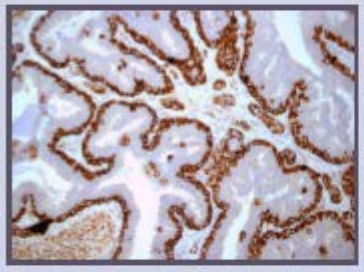
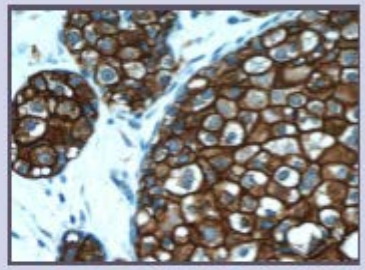




NordiQC

TATA MEDICAL CENTER

ISIMm
International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

Immunohistochemical principles
The technical test approach

Analytical parametres I

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

IHC – The Technical Test Approach

CME - DAY 1 Thursday - 04.01.2018	
09:00 – 13:00	***Morning session – the IHC and molecular laboratory. Moderator: CliveTaylor***
09:00 – 09:30	Søren Nielsen: Pre-analytical IHC parameters
09:35 – 10:10	Søren Nielsen: Analytical IHC parameters I (clone selection, protocol optimization, automation)
10:15 – 10:45	*Coffee break*
10:45 – 11:30	Mogens Vyberg: The impact of proficiency testing on lab immunoassays
11:35 – 12:10	Søren Nielsen: Analytical IHC parameters II (control selection)
12:15 – 12:55	T. S. Sridhar : Molecular studies on FFPE tissue
13:00 – 14.30	*Lunch break*
14:30 – 17:00	***Afternoon interactive Parallel IHC Session***
	IHC session, technicians | *IHC session, pathologists*
14:45 – 15:45	Søren Nielsen: Technical pitfalls, trouble shooting, internal quality control - **for technicians** Taylor, Bhargava, Vyberg: Diagnostic pitfalls, trouble shooting - **for pathologists**
15:45 – 16:15	*Coffee break*
16:15– 17:15	Søren Nielsen: Technical pitfalls, trouble shooting, internal quality control (cont'd) - **for technicians** Taylor, Bhargava, Vyberg:Diagnostic pitfalls, trouble shooting (cont'd) - **for pathologists**

Automation next session

Analytical IHC parametres II

IHC – The Technical Test Approach

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

Fixation
Time, Type, Volume

Decalcification
Preparation

Pre-analytic

Tissue
Type, Dimension,
Laser resection,
De-differentiation

Section
Thickness
Storage
Drying

Pre-treatment

Primary antibody
Clone, Dilution
Buffer, Time, Temp

Manual
Stainer

Analytic

Visualization
Sensitivity, Specificity

Development
Sensitivity,
Localization

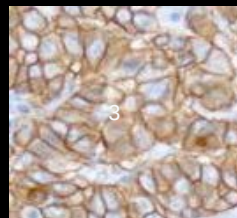
With 3 choices for 5 variables in each phase = > 4 million protocols….

Controlment

Quantification
Reporting

Post-analytic

Interpretation
Localization
Positive/Negative - cut-off level



IHC – The Technical Test Approach

Issues to be adressed for IHC assay implementation:

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

<u>Tissue controls are key element</u>

IHC – The Technical Test Approach

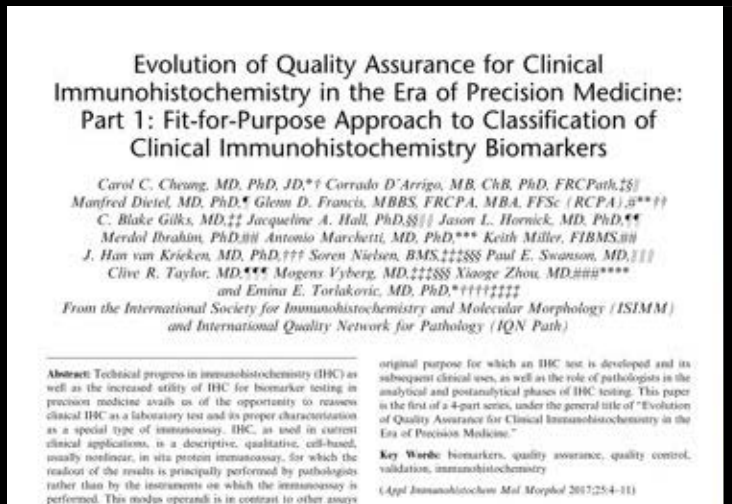
External tissue control tool-box:

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	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility Method of transfer proof
High expression Low expression No expression	With expression No expression		With expression No expression	High expression Low expression No expression	
			20/40 of each Type I/II IHC	+ relevant cut-off	

*Immunohistochemical critical assay performance controls

IHC – The Technical Test Approach

AIMM: January to April 2017



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

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,¶¶ Merdol Ibrahim, PhD,## Antonio Marchetti, MD, PhD,*** Keith Miller, FIBMS,## J. Han van Krieken, MD, PhD,††† Soren 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.[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 2: Immunohistochemistry Test Performance Characteristics

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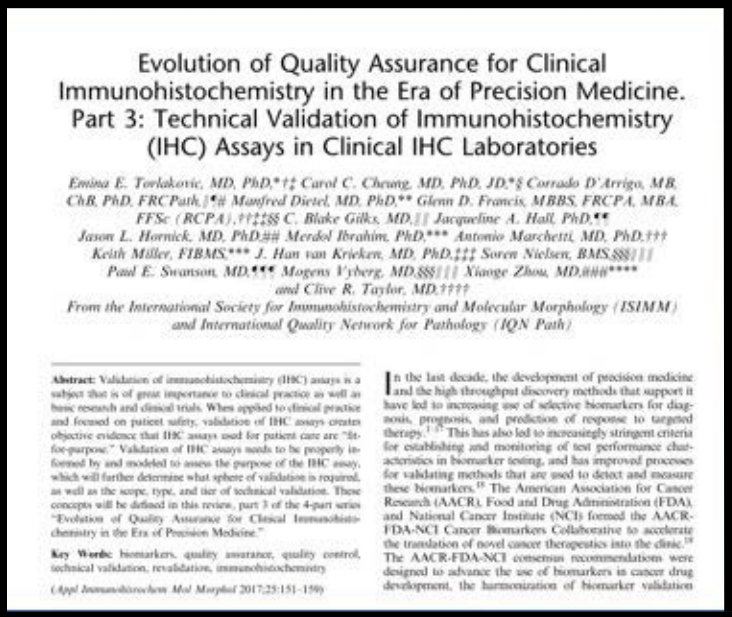
Abstract: All laboratory tests have test performance characteristics (TPCs), whether or not they are explicitly known to the laboratorian or the pathologist. TPCs are thus also an integral characteristic of immunohistochemistry (IHC) tests and other in situ, cell-based molecular assays such as DNA or 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

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,‡‡ 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,‡‡‡ Soren Nielsen, BMS,§§§‖‖‖ 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 therapy.[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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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

IHC – The Technical Test Approach

Issues to be adressed for IHC assay implementation:

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

<u>Tissue controls are key element</u>

IHC – The Technical Test Approach

Issues to be adressed for IHC assay implementation:

1. Calibration of IHC assay and identification of best practice protocol – clone, titre, retrieval etc
    - Concentrated formats
        - Full test comprising various titres, retrieval settings, detection systems (stainer platform)
    - Ready-To-Use formats
        - Confirmatory test primarily using official recommendations and if needed modifications e.g. incubation times, detection system etc

IHC – The Technical Test Approach

Concentrated antibodies – VMS ULTRA

	1:25	1:100	1:400
A	None	None	None
B	Enzyme P1, 4 min	Enzyme P1, 4 min	Enzyme P1, 4 min
C	HIER CC1 pH 8.5*	HIER CC1 pH 8.5	HIER CC1 pH 8.5
D	HIER CC2 pH 6.0*	HIER CC2 pH 6.0	HIER CC2 pH 6.0
(E)	CC1 + Enzyme P3, 8 min	CC1 + Enzyme P3, 8 min	CC1 + Enzyme P3, 8 min
(F)	Enzyme P3, 8 min + CC1	Enzyme P3, 8 min + CC1	Enzyme P3, 8 min + CC1

*HIER time 48 min. at 99°C

OptiView DAB, Ventana BenchMark Ultra

Protocol A: 2 %
Protocol B: 3 %
Protocol C: 90 %
Protocol E: 3 %
Protocol F: 1 %
Others : 2 % (E.g. prolonged HIER, prolonged proteolysis)

IHC – The Technical Test Approach

Concentrated antibodies – Dako AS48 Link* or OMNIS**

	1:25	1:100	1:400
A	None	None	None
B	Proteinase K, 5 min	Proteinase K, 5 min	Proteinase K, 5 min
C	HIER TRS pH 9.0* **	HIER TRS pH 9.0* **	HIER TRS pH 9.0* **
D	HIER TRS pH 6.1* **	HIER TRS pH 6.1* **	HIER TRS pH 6.1* **
(E)	TRS 9.0 + Prot. K**, 5 min	TRS 9.0 + Prot. K**, 5 min	TRS 9.0 + Prot. K**, 5 min
(F)	Prot. K**,5min + TRS 9.0	Prot. K**,5min + TRS 9.0	Prot. K**,5min + TRS 9.0

*HIER time 20 min. at 97°C
** HIER time 25-30 min. at 97°C
** Proteinase K S3020, 1:50

EnVision FLEX+

A	app. 1-2%
B	app. 1-2%
C	app. 90%
D	app. 2-5%
E+F	app. 1-2%

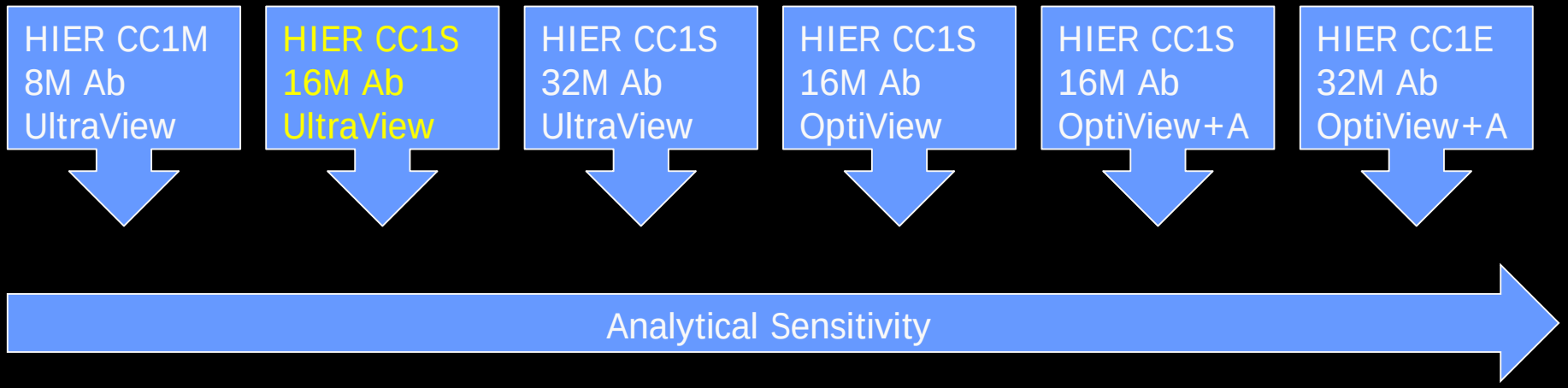
IHC – The Technical Test Approach

Ready-To-Use – VMS ULTRA

RTU

Typical protocol:

A: HIER in CC1 standard (64 min.), 16 min. Incubation time in primary Ab and UltraView-DAB



IHC – The Technical Test Approach

The basal fundament for a technical optimal IHC performance:

- Appropriate tissue fixation and processing
- Appropriate and efficient epitope retrieval
- Appropriate choice & titre of antibody/clone
- Robust, specific & sensitive detection system
- Appropriate choice of control material

IHC – The Technical Test Approach

Pre-treatment / Epitope retrieval:

Defined as an unmasking method for ”re-storing” blocked antigens in formaldehyde fixed tissue

The key to an optimal IHC reaction…

IHC – The Technical Test Approach

Heat Induced Epitope Retrieval

Optimized temperature-time-pH-buffer system

‘Heating condition’ = temperature × time:

121ºC/1 min 100ºC/20 min 95ºC/40 min 60ºC/24 h.

Device:
Stainer
Water bath
MWO
Pressure cooker
Pressure cooker & MWO
Autoclave
Steam

Considerations:
Efficiency
Standardization
Tissue damage
Performance

IHC – The Technical Test Approach

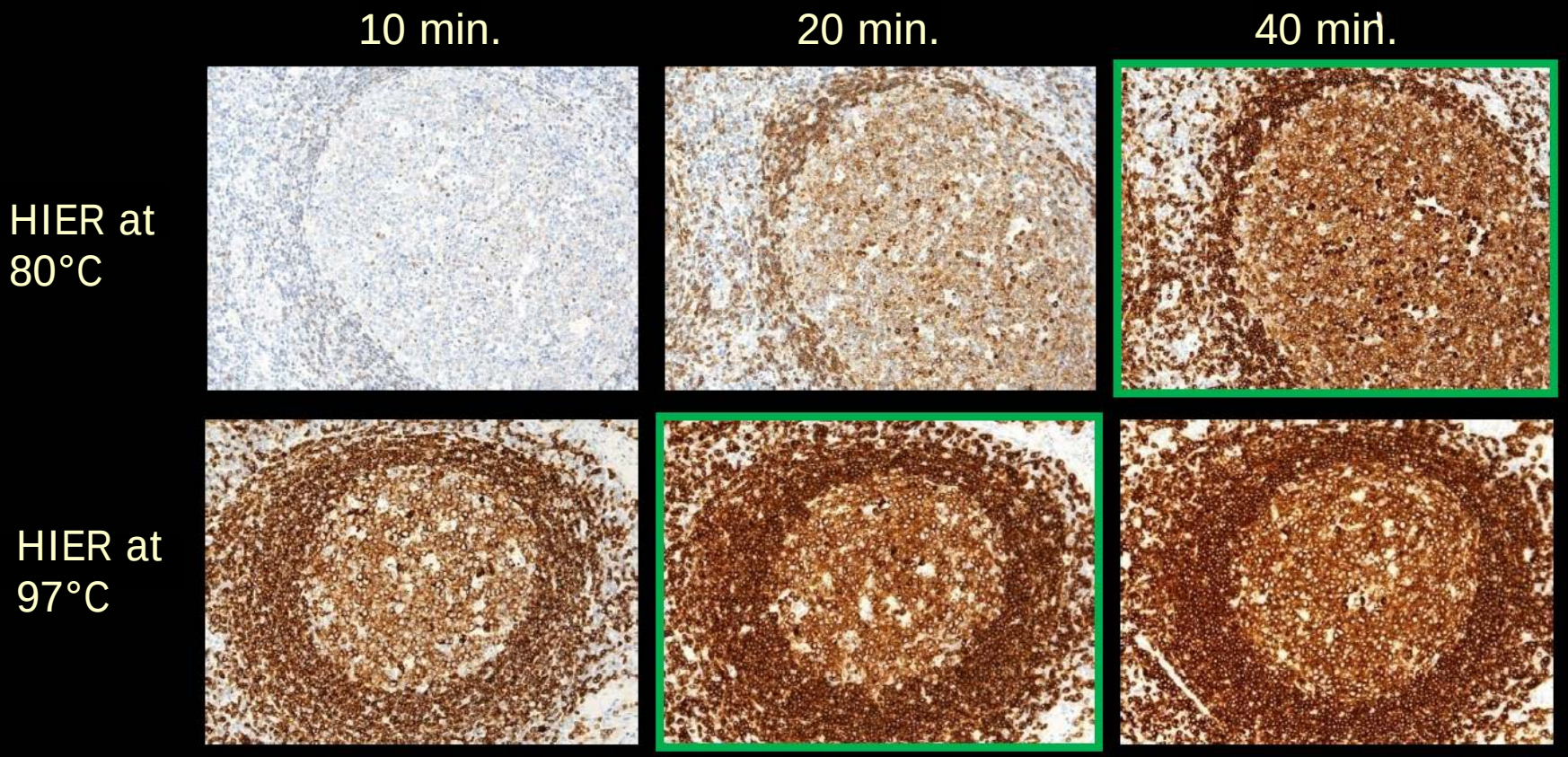
HIER; Influence of heating time and temperature

Tonsil fixed 48 hours in 10% NBF

IHC for CD79a (mAb clone JCB117)

HIER performed in PT Link using TRS High pH 9 (Dako)

Identify HIER settings to obtain a consistent level – maximum sensitivity and preserved morphology



IHC – The Technical Test Approach

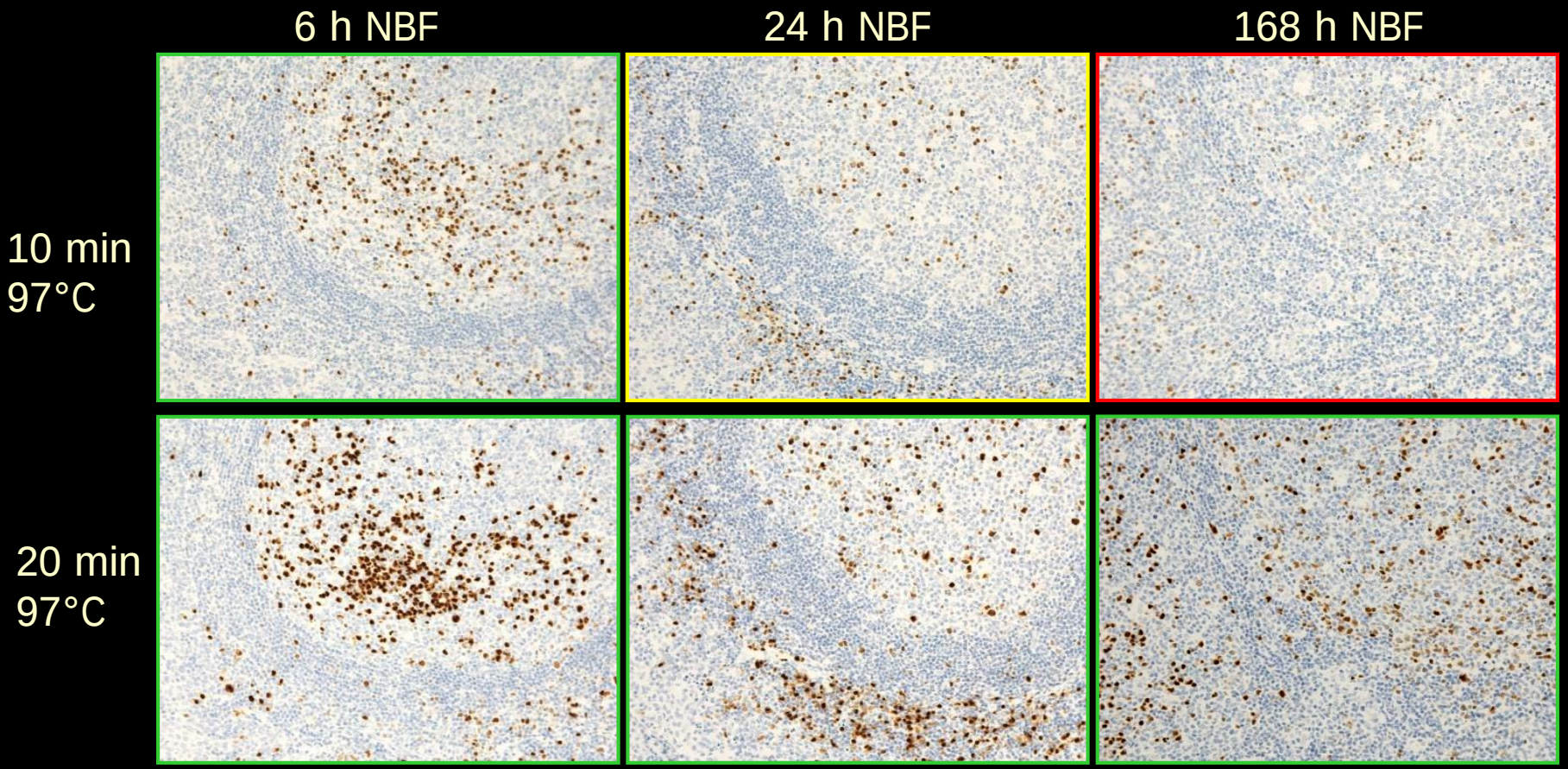
HIER; Influence of heating time and fixation time

Tonsil fixed 48 hours in 10% NBF

IHC for MUM1 (mAb clone MUM1p)

HIER performed in PT Link using TRS High pH 9 (Dako)

Identify HIER settings to obtain a consistent level – not being influenced by fixation time



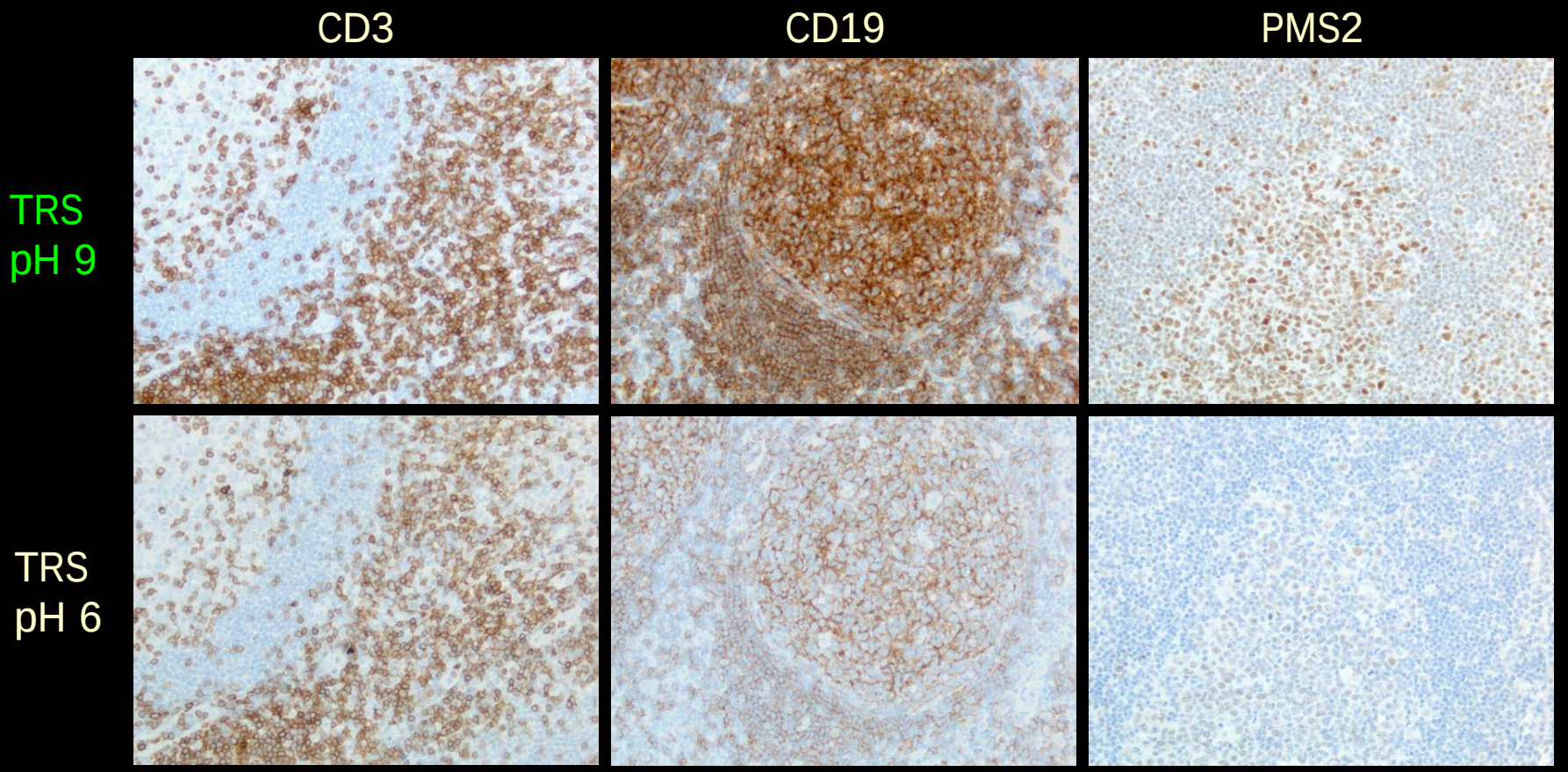
IHC – The Technical Test Approach

HIER; Influence of pH and chemical composition of HIER buffer

Identify HIER buffer to obtain maximum and consistent level of sensitivity
90% High pH buffers

Tonsil fixed 48 hours in 10% NBF - – HIER 20 min at 97°C

IHC for CD3 (mAb clone PS1), CD19 (mAb clone LE-CD19), PMS2 (mAb clone A16-4)



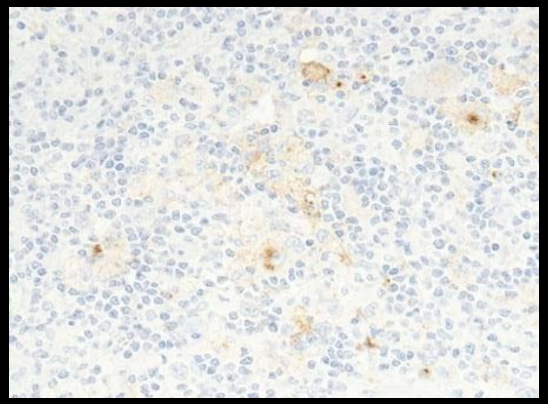
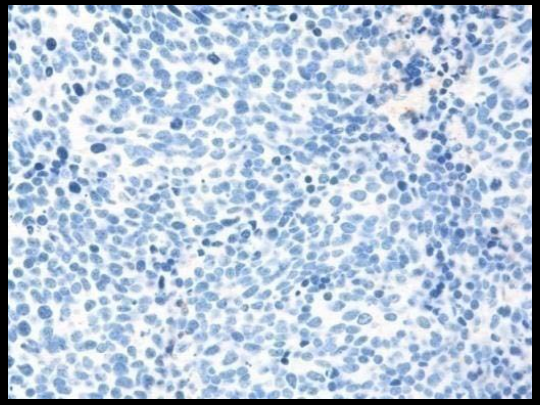
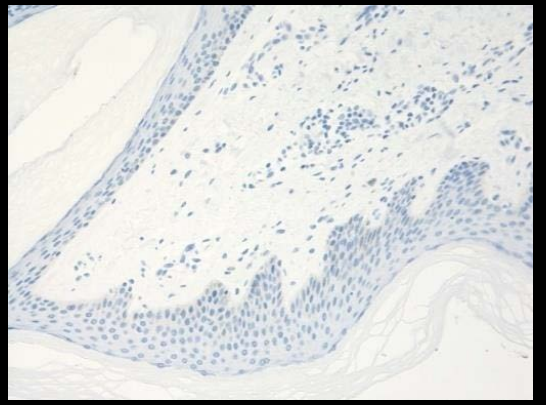
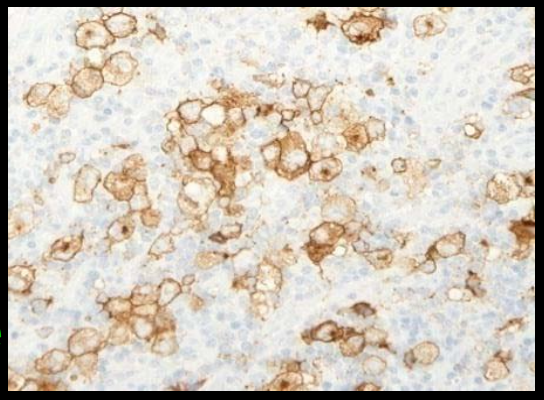
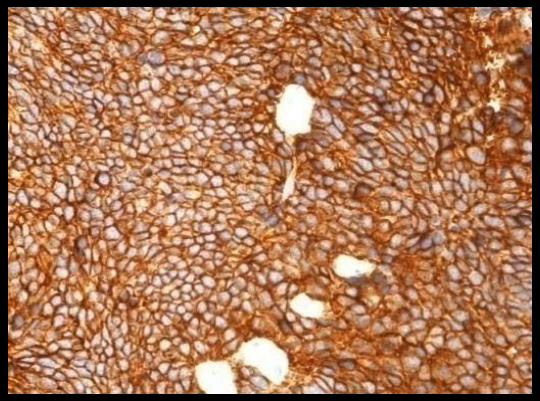
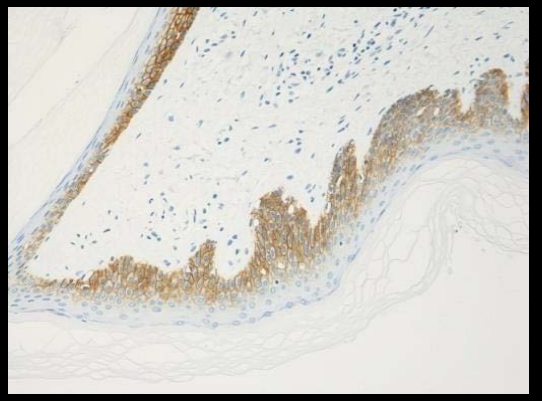
IHC – The Technical Test Approach

HIER; Influence of pH and chemical composition of HIER buffer

Identify HIER buffer to obtain maximum and consistent level of sensitivity
5% Low pH buffers

Material fixed in 10% NBF – HIER 20 min at 97°C

IHC; CD30 (mAb ConD6/B5), EPCAM (mAb MOC31), Desmoglein 3 (mAb clone BC11)

	CD30	EPCAM	Desg. 3
TRS pH 9			
TRS pH 6.1 Dako Or Biocare			

IHC – The Technical Test Approach

On board HIER :

General settings

XT / Ultra:	Cell Conditioning 1 pH 8.5 at 97°C - 99°C
	48-64 min. for OptiView DAB / UltraView DAB
Bond-max:	BERS2 (pH 9) at 100°C
	20 min. for Refine DAB
Omnis:	TRS High (pH 9) or TRS Low (pH 6.1) at 97°C
	25-30 min. for FLEX / FLEX+

IHC – The Technical Test Approach

Primary antibody

Optimal antibody-antigen reactions in formalin fixed tissue depends on:

Antibody clone/source – Sensitivity/Specificity

IHC – The Technical Test Approach

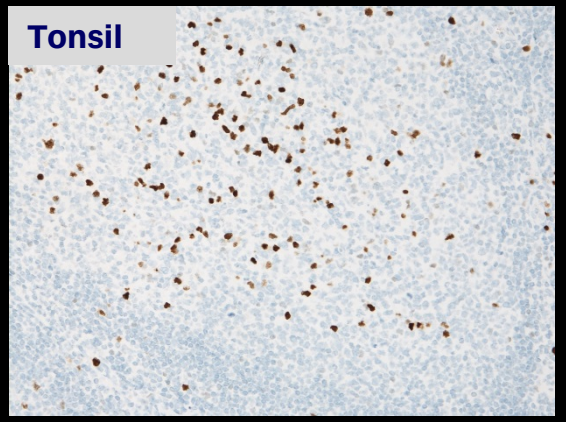
Table 1. **Antibodies and assessment marks for MUM1, run 48**

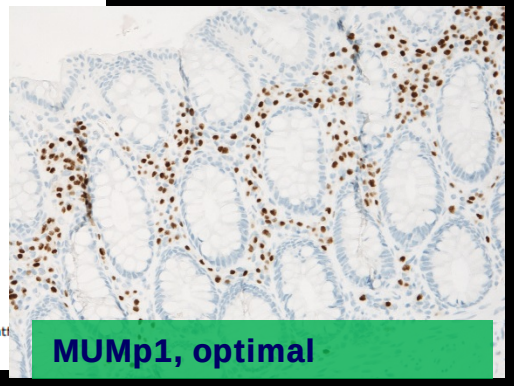
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MUMp1**	84 1 1	Agilent/Dako Diagnostic Biosystem GeneMed	52	19	11	4	83%	86 %
mAb clone **MRQ-8**	3	Cell Marque	0	0	2	1	-	-
mAb clone **BC5**	3	Biocare Medical	0	0	3	0	-	-
mAb clone **EAU32**	3	Leica/Novocastra	0	2	1	0	-	-
rmAb clone **MRQ-43**	5 1 1	Cell Marque Menarini Zeta	0	0	3	4	-	-
rmAb clone **SP114**	1	Thermo S./ LabVision	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **MUMp1 GA644**	18	Agilent/Dako	8	7	2	1	83%	88 %
mAb clone **MUMp1 IR/IS644**	28	Agilent/Dako	13	12	3	0	89%	88 %
mAb clone **MUMp1 GA644, IR/IS644**[3]	5	Agilent/Dako	3	0	2	0	-	-
mAb clone **MUMp1 MAD-000470QD**	3	Master Diagnostica	1	1	1	0	-	-
mAb clone **MUMp1 MAB-0573**	1	Maixin	1	0	0	0	-	-
mAb clone **EAU32 PA0129**	6	Leica Biosystems	5	1	0	0	100%	100%
rmAb clone **MRQ-43 760-4529**	31	Ventana/Roche	0	0	25	6	0%	0%
rmAb clone **MRQ-43 358R-77/78**	15	Cell Marque	0	0	13	2	0%	0%
rmAb clone **EP190 358R-17/18**	1	Cell Marque	1	0	0	0	-	-
Total	211		84	43	66	18	-	
Proportion			40%	20%	31%	9%	60%	

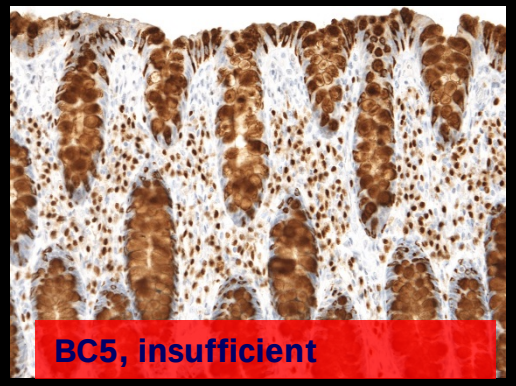
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU systems developed for Agilent/Dako`s automatic systems (Omnis/Autostainer) but used by laboratories off-label on the platf
Ventana Benchmark/Ultra or Leica BOND III.

MUM1

All abs:



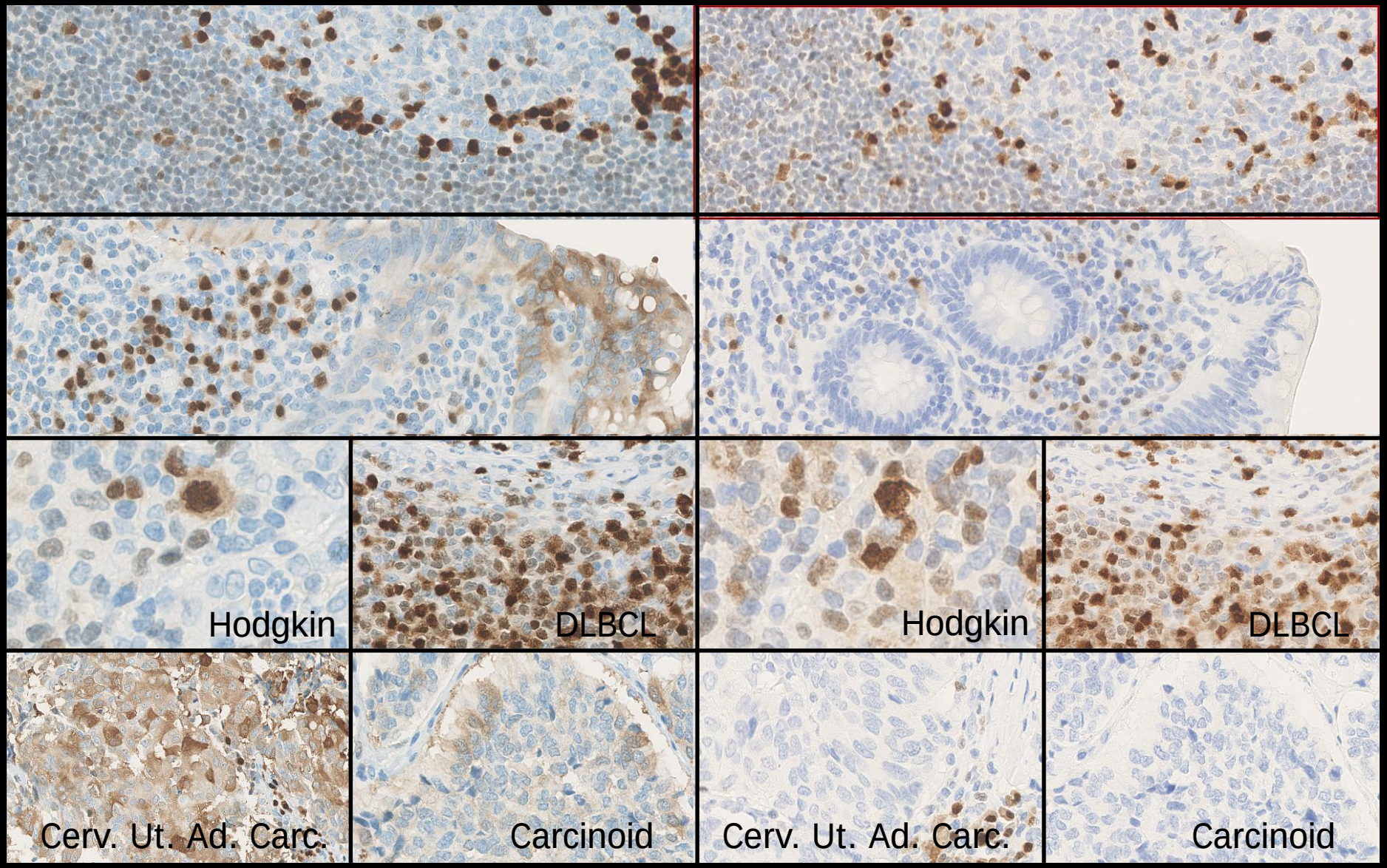




Primary antibodies providing low specificity and/or poor signal-to-noise ratio (NordiQC results/Latest run)

MUM1 clone MRQ-43 & BC5
CK-HMW clone 34βE12
PR clone 1E12
Many pAbs (e.g. p40 and SOX10)
……

IHC – The Technical Test Approach



MUM1 - mAb MRQ-43

MUM1 - mAb EUA32 & MUM1p

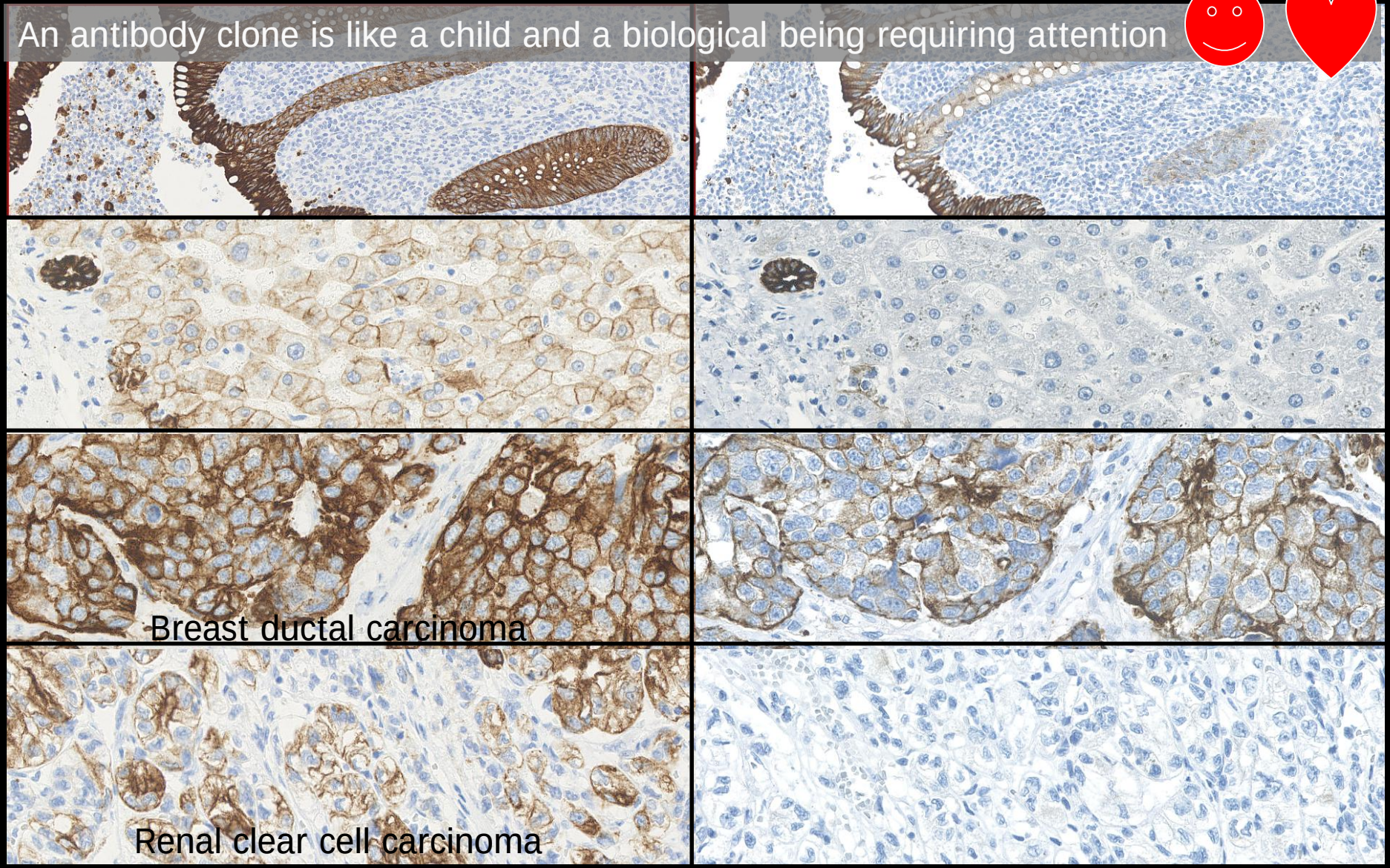
NordiQC – Platform depending antibodies

Antigen	Clone	XT / Ultra	Autostainer	Bond-max
BCL6	**PG-B6p**	**FN (3%H2O2)**	√	**FN (3%H2O2)**
BCL6	**GI191E/A8**	√	√	√
BSAP	**24**	**FN**	√	**(- Weak)**
BSAP	**SP34**	√	√	√
CD4	**1F6**	**FN (3%H2O2)**	√	**FN (3%H2O2)**
CD4	**SP35**	√	√	√
CD56	**123C3**	**FN**	√	**(-Weak)**
CD56	**MRQ-42**	√	√	**?**
CK LMW (8/18)	**5D3**	**(-Weak/FN)**	√	√
CK LMW (8/18)	**B22.1 / B23.1**	√	√	√
Melan A	**A103**	**(-Weak/FN)**	√	√
Melan A	**EP43**	√	√	√
Oct-2	**OCT-207**	**FN**	√	**?**
Oct-2	**MRQ-2**	√	**?**	**?**
SYP	**27G12**	**Weak**	√	√
SYP	**MRQ-40**	√	√	√

Automated Semi-Automated Automated

NordiQC

An antibody clone is like a child and a biological being requiring attention



CK LMW – mAb B22.1/B23.1

CK LMW – mAb 5D3

VMS Ultra - OptiView

IHC – The Technical Test Approach

Choice of detection system;

Fundament and backbone of an IHC assay and must provide high level technical sensitivity and specificity

The key to optimal antibody performance

IHC – The Technical Test Approach

Complexity				
3-step			OptiView+amp (M+R)	
			Quanto (M+R) Hi Def. (M+R) EnV.Fl.+ (M+R) Refine (M) Super Sens. (M) OptiView (M+R) MACH 4 (M) UltraView+amp (M+R)	
2-step	Ult.Vis. ONE	EnV. Fl. UltraView		

Sensitivity

1:25 **1:50** **1:150** **1:500**

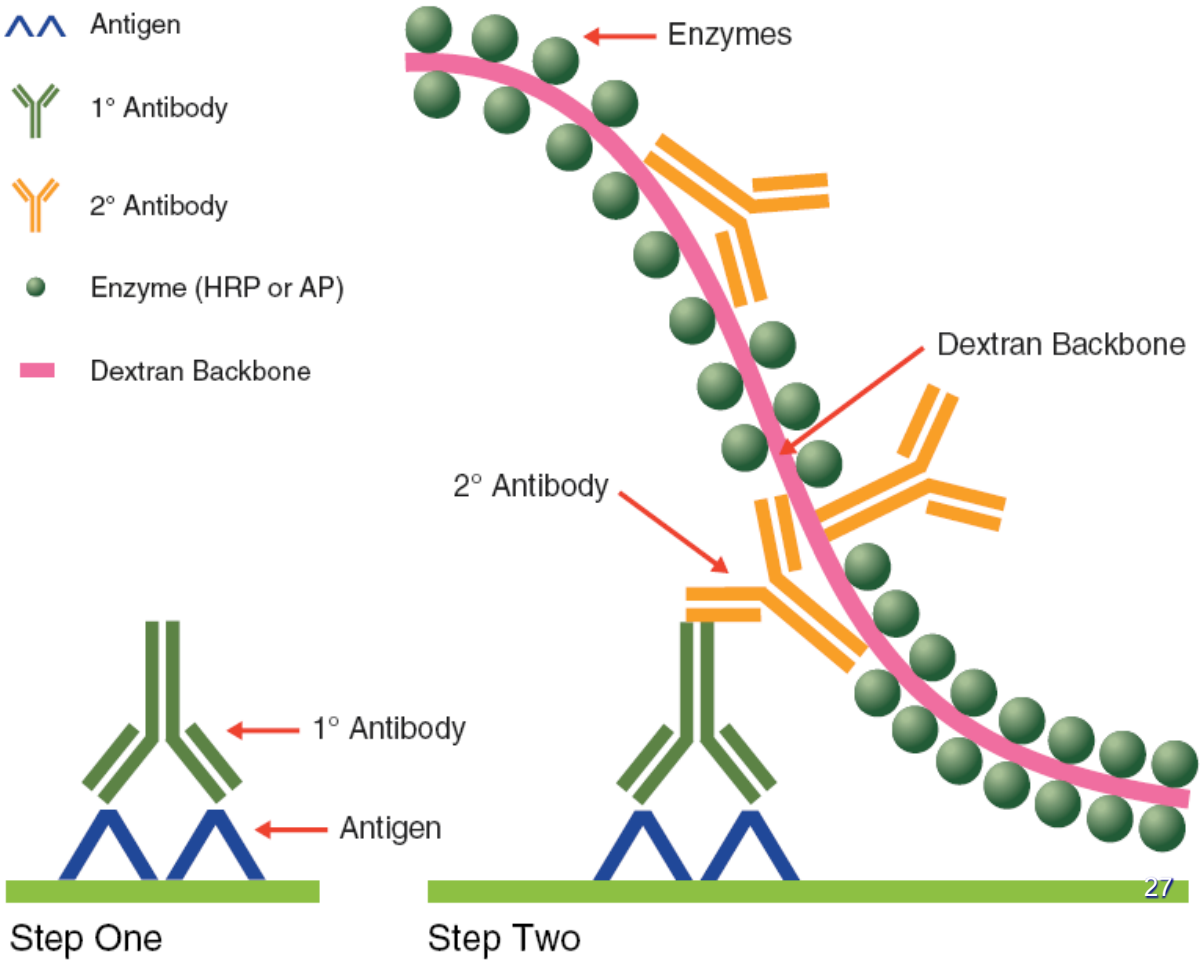
IHC – The Technical Test Approach

Indirect method: 2-step polymer method

EnVision+, Env. Flex
UltraView
UltraVision-one

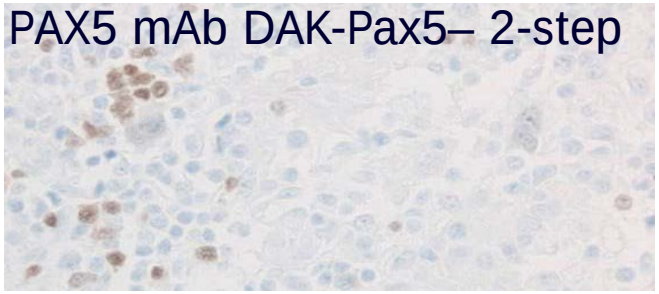
Cocktail of two polymers

1.Goat anti rabbit
2.Goat anti mouse IgG + IgM

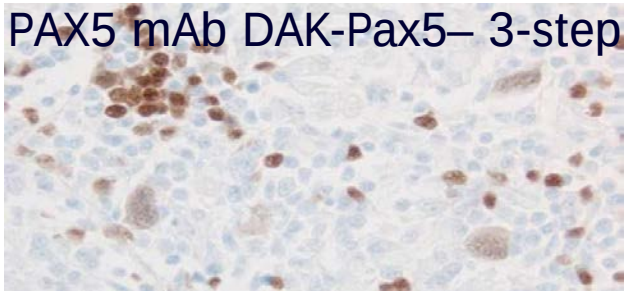


IHC – The Technical Test Approach

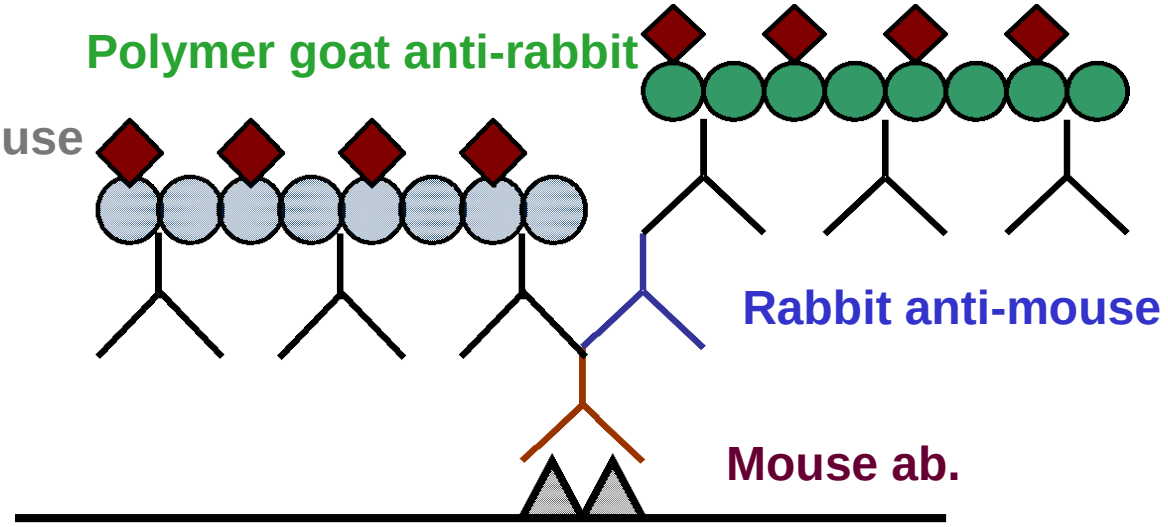
PAX5 mAb DAK-Pax5– 2-step



PAX5 mAb DAK-Pax5– 3-step



1. **Primary ab.**
2. **Link ab.**
3. **Polymer cocktail**

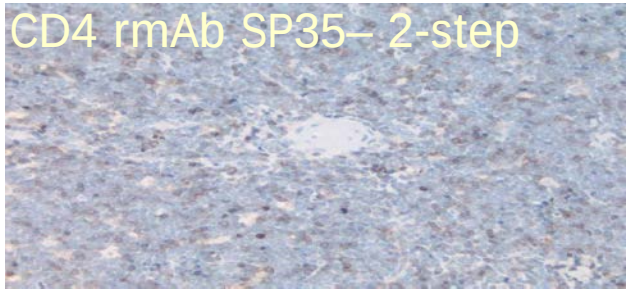


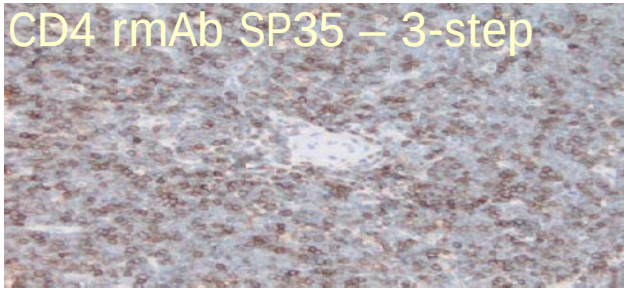
Traditionel 3-step polymer based detection system:

Ultravision LP, Refine, Super Sensitive, PowerVision+, Novolink – amp. mouse ab

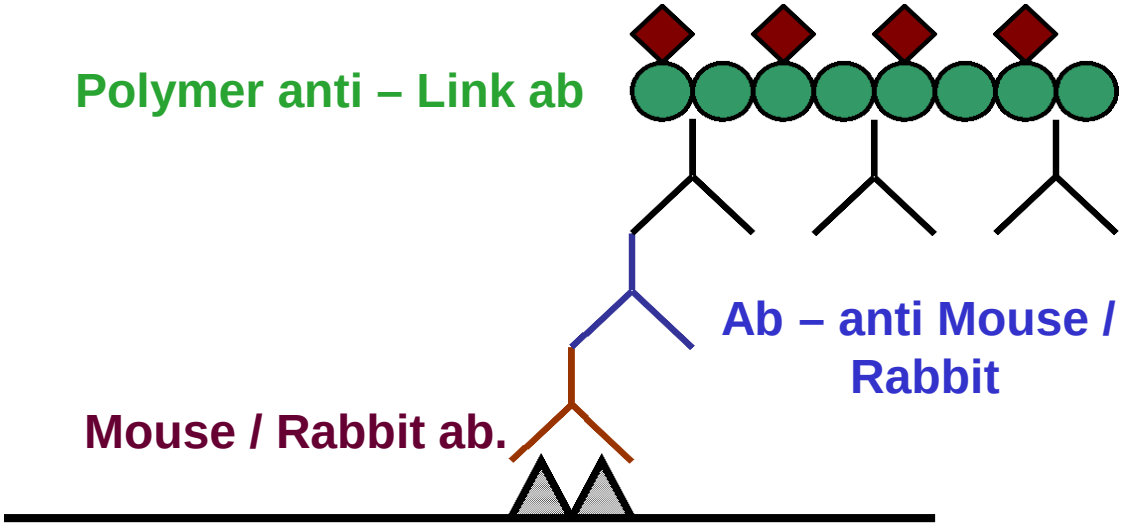
Will amplify the sensitivity 2-5 x for mouse antibodies compared to a 2-step method

IHC – The Technical Test Approach





1. **Primary ab.**
2. **Link ab.**
3. **Polymer (cocktail)**



Second generation 3-step polymer based detection systems:

EnVision Flex+, UltraView + amplification, OptiView, Quanto, – amp. mouse & rabbit ab

Will amplify the sensitivity 2-5 times for both mouse and rabbit abs compared to a 2 step method

IHC – The Technical Test Approach

Table 1. Abs and assessment marks for MLH1, run 30

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **G168-15**	33 8 1	BD Pharmingen Biocare Zytomed	10	11	15	6	50 %	70 %
mAb clone **ES05**	16 3	Novocastra/Leica Dako	11	5	2	1	84 %	100 %
mAb clone **G168-728**	1	BD Pharmingen	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **ES05, IR079**	7	Dako	7	0	0	0	100 %	100 %
mAb clone **ES05, PA0610**	1	Leica	1	0	0	0	-	-
mAb clone **G168-728, 760-4264**	11	Ventana	0	2	7	2	18 %	-
mAb, clone **G168-728, 285M & CMA869**	3	Cell Marque	1	0	2	0	-	-
mAb, clone **G168-15 PM220**	1	Biocare	0	1	0	0	-	-
Total	85		30	19	26	10	-	-
Proportion	-		35 %	22 %	31 %	12 %	57 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

HIER alkaline buffer + 2-step polymer: 32% sufficient, 13% optimal

HIER alkaline buffer + 3-step polymer: 89% sufficient, 67% optimal

IHC – The Technical Test Approach

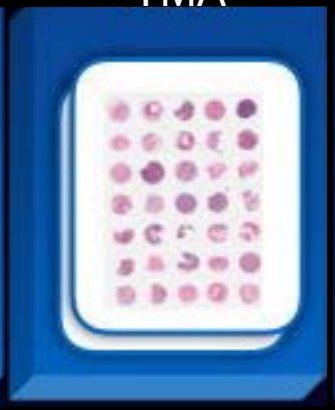
Issues to be adressed for IHC assay implementation:

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

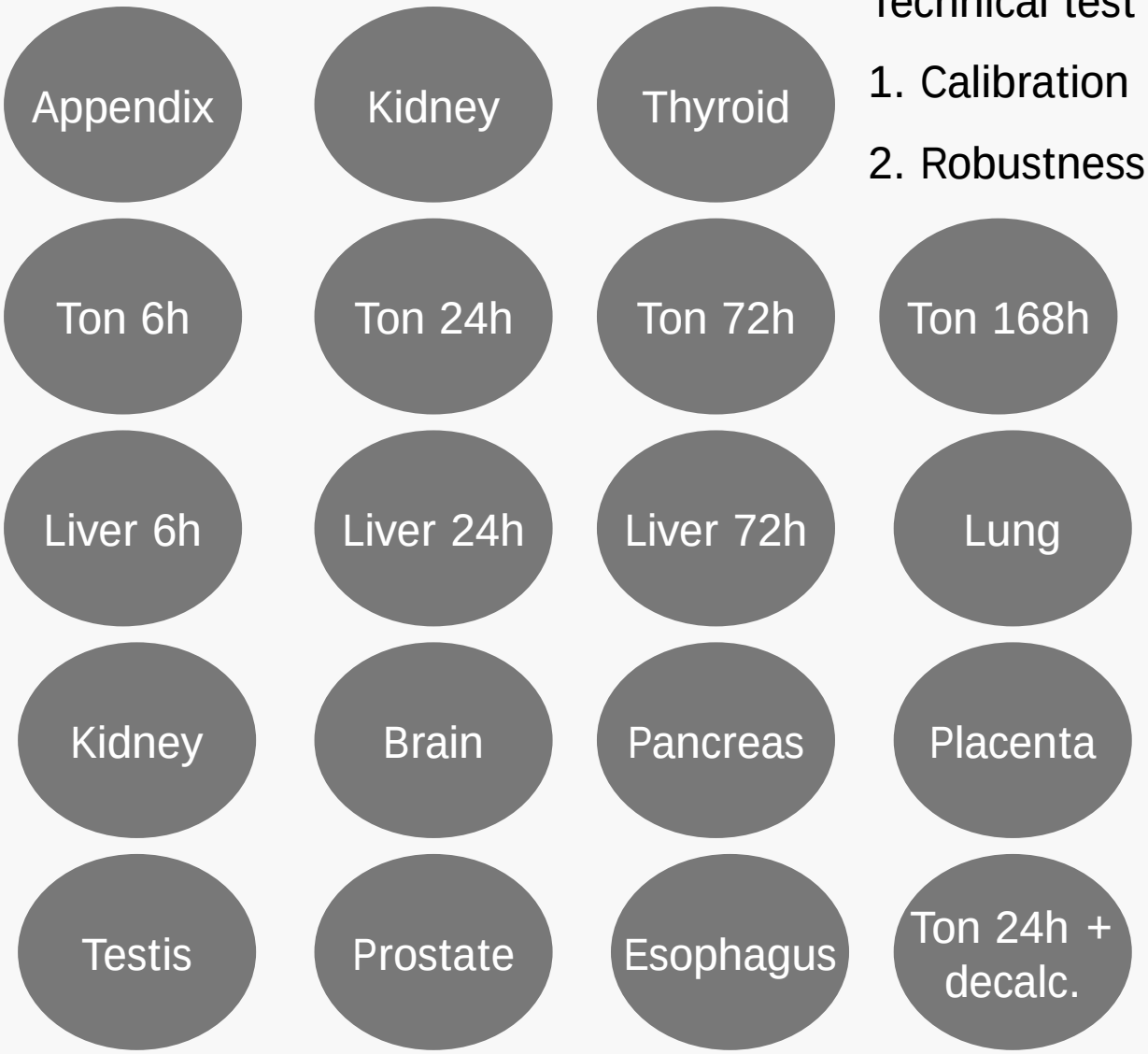
<u>Tissue controls are key element</u>

IHC – The Technical Test Approach

External tissue control tool-box:

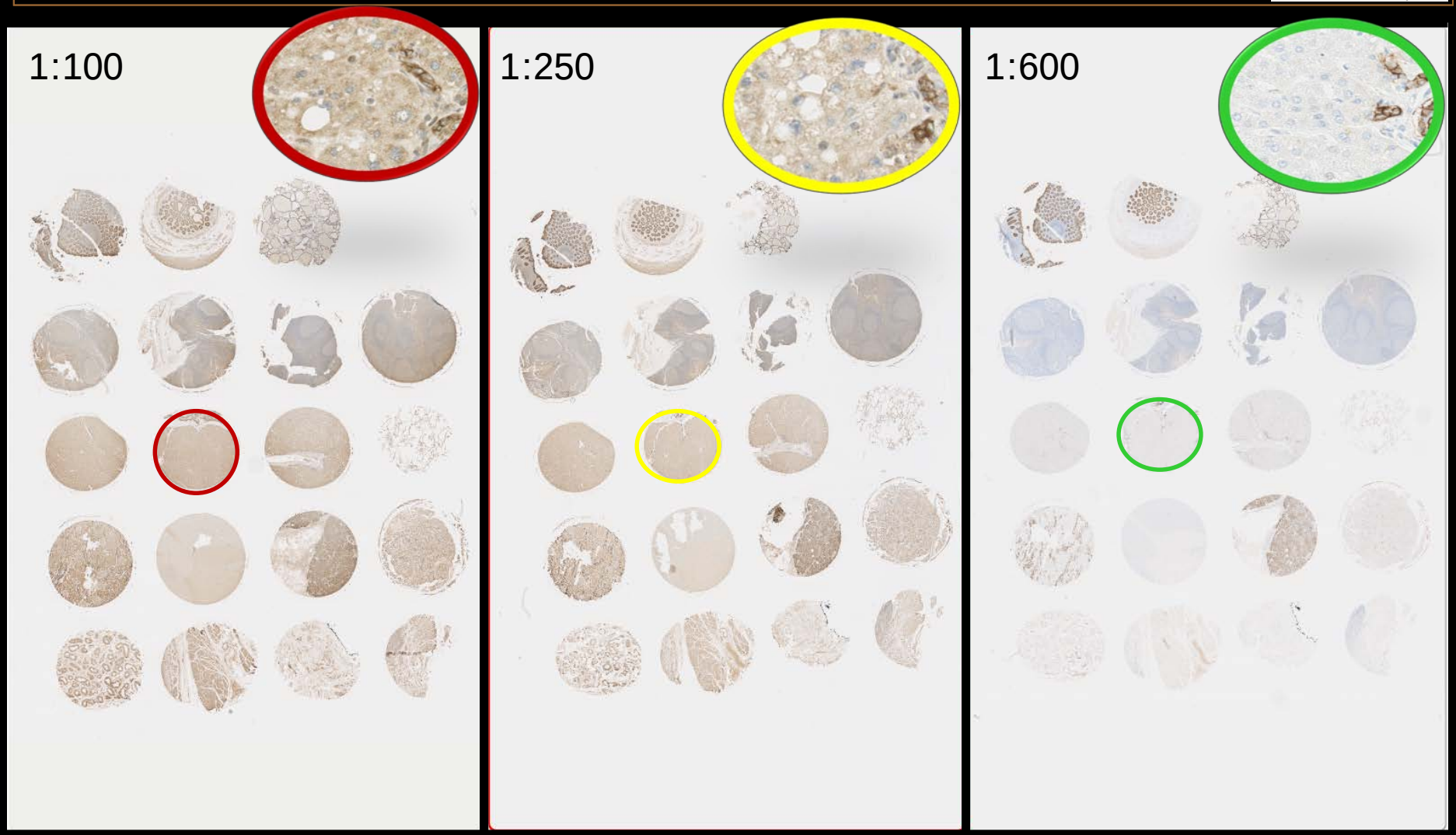
Calibration TMA's			Analytical "Validation" TMA's		Lab QC TMA
iCAPC TMA	Specificity TMA	Pre-analyt. TMA	Accuracy TMA	Index TMA	"Daily QC" TMA
					
"Gold standard" tissue controls	"Normal" tissues	iCAPCs processed as lab procedures	"Lesional" tissues	"Lesional" tissues	iCAPCs + selected tissues
IHC critical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

IHC – The Technical Test Approach



Protocol set-up: used as primary material for the calibration of 130 of 195 routine diagnostic markers, Aalborg University Hospital

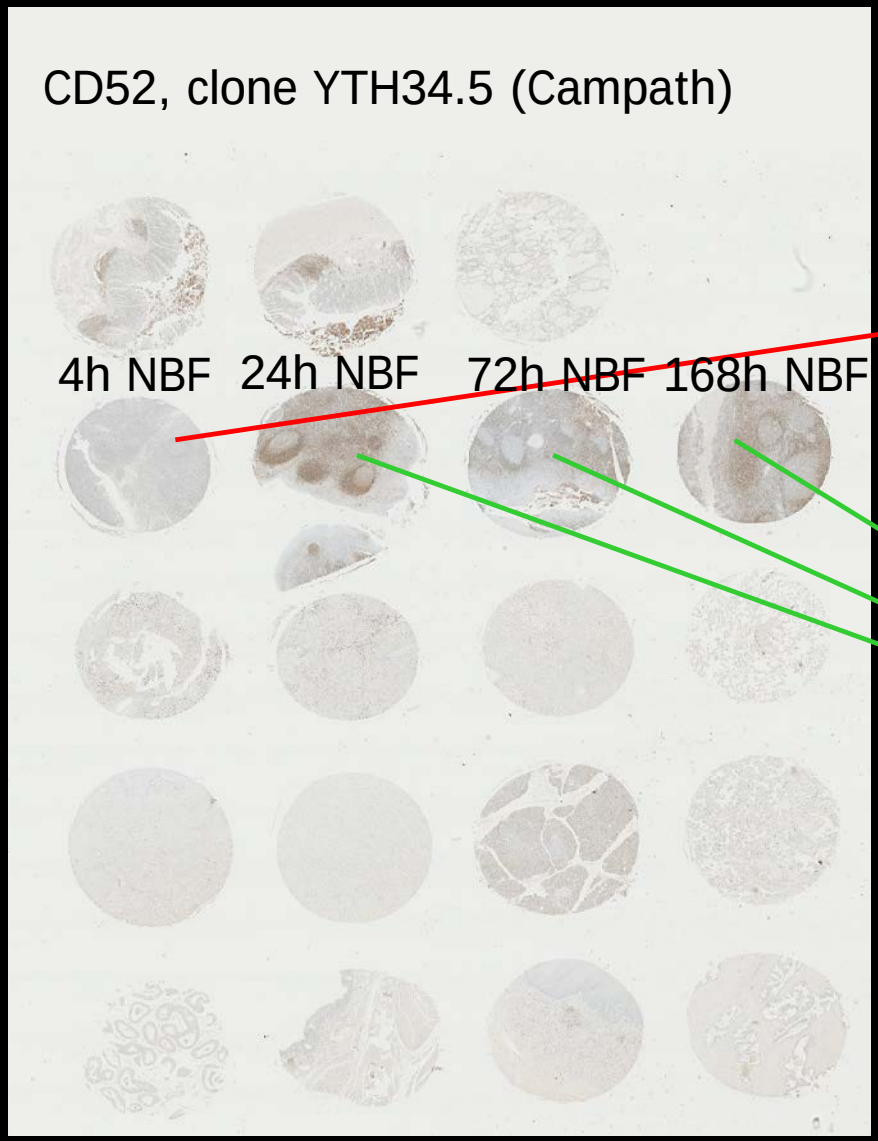
IHC – The Technical Test Approach

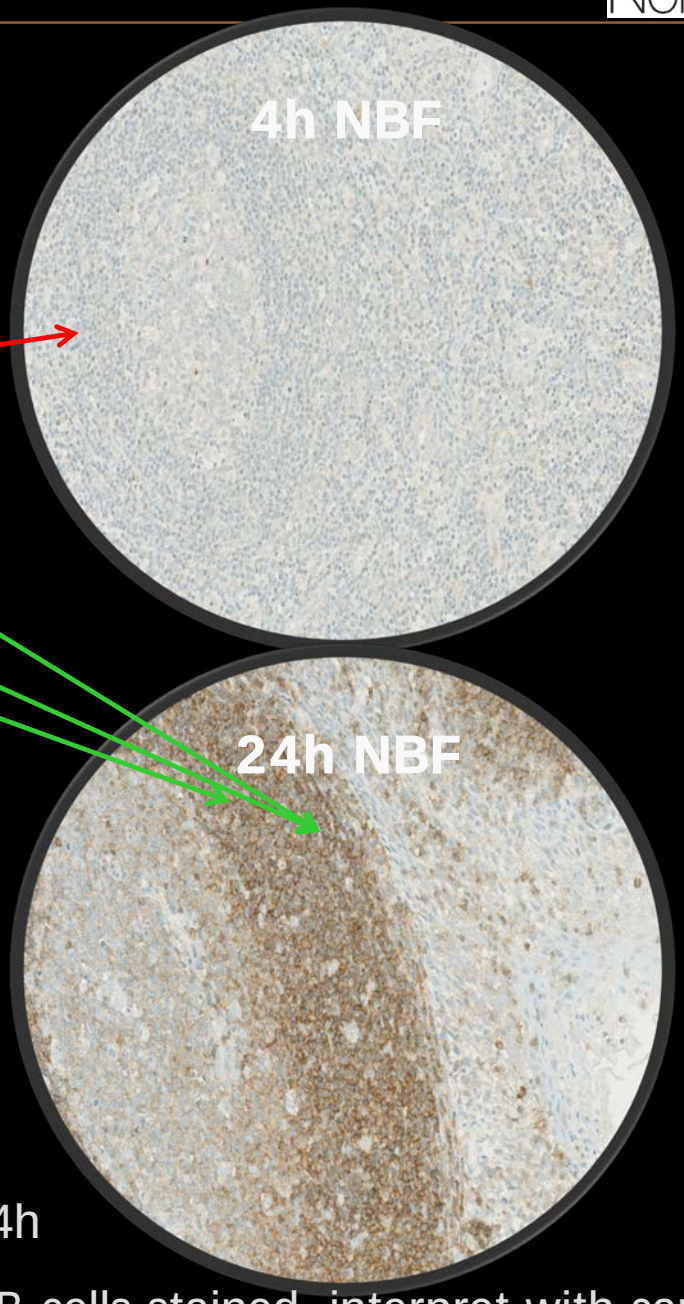


EPCAM calibration

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

IHC – The Technical Test Approach

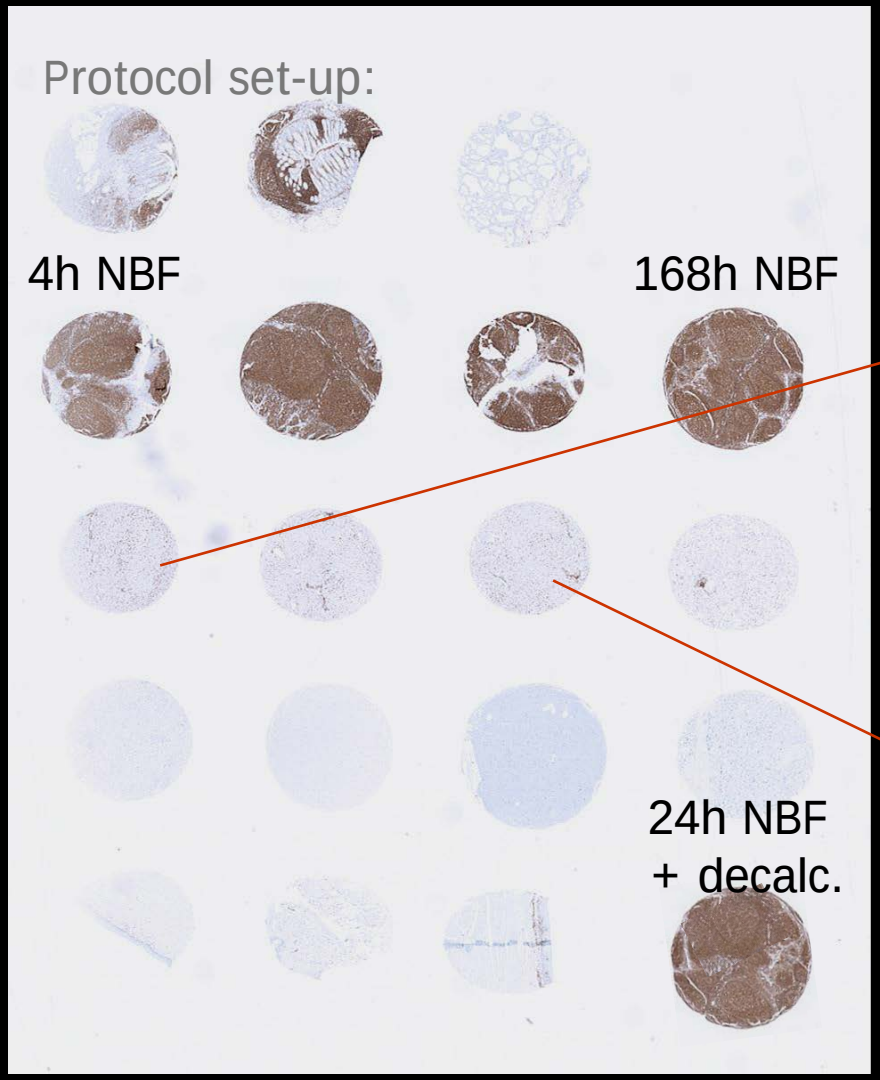


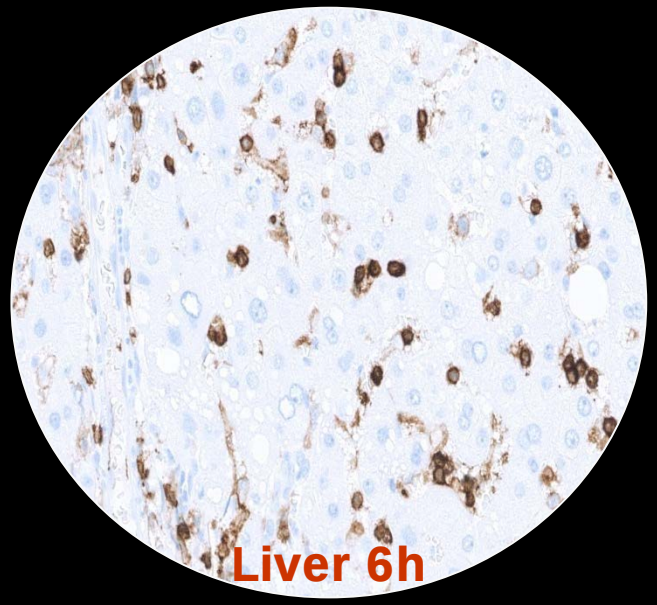


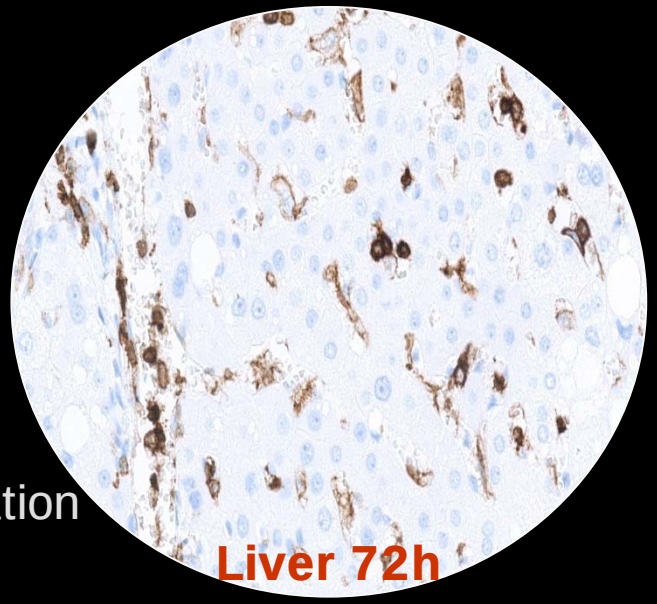
1. Influenced by fixation time – reduced in <24h
2. IHC protocol, 3. Control; Tonsil – cave if no B-cells stained, interpret with caution

IHC – The Technical Test Approach

Anti-CD45 test:

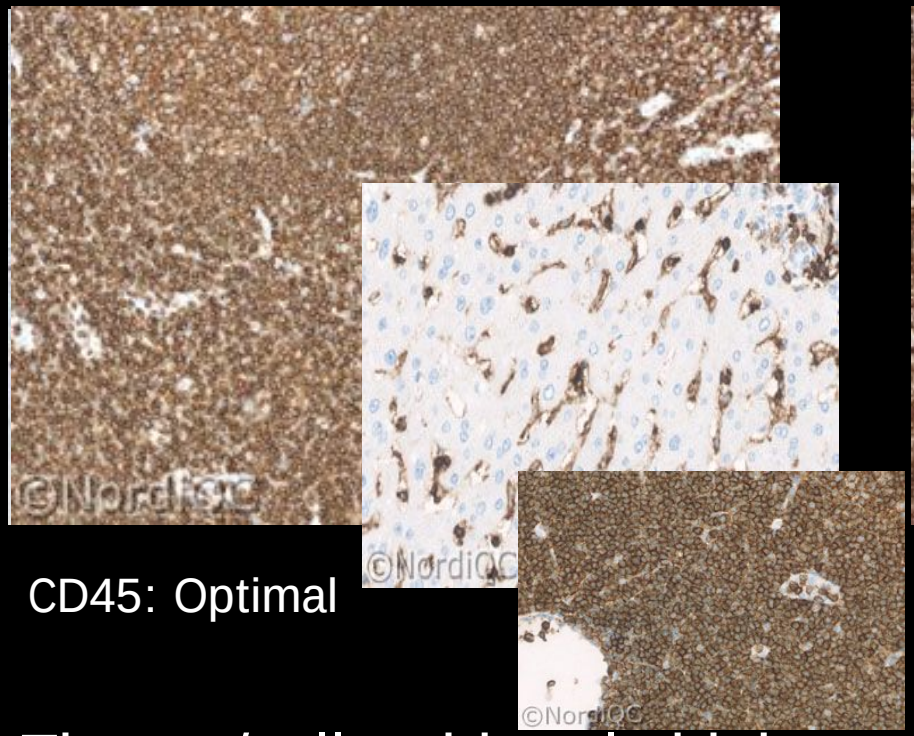




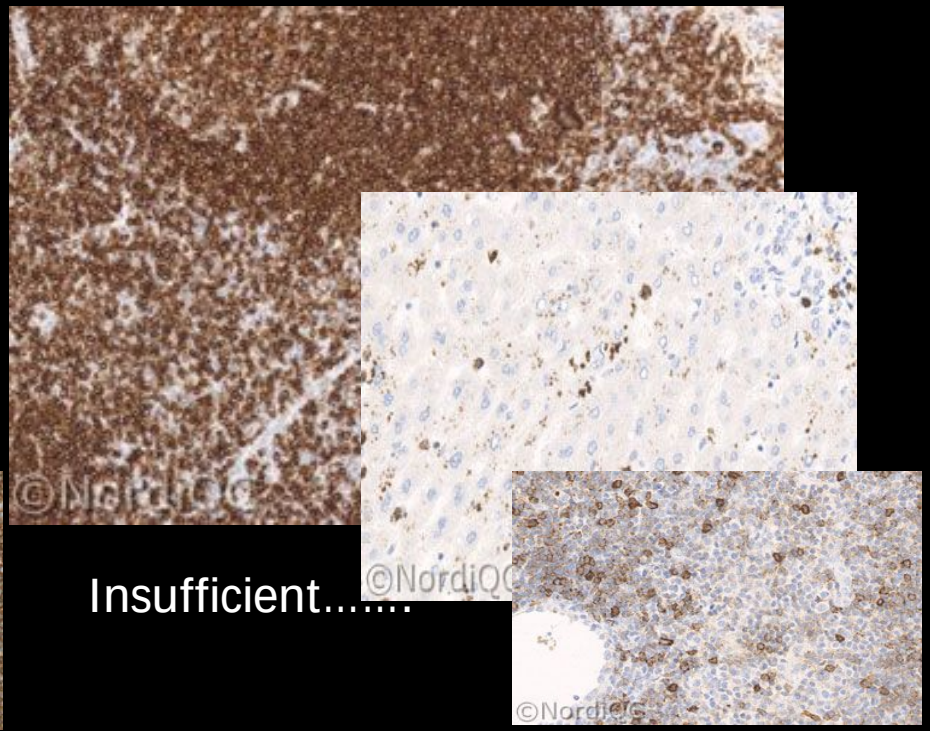


1. Not NBF dependent or influenced by decalcification
2. IHC protocol, 3. Control; Liver and Tonsil

IHC – The Technical Test Approach



CD45: Optimal



Insufficient.....

Tissues/cells with only high expression will not identify:

1. A poorly calibrated IHC assay
2. A reduced sensitivity in an optimally calibrated IHC assay

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

IHC – The Technical Test Approach

IHC Critical Assay Performance Controls (iCAPCs)

Which tissues are recommended ?

What is the expected staining pattern ?

Which tissues / cells are critical ?

Right antibody
Appropriate level of sensitivity
Guidance level of specificity

REVIEW ARTICLE

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

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

IHC – The Technical Test Approach

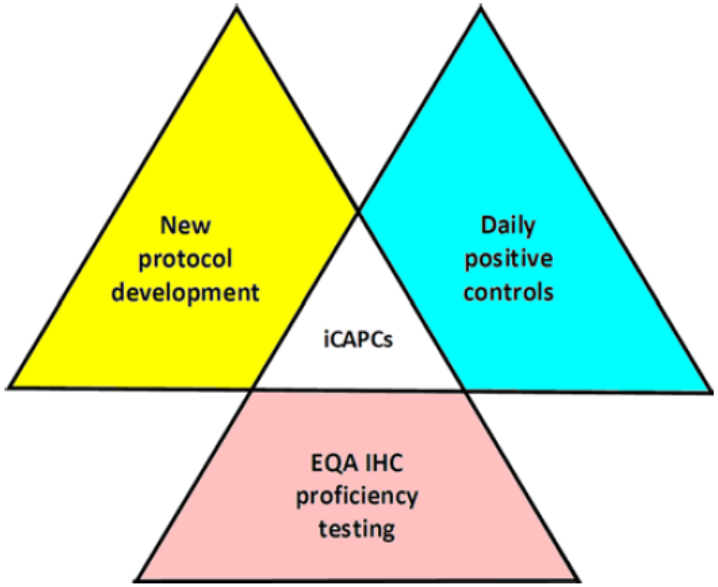


FIGURE 19. The roles of iCAPCs in clinical immunohistochemistry (IHC) laboratories. iCAPCs are an essential part of new protocol development, daily quality controls, and proficiency testing. EQA indicates External Quality Assurance; iCAPC, immunohistochemistry critical assay performance controls.

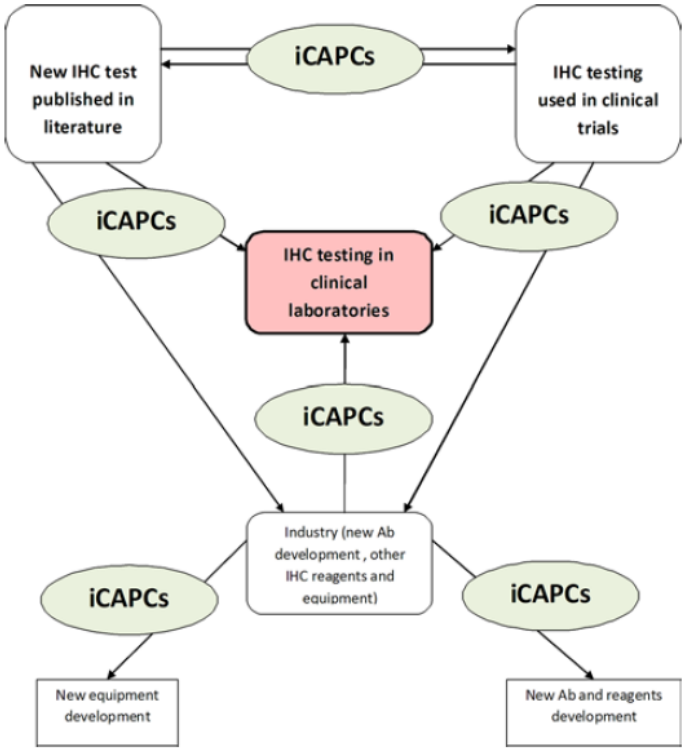


FIGURE 20. iCAPCs and Methodology Transfer. iCAPCs are proposed as important elements for harmonization of immunohistochemistry (IHC) testing between clinical research, product development, and clinical IHC testing. iCAPCs enable IHC harmonization of protocol transfer between research, industry, and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

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

Expected level – calibration
Analytical sensitivity and specificity

IHC – The Technical Test Approach

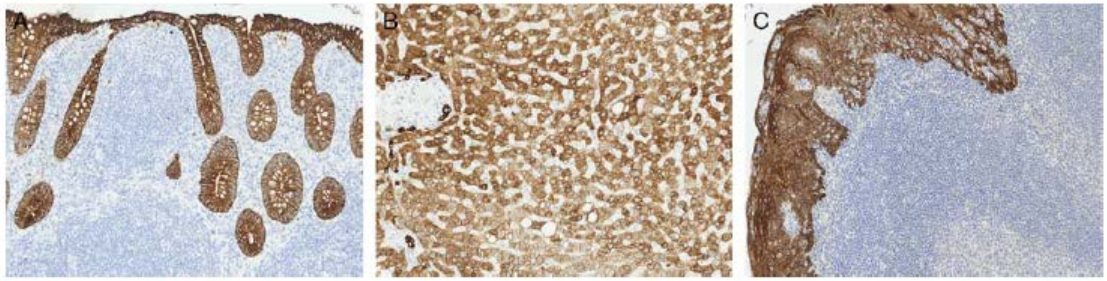


FIGURE 1. Pan-keratin iCAPC. A, Appendix: virtually all columnar epithelial cells must show a moderate to strong predominantly cytoplasmic staining reaction (a membranous accentuation will typically be seen). B, Liver: the vast majority of hepatocytes must show at least weak to moderate cytoplasmic staining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

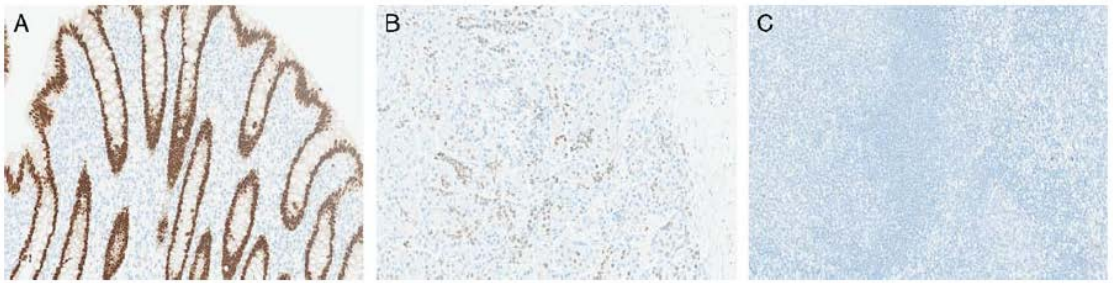


FIGURE 8. CDX-2 iCAPC. A, Appendix: virtually all epithelial cells must show a strong nuclear staining reaction. A weak cytoplasmic staining reaction in addition to strong nuclear staining is often present. B, Pancreas: the majority of epithelial cells of intercalated ducts must show a weak to moderate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

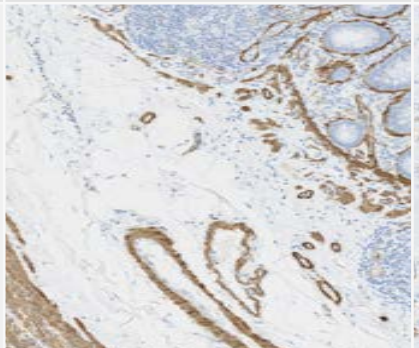
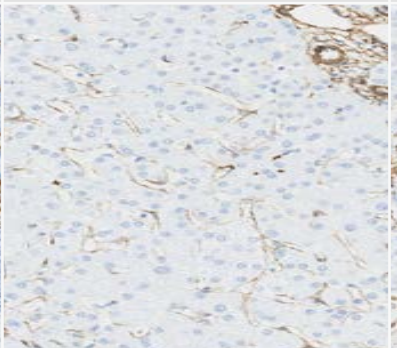
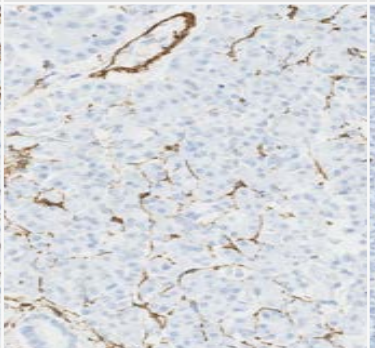
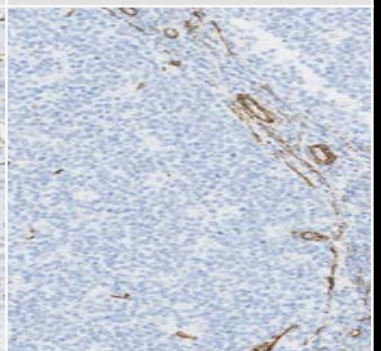
No expression
(Appropriate specificity)

Which tissue
Which cells
Which extension
Which intensity

IHC – The Technical Test Approach

	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	Tonsil	Tonsil	Tonsil	Different comp.

IHC – The Technical Test Approach

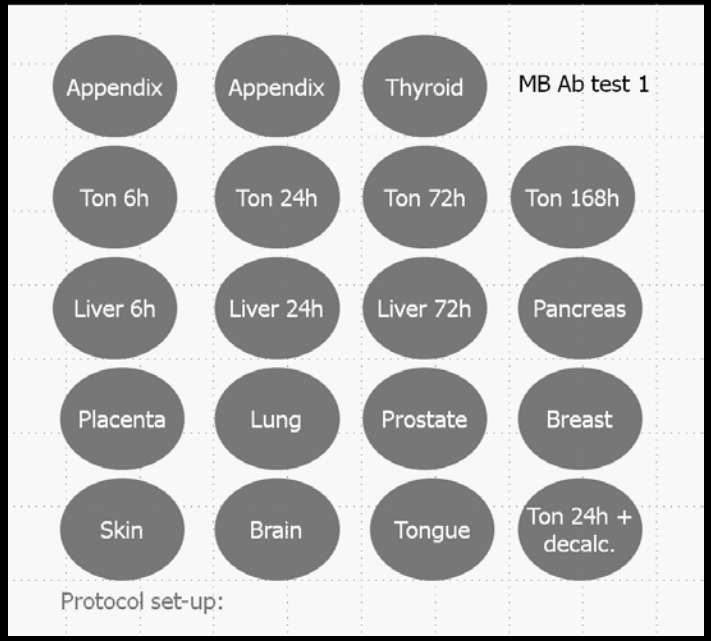
ASMA (C)	Appendix	Liver	Pancreas	Tonsil
High expression (right ab)	A moderate to strong staining reaction in virtually all smooth muscle cells in muscularis mucosae	A moderate to strong staining reaction in the smooth muscle cells in vessels	A moderate to strong staining reaction in the smooth muscle 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
				

IHC – The Technical Test Approach

Concentrated antibodies – Aalborg Hospital (app. 200 Abs) – VMS ULTRA

	1:25	1:100	1:400
A	None	None	None
B	Enzyme P1, 4 min	Enzyme P1, 4 min	Enzyme P1, 4 min
C	HIER CC1 pH 8.5*	HIER CC1 pH 8.5	HIER CC1 pH 8.5
D	HIER CC2 pH 6.0*	HIER CC2 pH 6.0	HIER CC2 pH 6.0
(E)	CC1 + Enzyme P3, 8 min	CC1 + Enzyme P3, 8 min	CC1 + Enzyme P3, 8 min
(F)	Enzyme P3, 8 min + CC1	Enzyme P3, 8 min + CC1	Enzyme P3, 8 min + CC1

*HIER time 48 min. at 99°C
OptiView DAB



1. Technical calibration

2. Diagnostic / analytical evaluation

IHC – The Technical Test Approach

External tissue control tool-box:

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	Fixation time Fixative(s) Decalcification
High expression Low expression No expression	With expression No expression	

Analytical "Validation" TMA's

Accuracy TMA	Index TMA
	
"Lesional" tissues	"Lesional" tissues
Range of relevant expression levels	Range of relevant expression levels
With expression No expression	High expression Low expression No expression
20/40 of each Type I/II IHC	+ relevant cut-off

Lab QC TMA

"Daily QC" TMA

iCAPCs + selected tissues

Reproducibility

Method of transfer proof

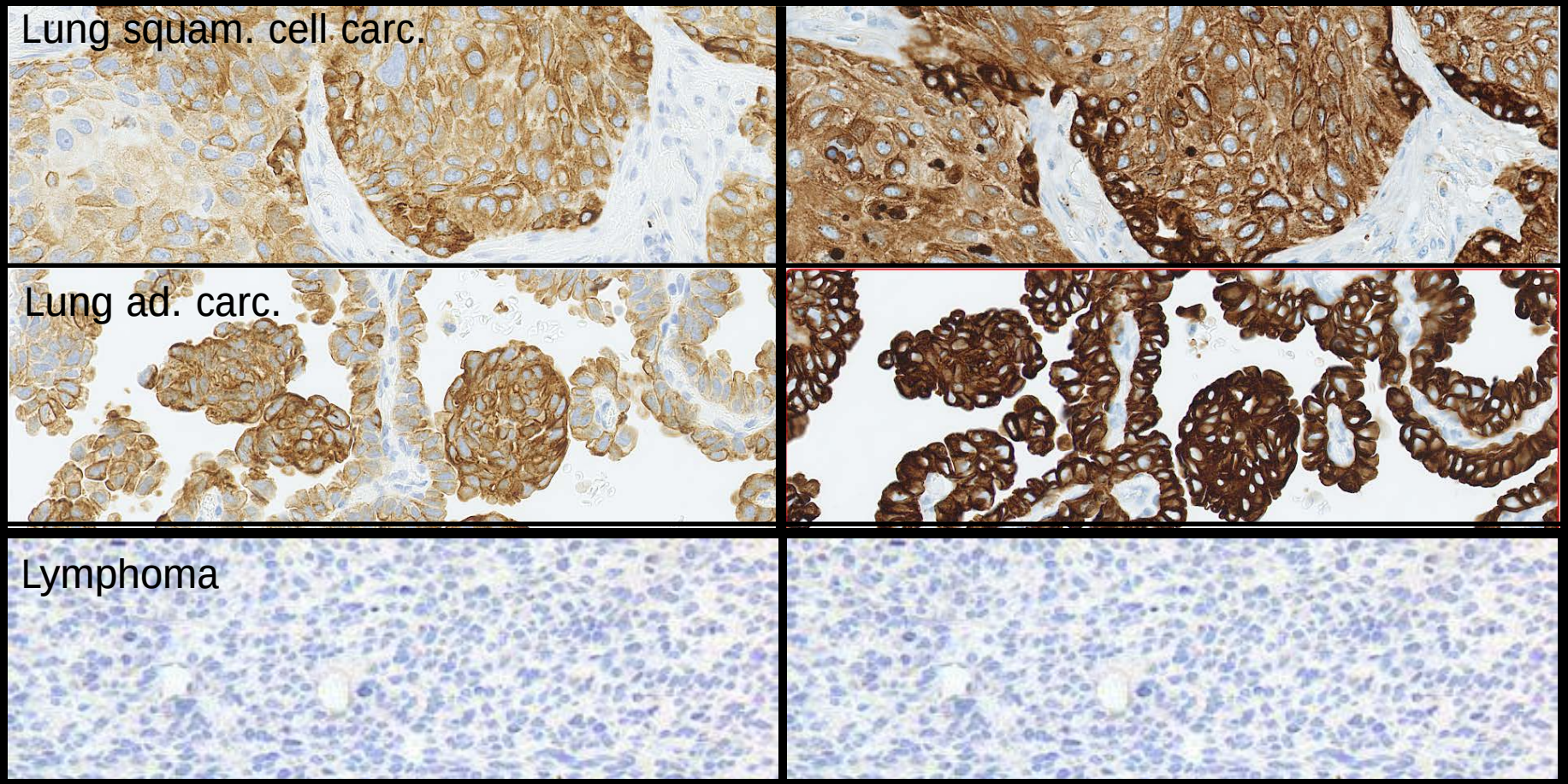
IHC – The Technical Test Approach

- 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 less important compared to use of tissue with full range of expression patterns reflecting the diagnostic use

IHC – The Technical Test Approach



3 x 10 samples

Mission completed.....

CK-PAN - mAb AE1/AE3 – Prot. 1

CK-PAN - mAb AE1/AE3 – Prot. 2

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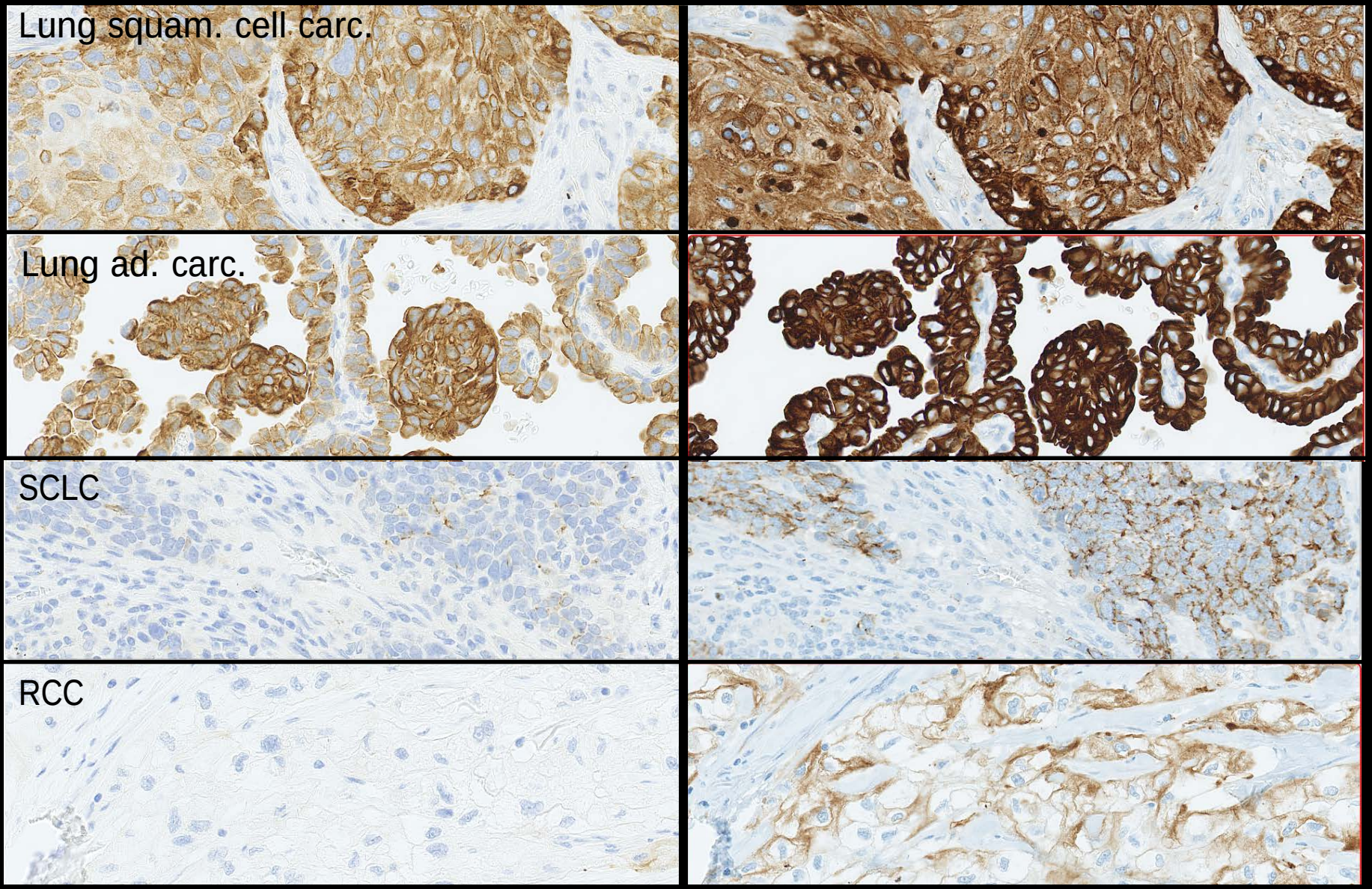

CK-PAN - mAb AE1/AE3 – Prot. 1

CK-PAN - mAb AE1/AE3 – Prot. 2

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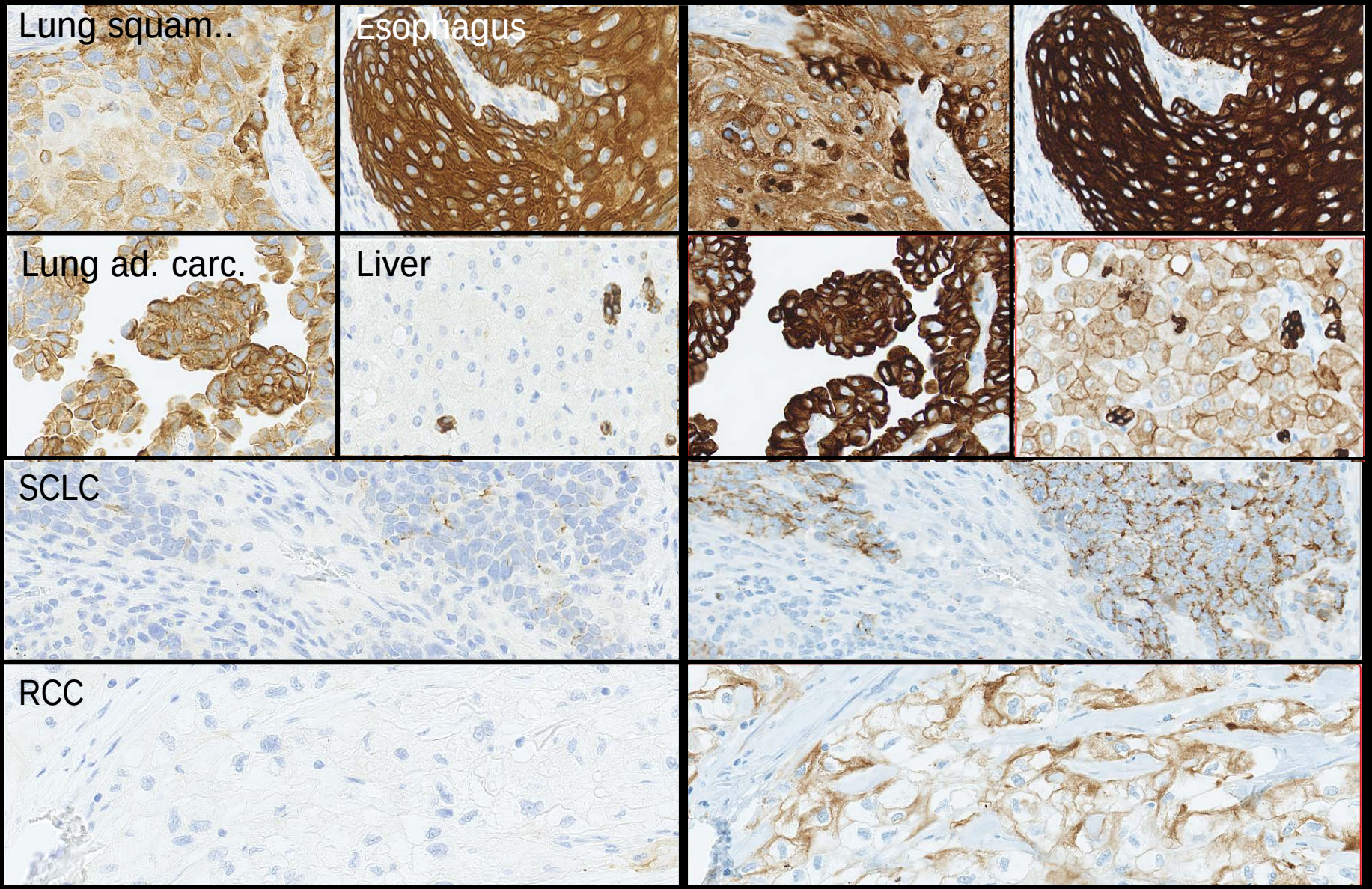

CK-PAN - mAb AE1/AE3 – Prot. 1

CK-PAN - mAb AE1/AE3 – Prot. 2

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TMA Neoplasia

Analytical Index / sensitivity TMA

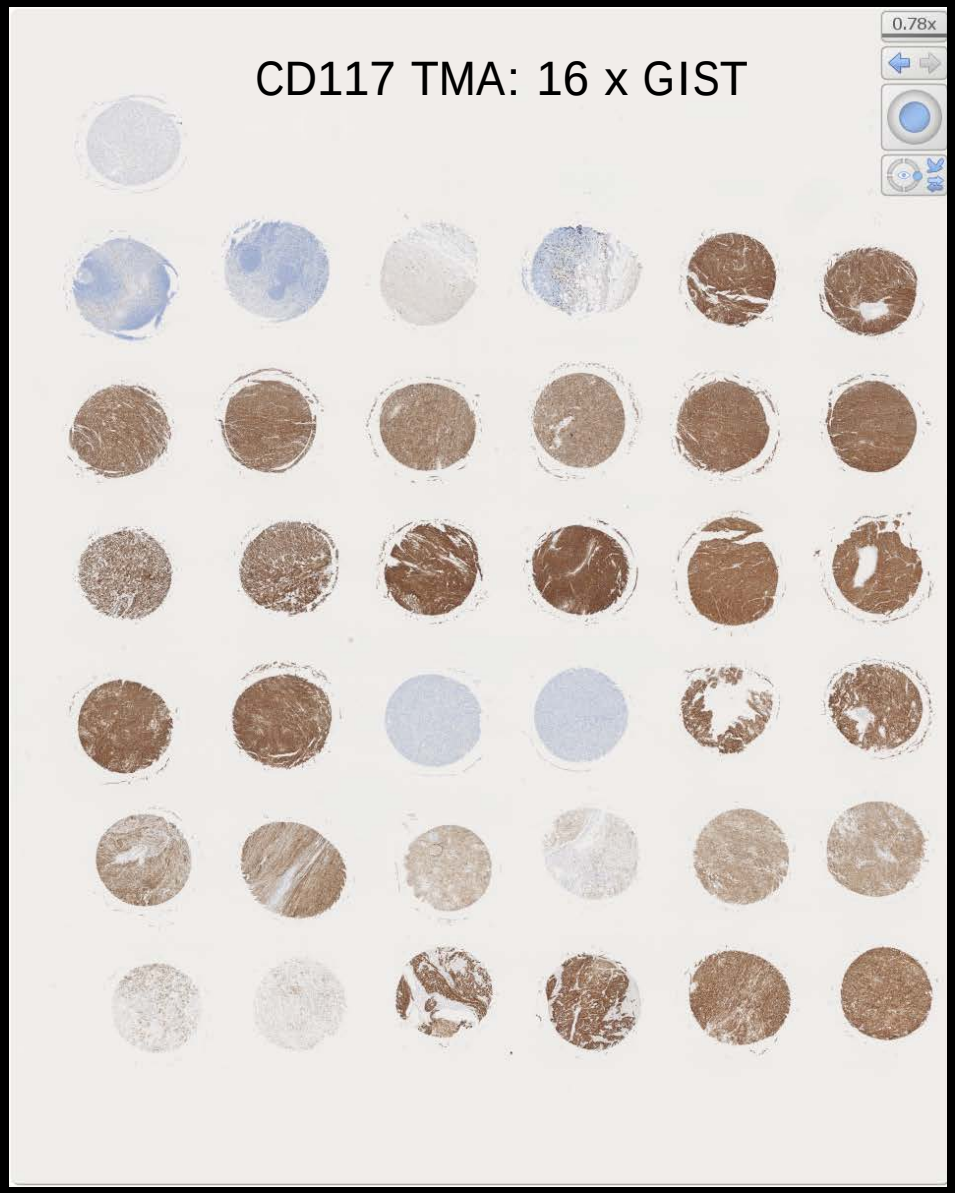
Diagnostic potential:

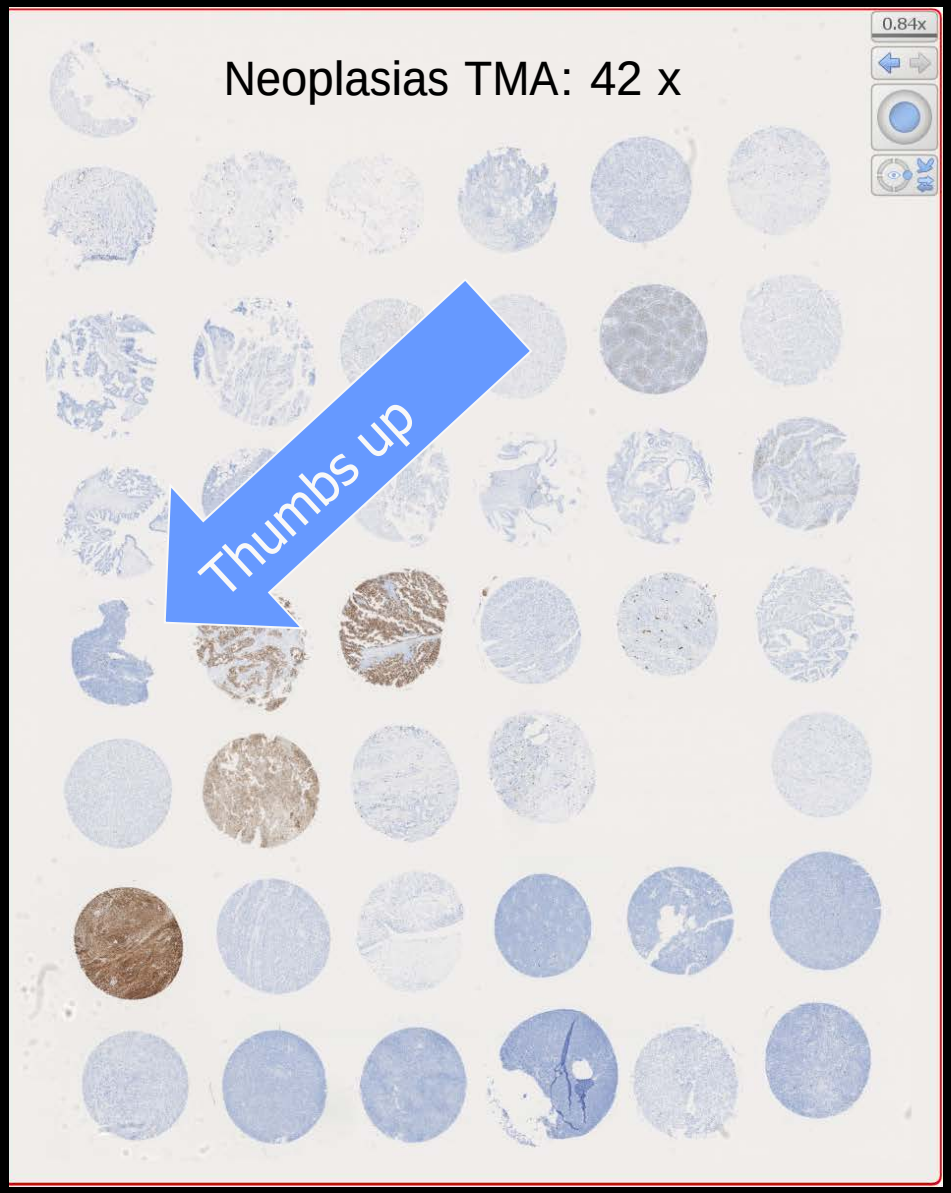
Index and accuracy TMA's

Analytical accuracy / specificity TMA

Liver					
Mamma ductal carc.	Mamma ductal carc.	Mamma Lobular carc.	Lung adeno carc.	Lung adeno carc.	Lung squam. carc.
Colon adeno carc.	Colon adeno carc.	Kidney clear c carc.	Kidney clear c carc.	Thyroid. follic. carc.	Thyroid. Medul. carc.
Ovary. Serous I carc.	Ovary. Serous I carc.	Ovary. Clear carc.	Ovary. Endom. carc.	Corpus Uteri Endom. carc.	Cerxix Uteri adeno carc.
Tonsil	Testis Semin.	Testis Semin.	Prostate adeno carc.	Prostate adeno carc.	Intest Carcinoid
Melanom	Melanom	Pancr. adeno carc.	Pancr. adeno carc.	Uroth. carc.	Uroth. carc.
GIST	Leio myo sarcoma	Rhabdo myo sarcoma	Hodgkin Classic	Hodgkin mixed	Diffuse large B lymph.
Diffuse large B lymph.	B-CLL	Follik. lymph	Mantle cell lymph.	T-cell lymph. perip.	T-cell lymph. Anapl.

IHC – The Technical Test Approach





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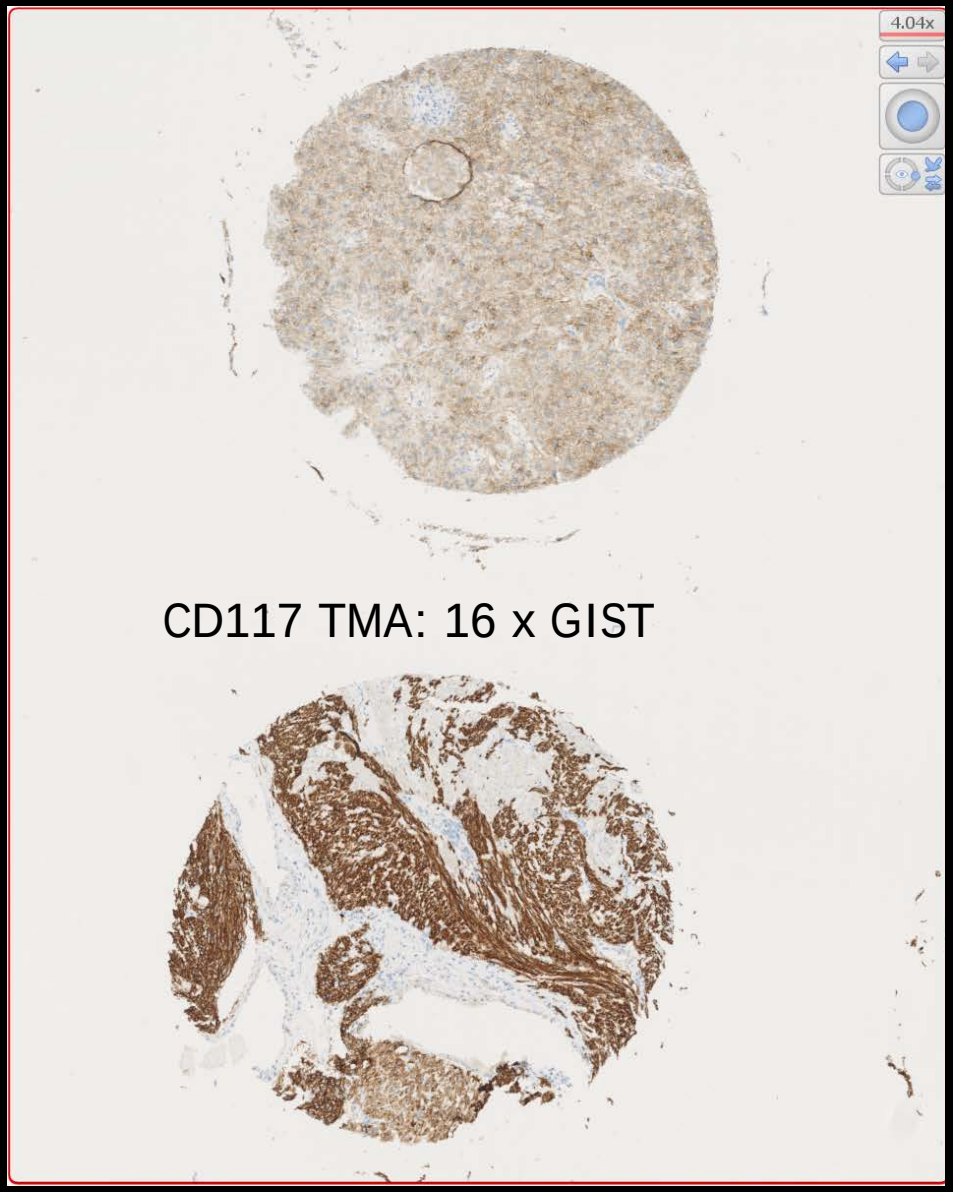
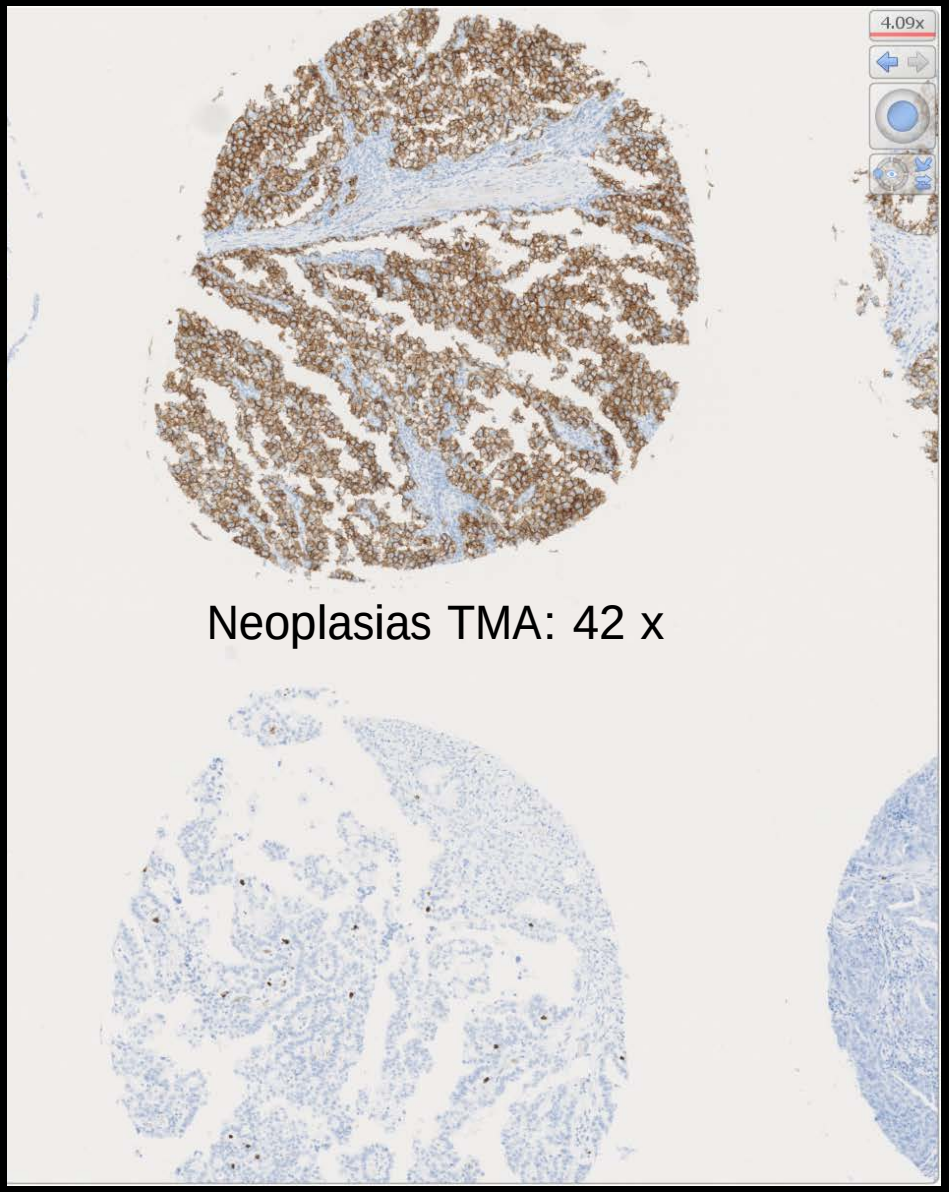


CD117 TMA: 16 x GIST



Neoplasias TMA: 42 x

NordiQC – Antibodies giving different patterns

Antigen	Clone	High expressor	Low expressor	Non expressor
CD3	LN10, 2GV6	√	√	–
CD3	Poly A0452	√	√	(+) – (epith.)
CD5	SP19	√	√	–
CD5	4C7	√	√	(+) – (epith.)
CD8	4B11,C8/144B	√	√	–
CD8	SP57	√	√	(+) – (epith.)
MUM1	EUA32, MUM1p,	√	√	–
MUM1	MRQ-43	√	√	(+) – (epith.)
OCT 3/4	C10, N1NK	√	√	–
OCT 3/4	MRQ-10	√	√	+ – (neuroendo.)
PLAP	NB10	√	√	–
PLAP	8A9	√	√	+ – (muscle)
WT1	WT49	√	√	–
WT1	6F-H2	√	√	+ – (epith.)

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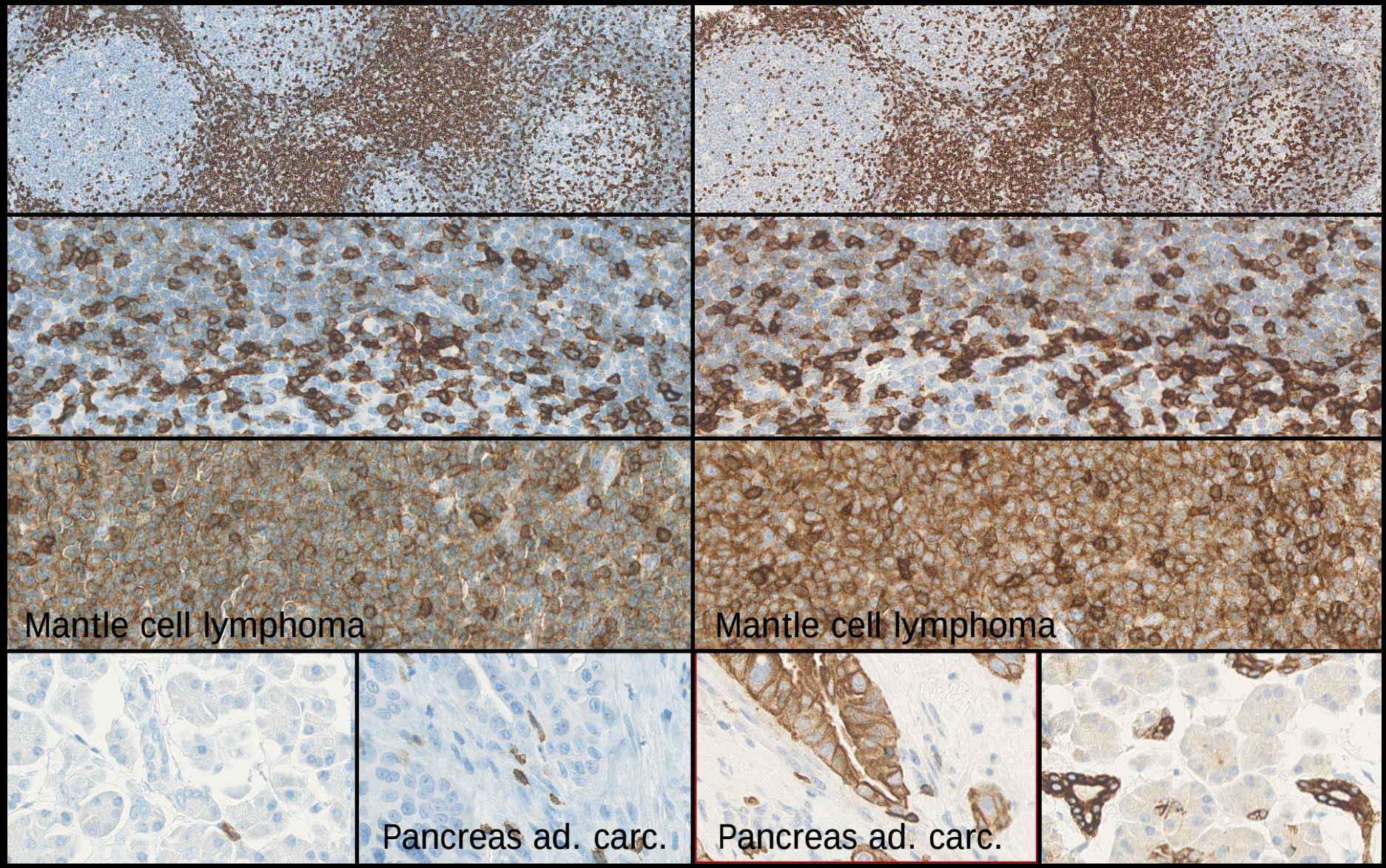

CD5 - rmAb SP19

CD5 - mAb 4C7

NordiQC – Less successful antibodies

Antigen	Clone	High expressor	Low expressor	Non expressor
CD5	CD5/54/F6	√	FN	–
CD23	MHM6	√	FN	–
CD31	1A10	(√)	FN	–
CD31	SP38	(√)	FN	–
CD138	5F7	(√)	FN	–
CDX2	SP54	(√)	FN	FP
CEA	TF-3H8-1	√	√	FP
CGA	DAK. A3	√	FN	–
CK20	PW31	√	(√)	–
CK-LMW	35BH11	√	FN	–
MLH1	EPR3894	√	√	FP
MSH2	EPR3943	√	√	FP
MSH6	44	√	FN	XB
SYP	SY38	√	FN	XB

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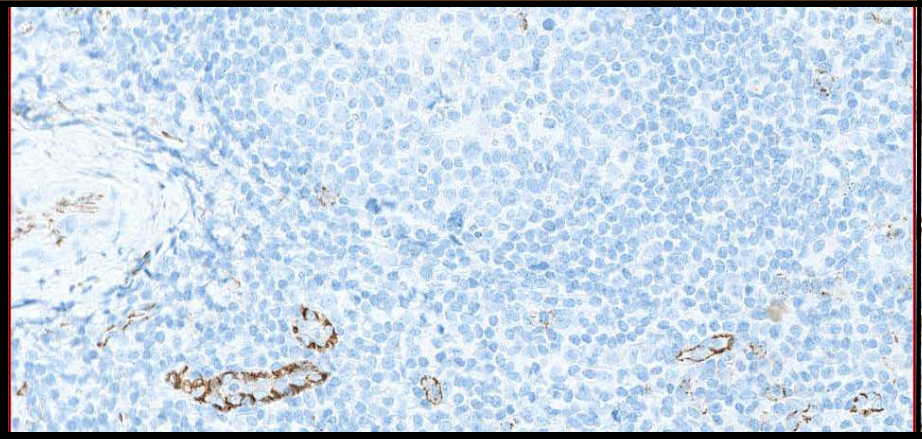

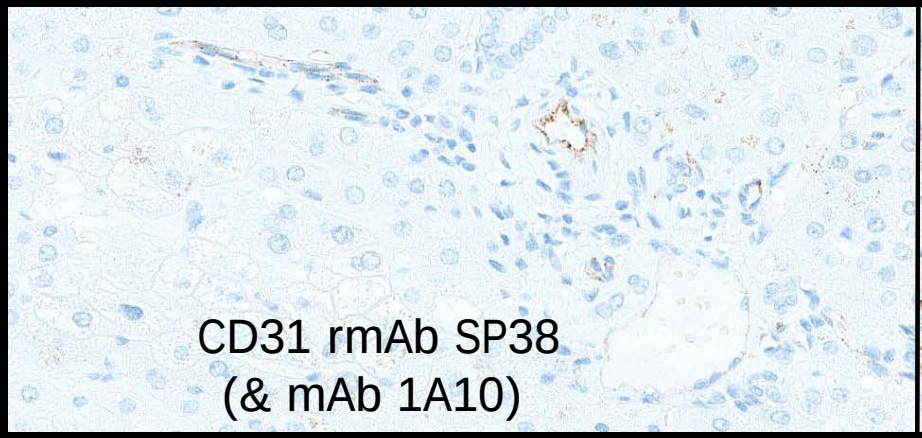

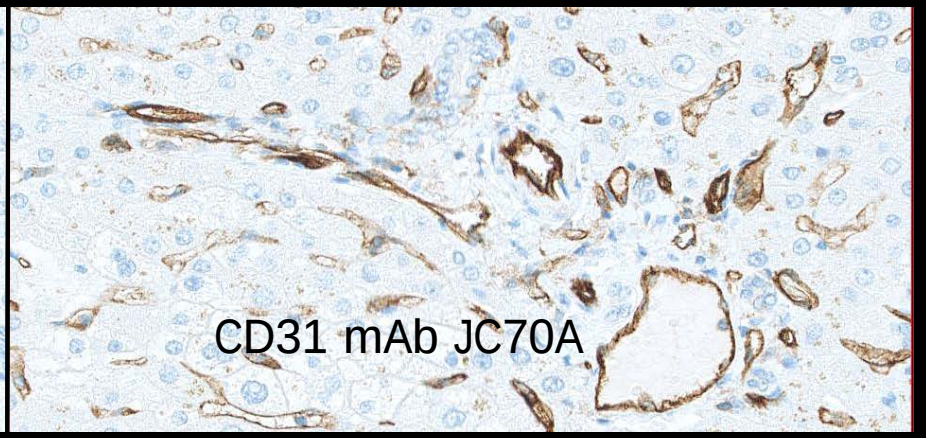

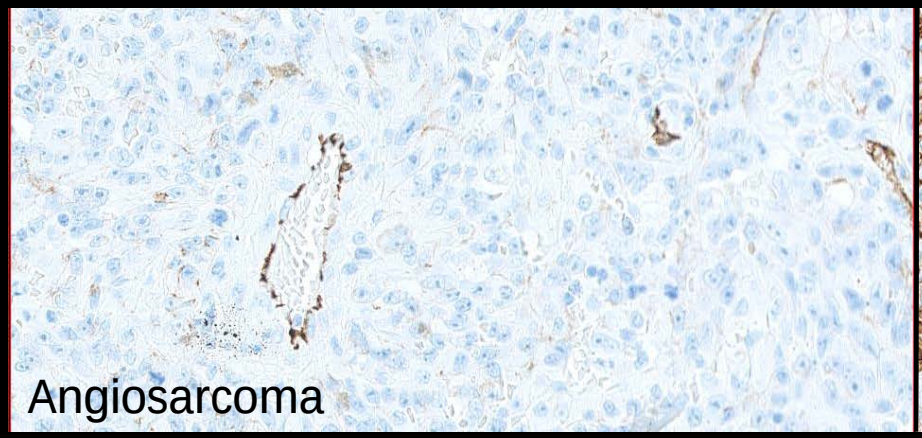

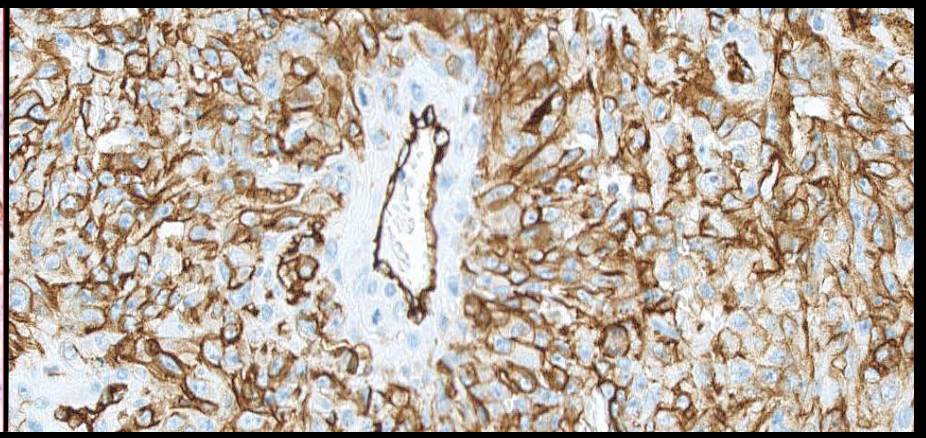

IHC – The Technical Test Approach

External tissue control tool-box:

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	Fixation time Fixative(s) Decalcification	Range of relevant expression levels	Range of relevant expression levels	Reproducibility Method of transfer proof
High expression Low expression No expression	With expression No expression		With expression No expression	High expression Low expression No expression	
			20/40 of each Type I/II IHC	+ relevant cut-off	

*Immunohistochemical critical assay performance controls

IHC – The Technical Test Approach