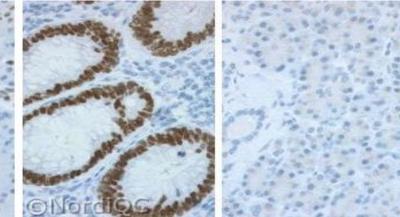


Fig. 1a. Optimal staining for CDX2 using the mAb clone CDX2-88.

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 Right, pancreas: No nuclear staining is seen in the ductal 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.

epithelial cells. Also compare with Fig 2b - same protocol.

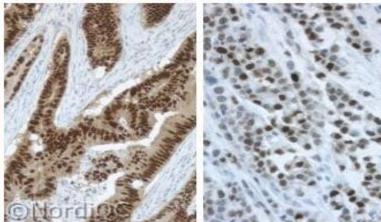


Fig. 2a. Optimal staining for CDX2 using same protocol as in Fig. 1a.

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.

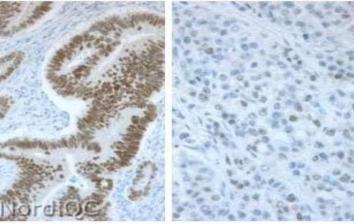


Fig. 2b. Insufficient staining for CDX-2 using same protocol as in Fig. 1b.

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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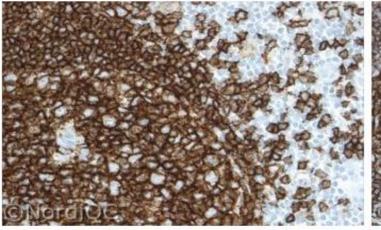


Fig. 1a. Lymphatic tissue in the appendix showing an optimal staining reaction for CD20 using the mAb clone L26 in a RTU format on the BenchMark platform. HIER was performed using Cell Conditioning 1. A very strong membranous staining reaction is seen in virtually all the Bcells.

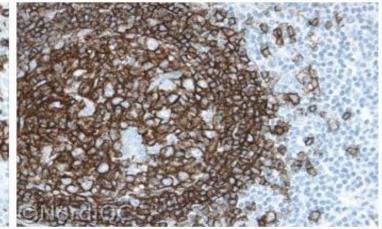


Fig. 1b. Lymphatic tissue in the appendix. Same field as in Fig. 1a. Insufficient staining for CD20 using the mAb clone L26 in a RTU format at the BenchMark platform. No HIER was performed. A moderate to strong staining reaction is seen in virtually all the B-cells. The normal B-cells are high expressors 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).

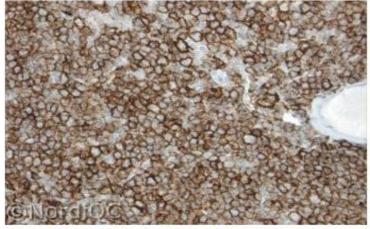


Fig. 2a. B-CLL. Optimal staining reaction for CD20. Same protocol as in Fig. 1a. A moderate to strong membranous staining is seen in virtually all the neoplastic cells.

C NordiOC

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:

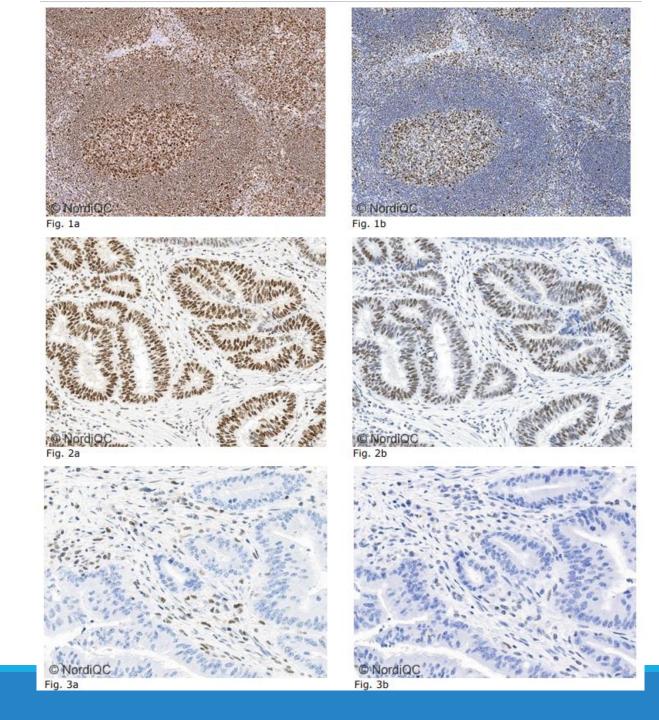
iCAPCs: ????

Tonsil;

B-cells to be ASAP....

As strong as possible...

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MMR:

iCAPCs:

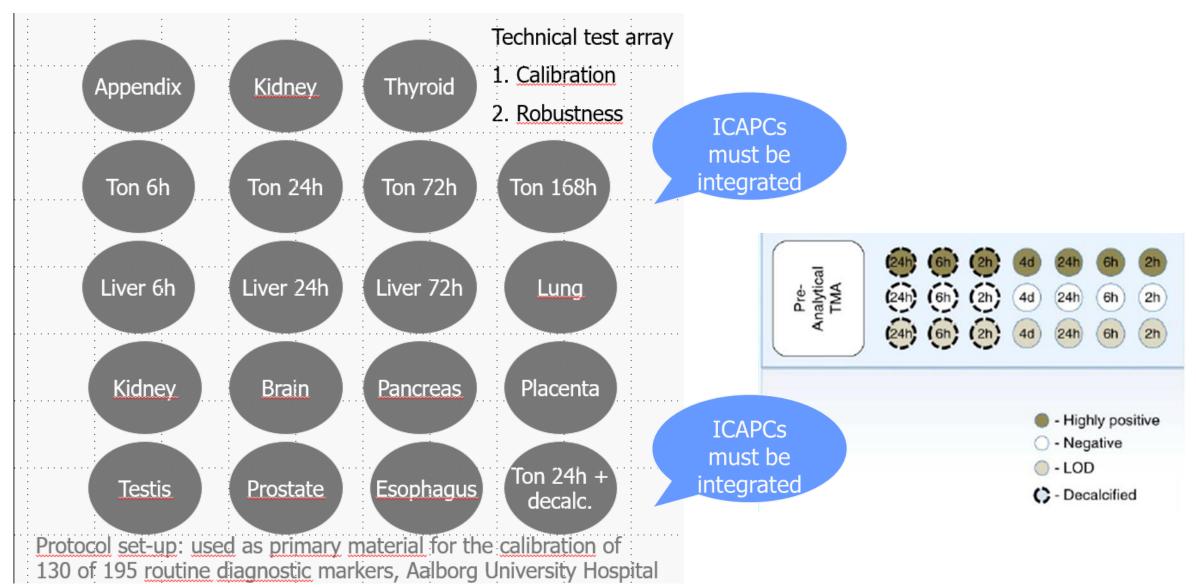
External tissue control Mantle zone B-cells in tonsil Assay run-to-run consistency

Internal tissue control

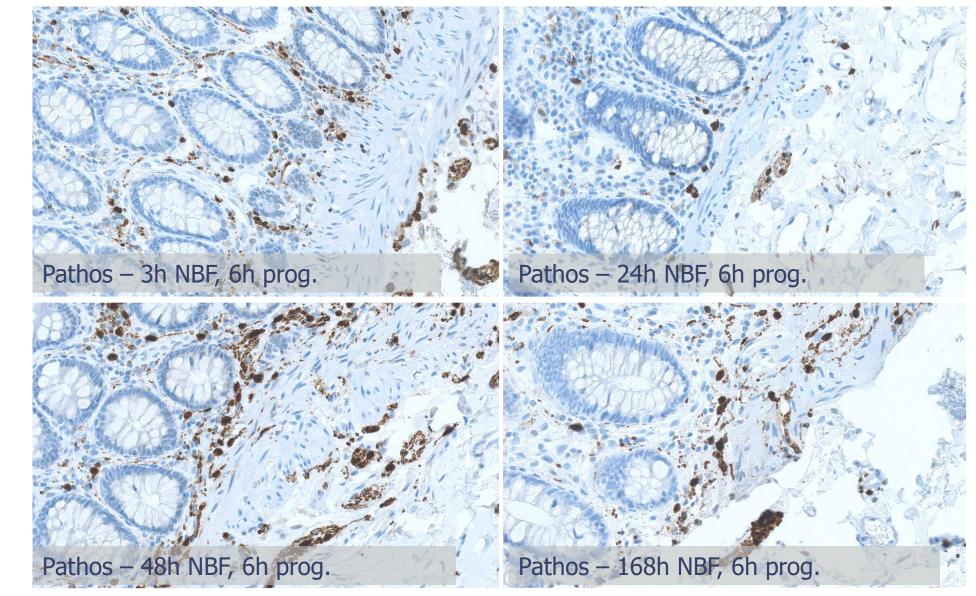
Stromal cells!!

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"Poor mans" specificity and pre-analytical TMAs



"Poor mans" specificity and pre-analytical TMAs

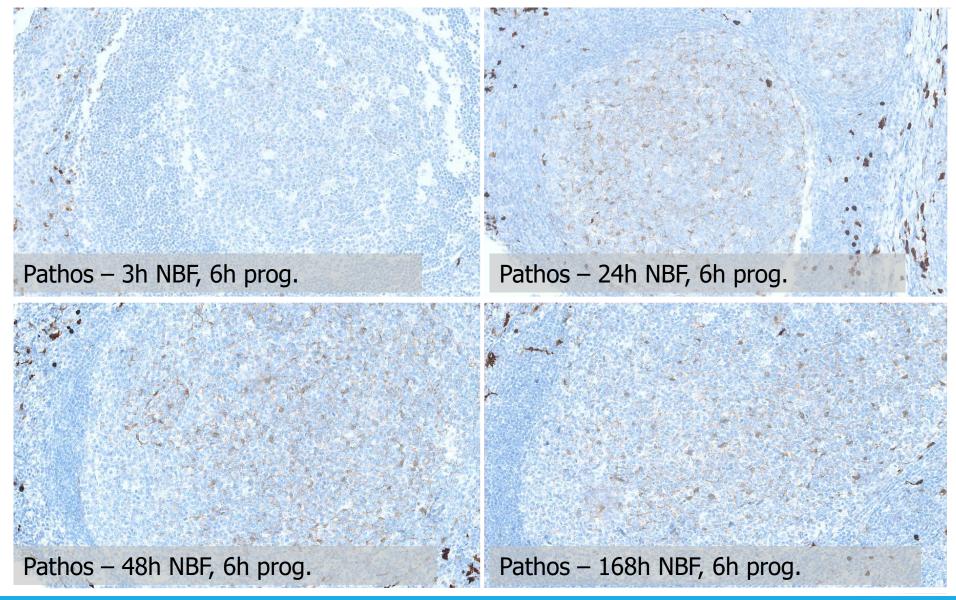


Colon: S100, polyclonal

"Poor mans" specificity and pre-analytical TMAs



Tonsil: S100, polyclonal



Main elements to develop & validate IHC assays

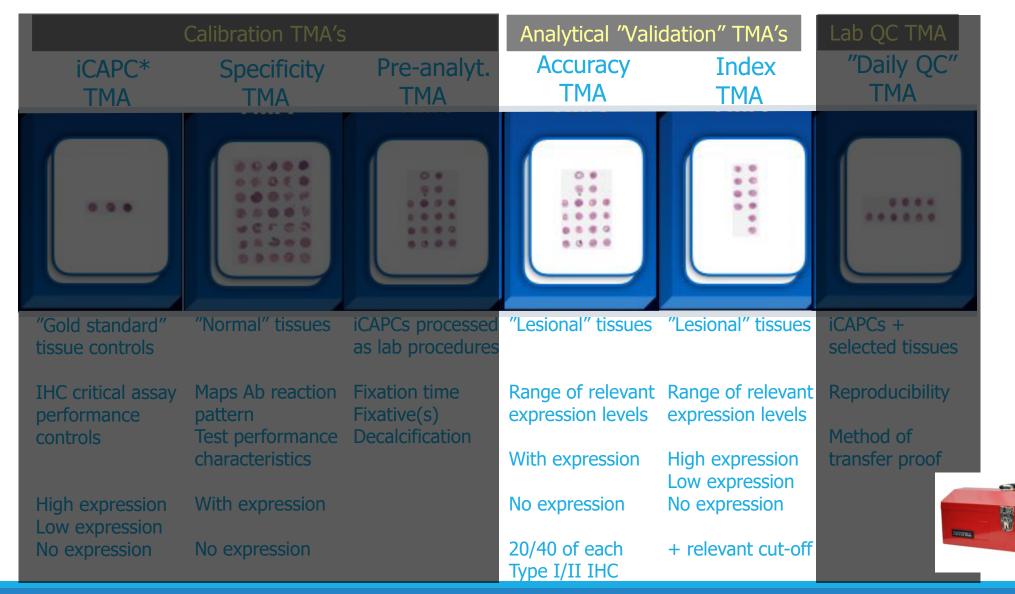
The journey from an antibody to a diagnostic IHC assay with a specific purpose

- 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



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.

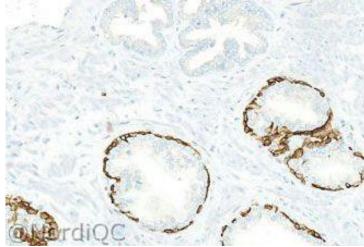
Number perhaps less important compared to use of tissue with full range of expression patterns reflecting the diagnostic use and purpose of test

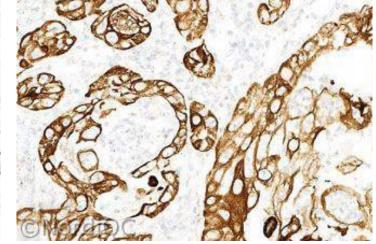
E Torlakovic et al. AIMM 2017;25:4-11 Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 1

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

IHC for CK5

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





Prostate sample

Lung sample

Same protocol applied for different purposes and meeting the requirements

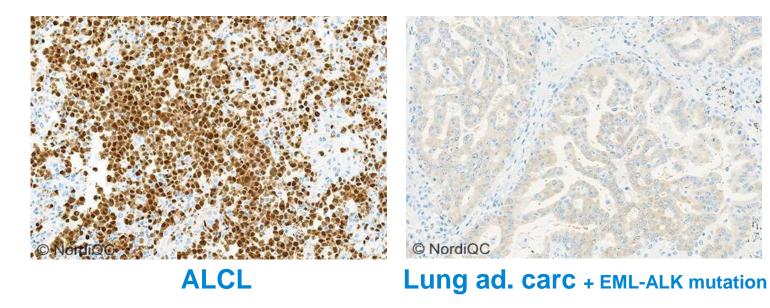
(source; www.nordiqc.org)

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

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

IHC for ALK

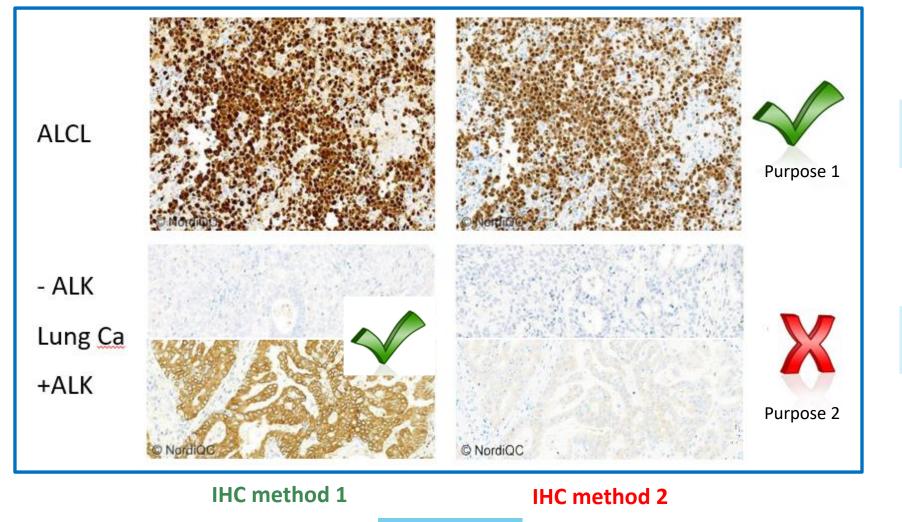
- 1. To identify anaplastic large cell cell lymphoma
- 2. To identify lung adenocarcinoma with ALK mutation
- 3.



Same protocol applied for different purposes **not** meeting the requirements

(source; www.nordiqc.org)

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 for ALK

Typically <u>high</u> antigen expression level

Typically <u>low</u> antigen expression level

49

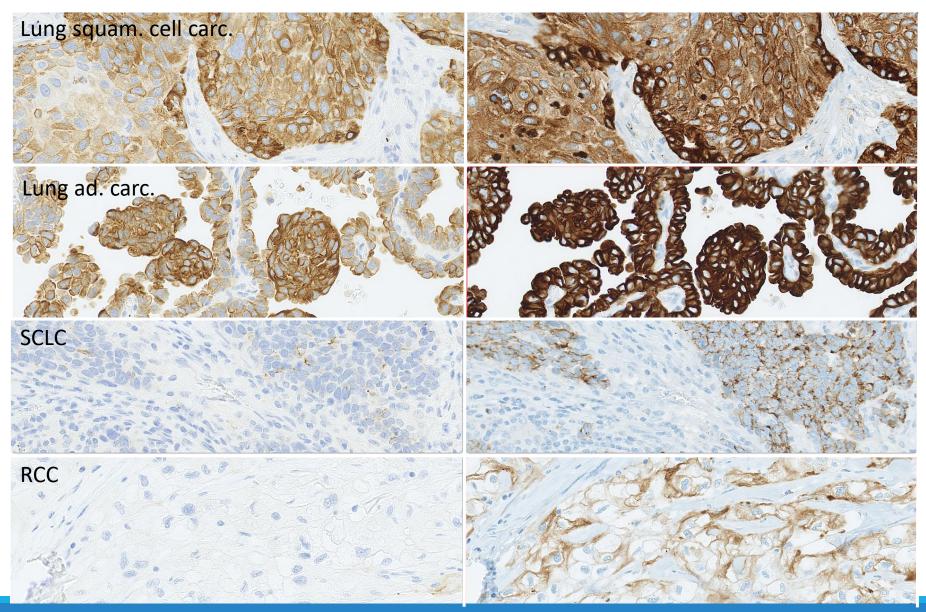
IHC tests must be fit-for-purpose....

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 squamus cell carc vs adenocarcinoma	
CK-PAN	CUP*	Sentinel node status - carcinoma	
GATA3	Breast carcinoma – CUP	Urothelial carcinoma - CUP	
lgK / lgL	Clonality myeloma (Cytopl)	Clonality lymphoma (Membrane)	
Melan A	Melanoma	Sex cord tumours [¤]	[¤] mAb A103 only
PAX5	B-cell lineage marker (Lymphoma)	Hodgkin	
SOX10	Melanoma - CUP	TNBC - CUP	
TTF1	Lung ad. carc CUP	Lung squamus cell carc vs adenocarcinoma	

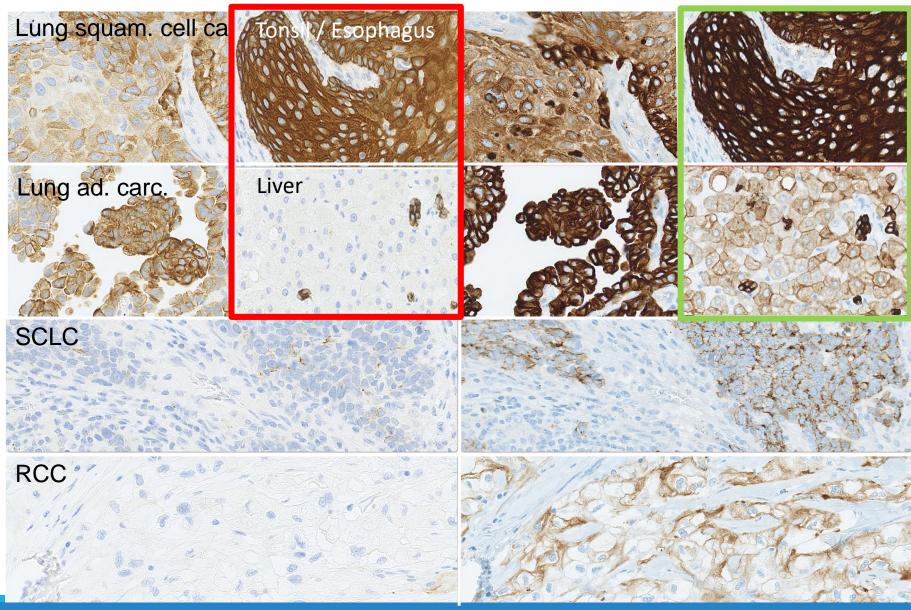
Use of samples for technical / analytical validation of IHC



CK-PAN - mAb AE1/AE3 – Prot. 1

CK-PAN - mAb AE1/AE3 – Prot. 2

Use of samples for technical / analytical validation of IHC



CK-PAN - mAb AE1/AE3 – Prot. 1 CK-PAN - mAb AE1/AE3 – Prot. 2

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 l	Purpose II	Influenc. factors
CK-Pan	CUP - carcinoma lineage	Sentinel node – carcinoma metastatis	Clone, titer, retrival
СК 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

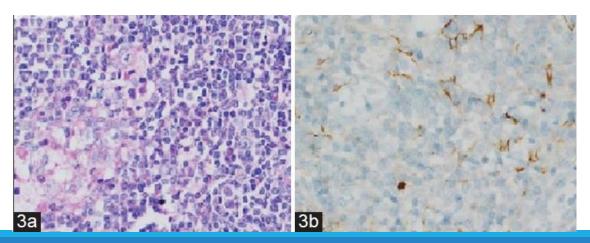
Protocol developed, optimized and validated for purpose II will most likely compromise use for purpose I due to a reduced level of analytical sensitivity



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

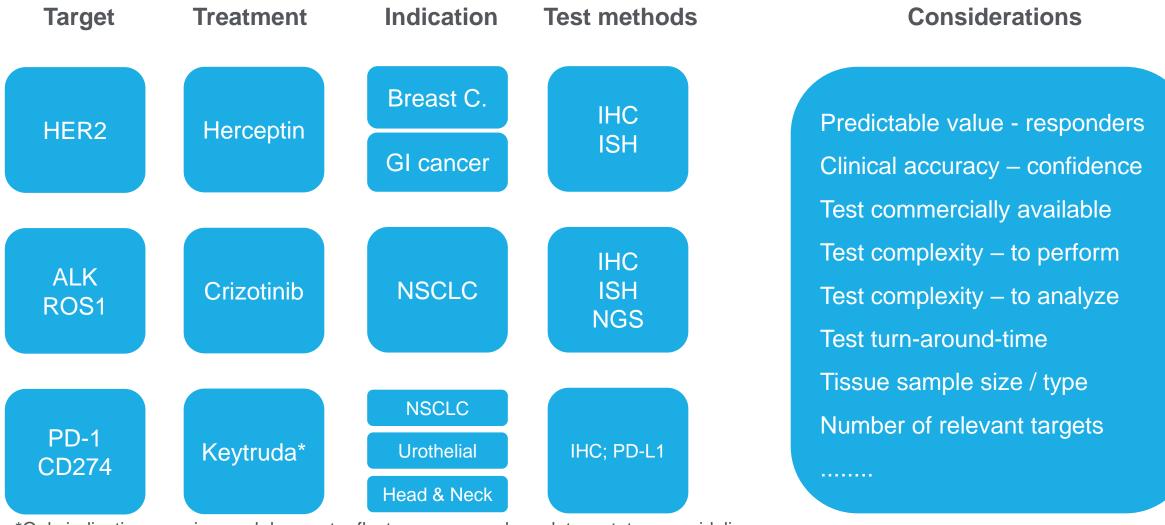
Can and will provide interpretational challenges in SN due to labelling of specialized macrophages with CK8/18

CK19 more selective (CK19 mRNA applied for OSNA technique)

Conclusions for technical / analytical validation of IHC

- IHC assay is calibrated (LD assay) / verfied (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.
- IHC assay is validated on TMAs with e.g. 30-45 commonly seen neoplasias and on TMAs with the target of interest – 10/10 or 20/20 neoplasias expected to be pos./neg. (accuracy) covering the dynamic range of expression and cut-off's (index) – note not all markers are reliable if only TMA's are used (e.g. heterogene expression)
- 3. Results compared to literature, reference clone etc and conclusion made.

Role of non-IHC methods to guide quality / accuracy



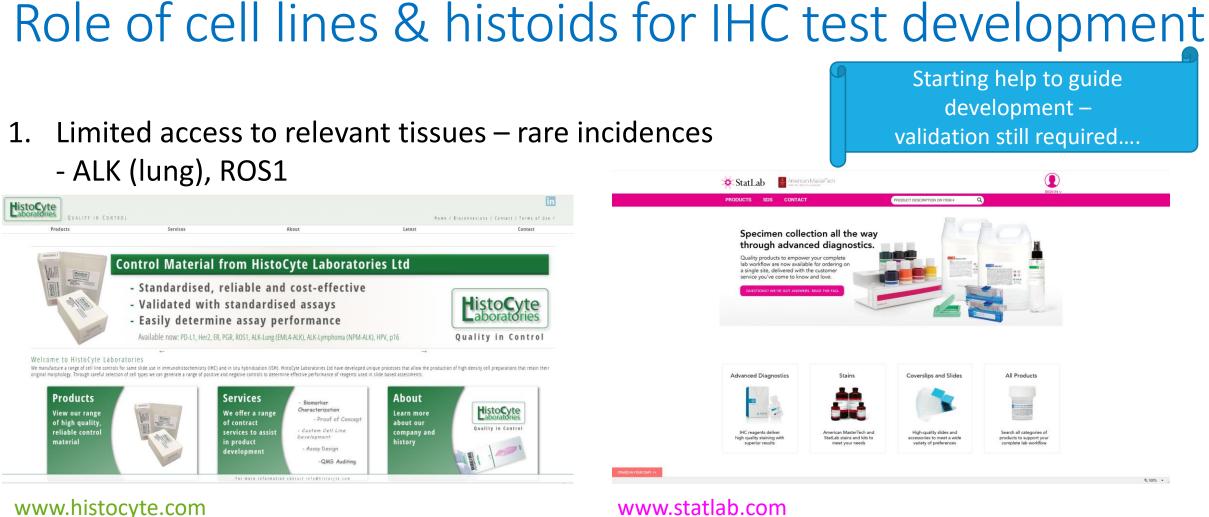
*Only indicative overview and does not reflect any approved regulatory status or guidelines

Challenges for technical / analytical validation of IHC

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

 PD-L1 IHC





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

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

Online ressources – "www.antibodypedia.com"

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

GeneTex GeneTex

antil	oodypedia		About Us Contact	FAQ For Providers Sign in
Q Explore	L Validate	Learn		
			Application	Search help
Search for	1		Any	▼ Search Advanced search ▼
e.(g. Her2, Transcription factors, Chromos	ome X		

A portal for validated antibodies

Antibodypedia scores antibodies to guide researchers to choose an appropriate antibody for a particular application. The resource contains information about more than three milion publicly available research antibodies towards over 19,000 human protein targets from more than 80 providers.

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Featured Validations

Immunchistochemistry-Paraffin: MEKK4 Antibody (6C6) [H00004216-M02] - Analysis of monoclonal antibody to MAP3K4 on formalin-fixed paraffinembedded human testis. Antibody concentration 3 ug/ml. More info

Home > Search result About Us Contact FAQ For Providers Sign in antibody Learn Explore Validate Search heln Application IHC Search for satb2 Search Advanced search Found 1 gene products for 'satb2' DESCRIPTION FAMILY CHROMOSOME UNIPROT MOUSE ORTHOLOG NAME 1 SATB2 Q9UPW6 Satb2 SATB homeobox 2 D 2:q33.1 126 of 256 antibodies matching filte Synonyms: FLJ21474, KIAA1034 Up from 25 of 34 providers Za Eb Ea Tf Home > Search result > SATB2 About Us Contact FAQ For Providers Sign in antibody Validate Learn Explore SATB2 gene product FLJ21474, KIAA1034 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] More gene data FEATURED ANTIBODIES WB 🔘 TATLAS ANTIBODIES Atlas Antibodies AMAb90635 4 references Monoclona

GTX30894

0 references

Polyclonal

Content updated 2019-08-08 4008379 reviewed antibodies from 85 providers, covering gene-products encoded by 19165 genes (approximately 94% of all human genes). Primary data available for 1972133 experiments.

59

IHC ★ WB ● ICC ●

EL 🕘 IHC 🖲

Online ressources – "www.antibodypedia.com"

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

Validate TB2 antibody from Allas A I21474, KIAA1034 Eligible for validation within			Search	C
121474, KIAA1034				
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SKOV3 xenograft

Figure 1

From: Evaluating real-time immunohistochemistry on multiple tissue samples, multiple targets and multiple antibody labeli

Mouse live

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Heart musc

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 ressources – "www.proteinatlas.com"

THE HUMAN PROTEIN ATLAS 🏞	THE HUMAN PROTEIN ATLAS Sate2 EMENU Help News
SATB2 e.g. ACE2, GFAP, EGFR	1 GENE FOUND ⁱ Show / hide columns ▼ Download: XML RDF TSV JSON Custom TSV/JSON ▼ Page 1 of 1 Gene ⁱ Gene description ⁱ × Evidence ⁱ × Trissue ⁱ Cell type ⁱ Pathology ⁱ Blood ⁱ Cell ⁱ SATB2 SATB homeobox 2 ■ ↓↓↓↓↓ RNA ↓↓↓↓↓ RNA ↓↓↓↓↓
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Online ressources – "www.proteinatlas.com"

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		Contractor					63

Main elements to develop & validate IHC assays

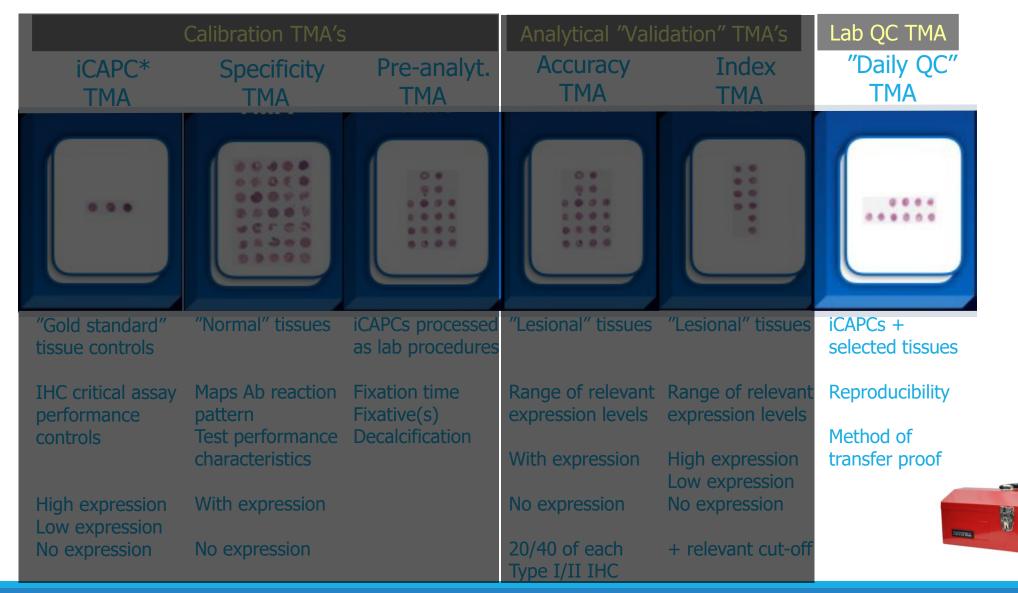
The journey from an antibody to a diagnostic IHC assay with a specific purpose

- 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 <u>Method transfer</u>.

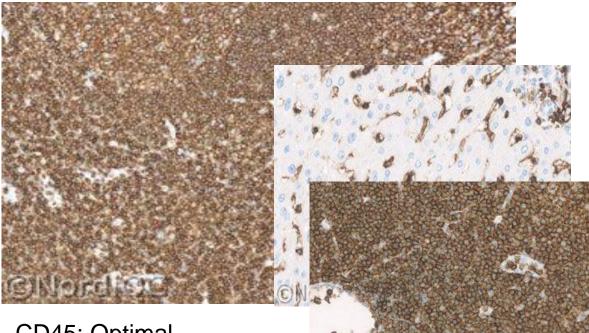
Based on selection and use of appropriate external tissue controls

External tissue control tool box

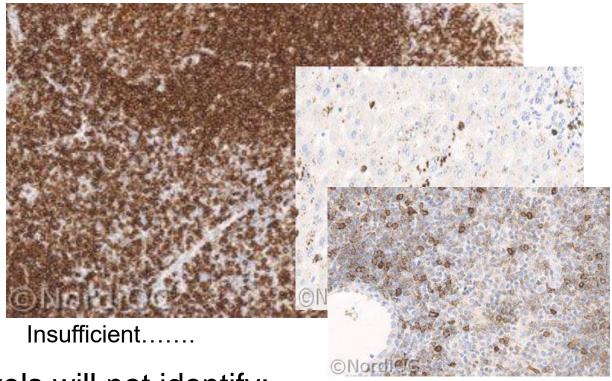
E Torlakovic et al. AIMM, 2017; 25:227-230 Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine: Part 4



Tissue controls; Fit for purpose - relevant range of analyte



CD45: Optimal



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 !

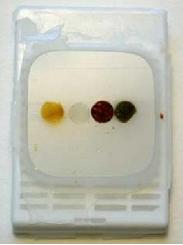
Composition of TMA for QC of diagnostic IHC

B1:	Appendix,	Hepar,	Tonsil,	Pan	creas
	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
	СК20	CK LMW	CD10		
	DOG1	CK8	CD20		
	MMR	CK18	CD21		6
	S100	HEPA	CD23		1
	SYP	Arginase	CD38		
			CD56		
Used together a	ddressing:		CD798	a	
High expression	•		CD13	8	14
- ·	 right sensitivity 		СК Ра	n	
	inglit sensitivity		CvD1		-

No expression – right specificity

CyD1

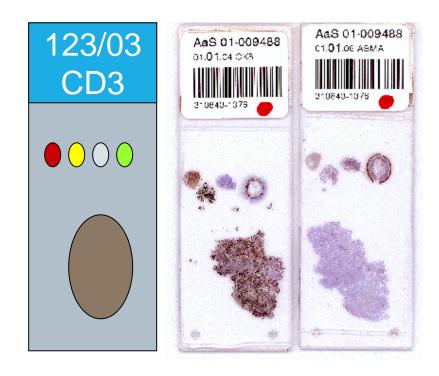
EMA



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, PhD⁵; Meredith M. Regan, ScD¹²; David L. Rimm, MD, PhD¹³; W. Fraser Symmans, MD¹⁰; Emina E. Torlakovic, MD, PhD^{14,13}; Leticia Varella, MD¹⁶; Giuseppe Viale, MD^{17,18}; Tracey F. Weisberg, MD¹⁹; Lisa M. McShane, PhD²⁰; and Antonio C. Wolff, MD²¹ "even for automated stainers, where it cannot be guaranteed that every slide in fact receives identical treatment".

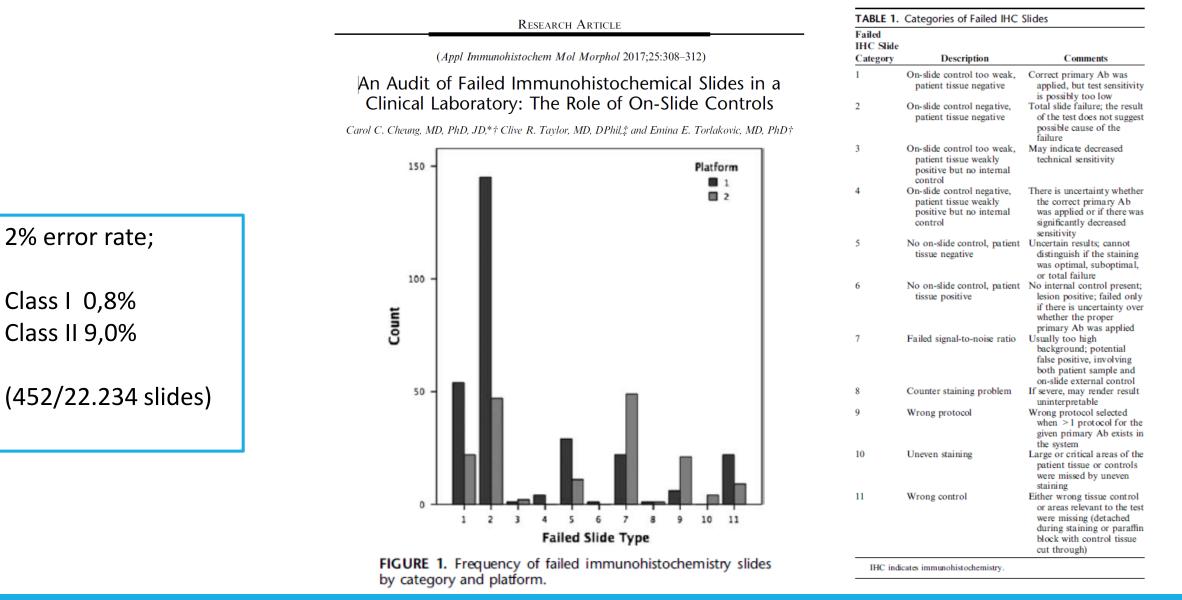


Target	2006	2020
Estrogen receptor	12%	46%
ALK	9%	60%

Participants using onslide controls for slide submitted to NordiQC

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

Application of TMA for QC of diagnostic IHC



⁷¹

Application of TMA for QC of diagnostic IHC

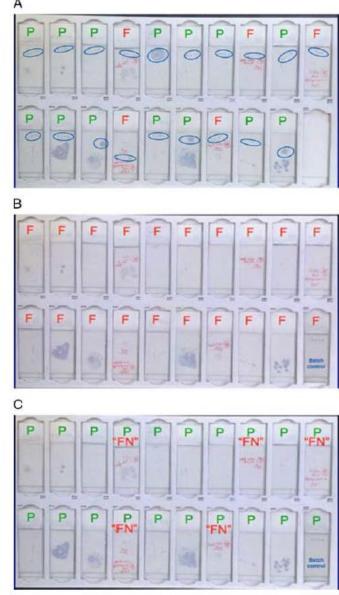
A: On-slide controls

IHC slides stained for ALK (Class II),same run, same instrument, same protocol14/19 passed5/19 failed (5 x 150 USD)

B: Batch-control - Theoretically:

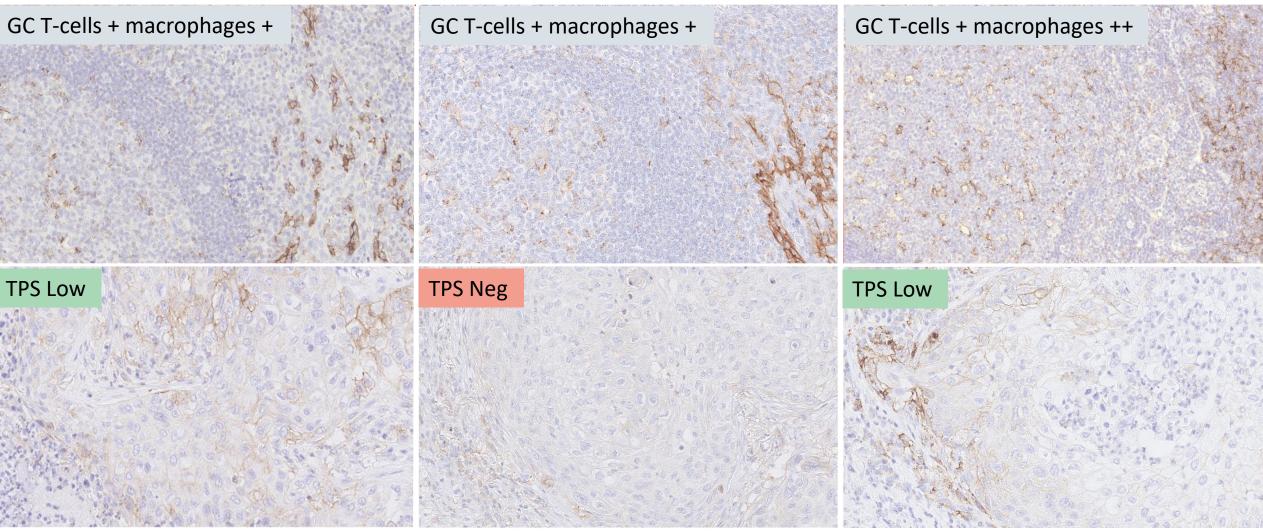
Batch control <u>failed</u> by same conditions as above 0/19 passed 19/19 failed (no consistent internal control...) (20 x 150 USD)

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...???)



Challenges; On-slide controls for accuracy / reproducibility

PD-L1 in NSCLC for KEYTRUDA

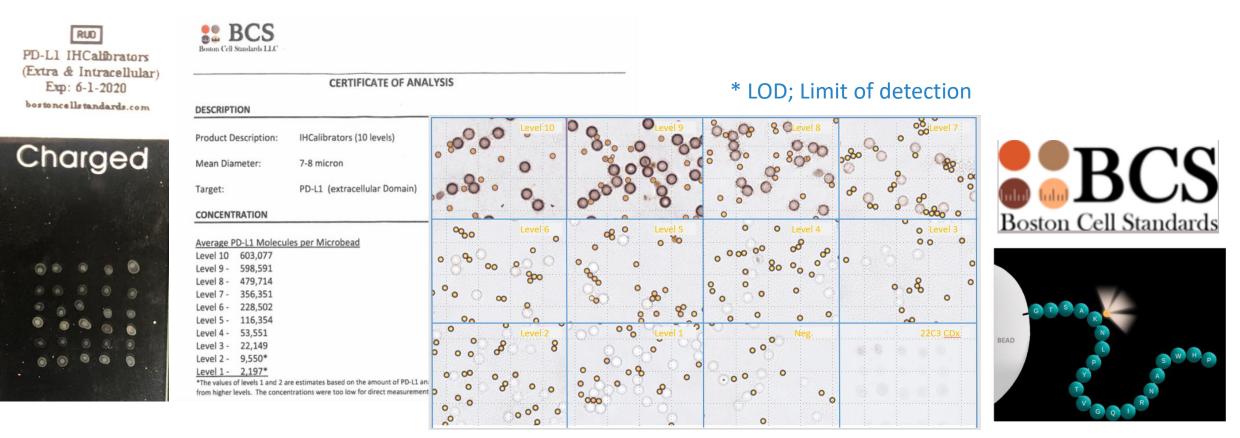


22C3 CDx – SK006

ZR3 – LDT method 1

ZR3 – LDT method 2

Reference standard materials for IHC; Calibrators – LOD*



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.

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

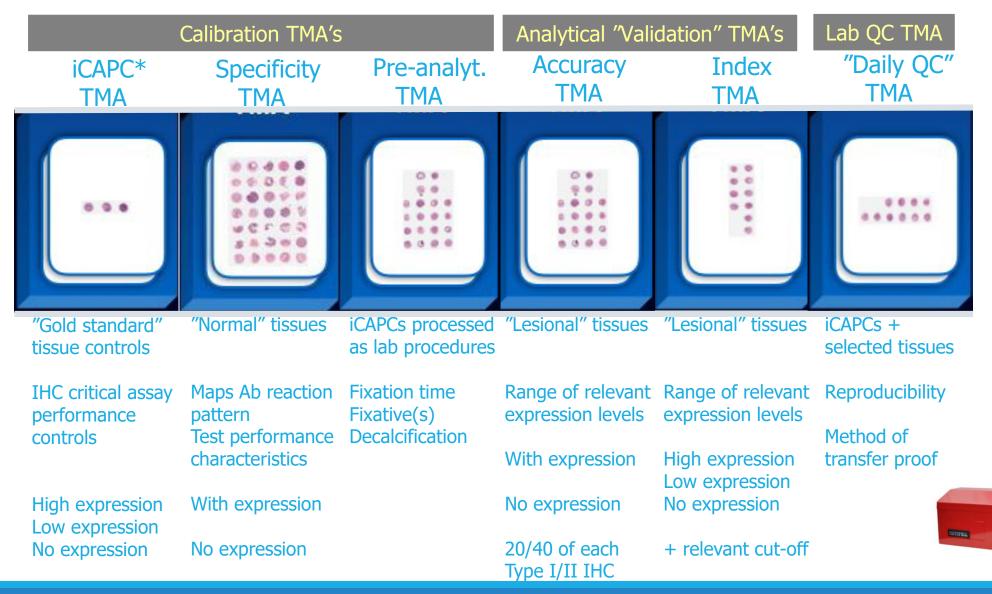
Conclusions

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

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

External tissue control tool box

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



Questions and/or comments



Thank You for the attention and.....