Nordic immunohistochemical Quality Control

EQALM Symposium 2025 – 16.-18. October – Vienna

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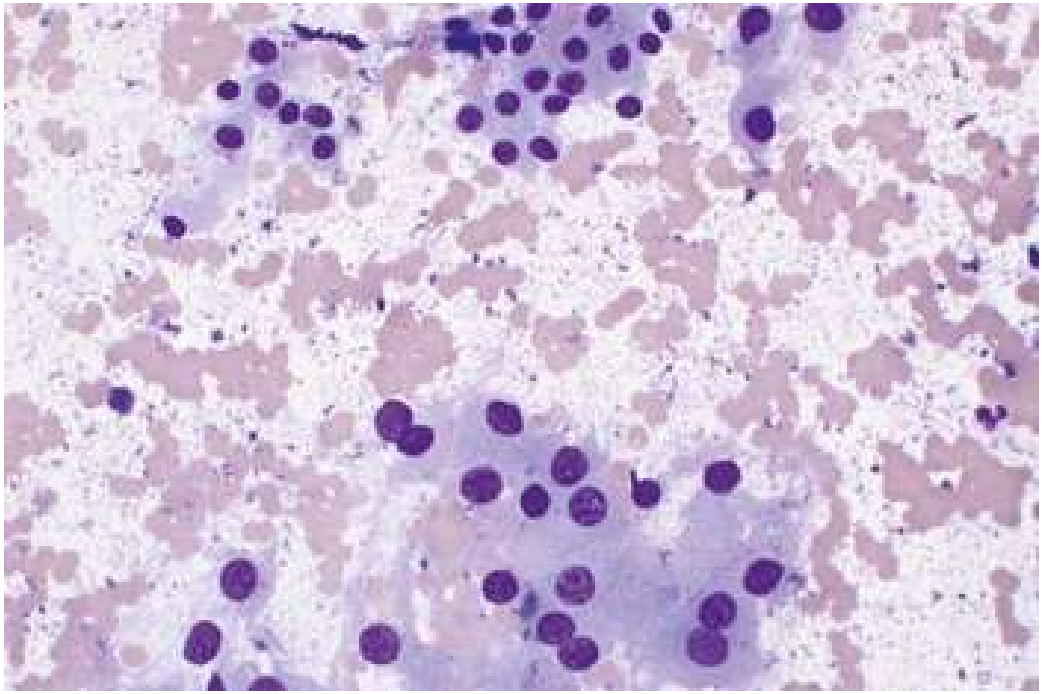
Department of Pathology
Aalborg University Hospital, Denmark



Cytology

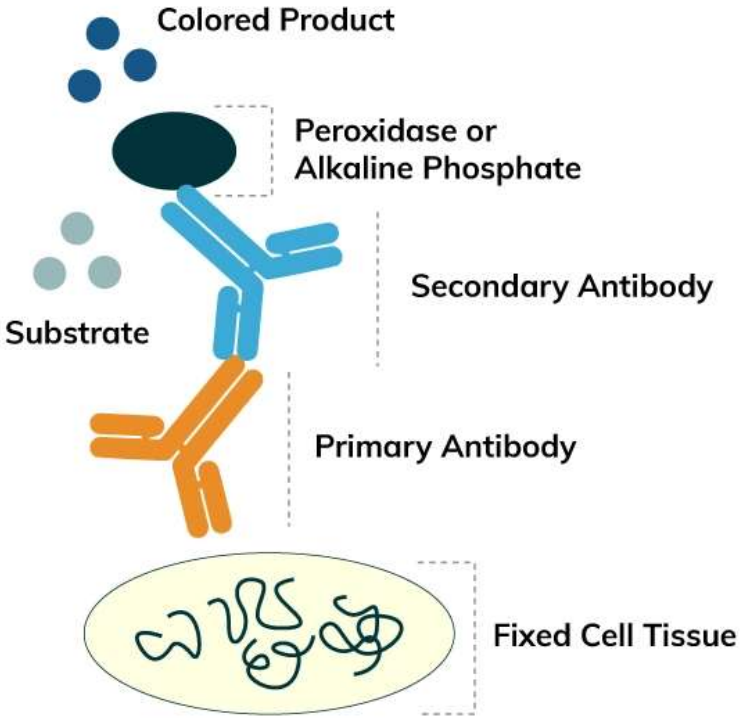
MGG

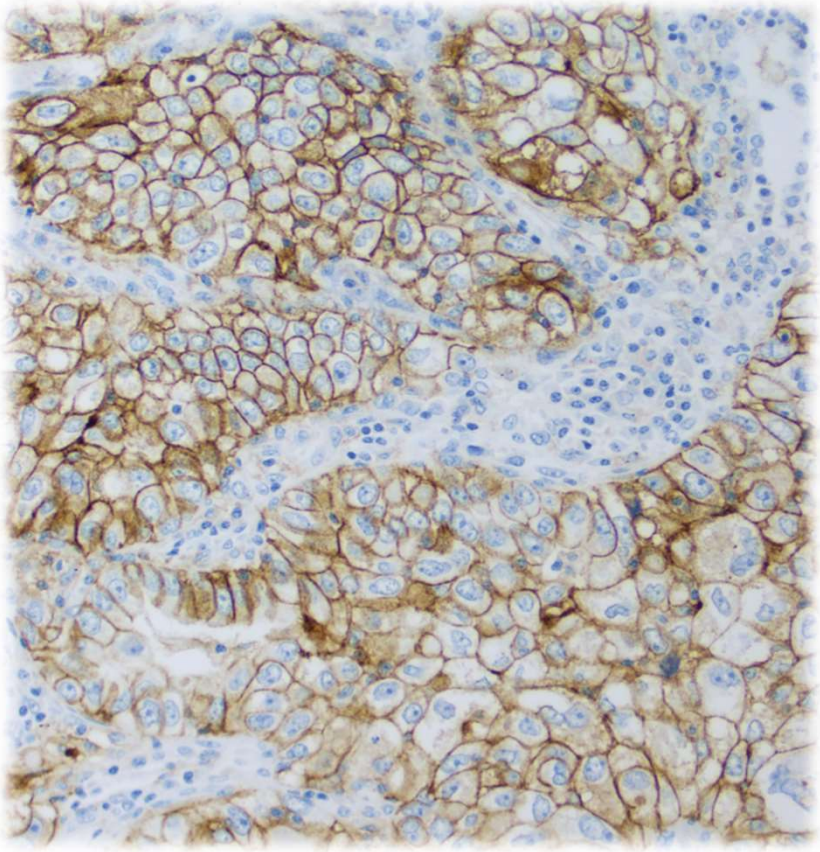
Histology

HE

NordiQC

Immunohistochemistry (IHC)





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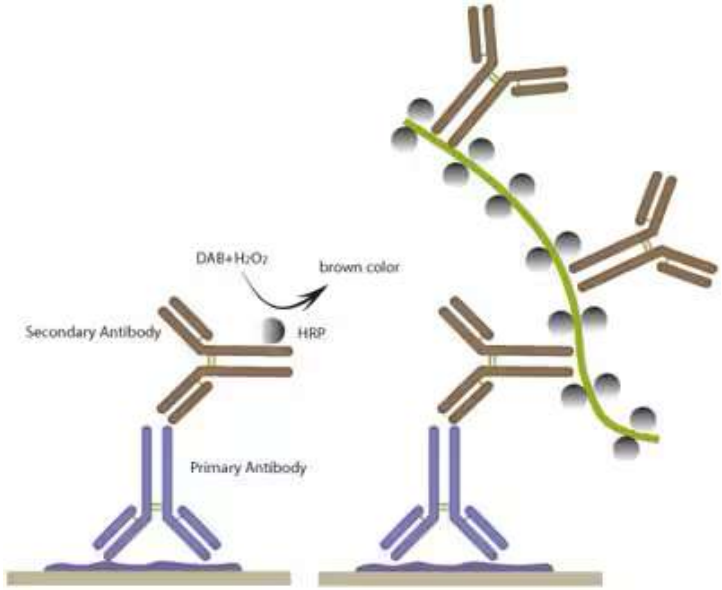
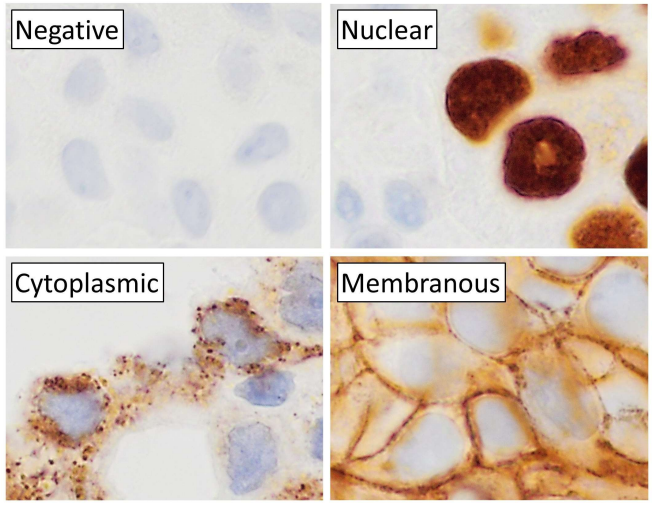
Immunohistochemistry (IHC)

- “Spatial proteomics”
- Most abundant “stain” after HE
- 100-300 different epitopes in most labs





Usages of IHC in clinical pathology

- Detection of specific proteins in patient materials that will help to confirm or disprove a diagnosis
- Identify origin of unknown primary or metastatic cancer
- Subclassify cancers (prognostic)
- Predictive biomarkers

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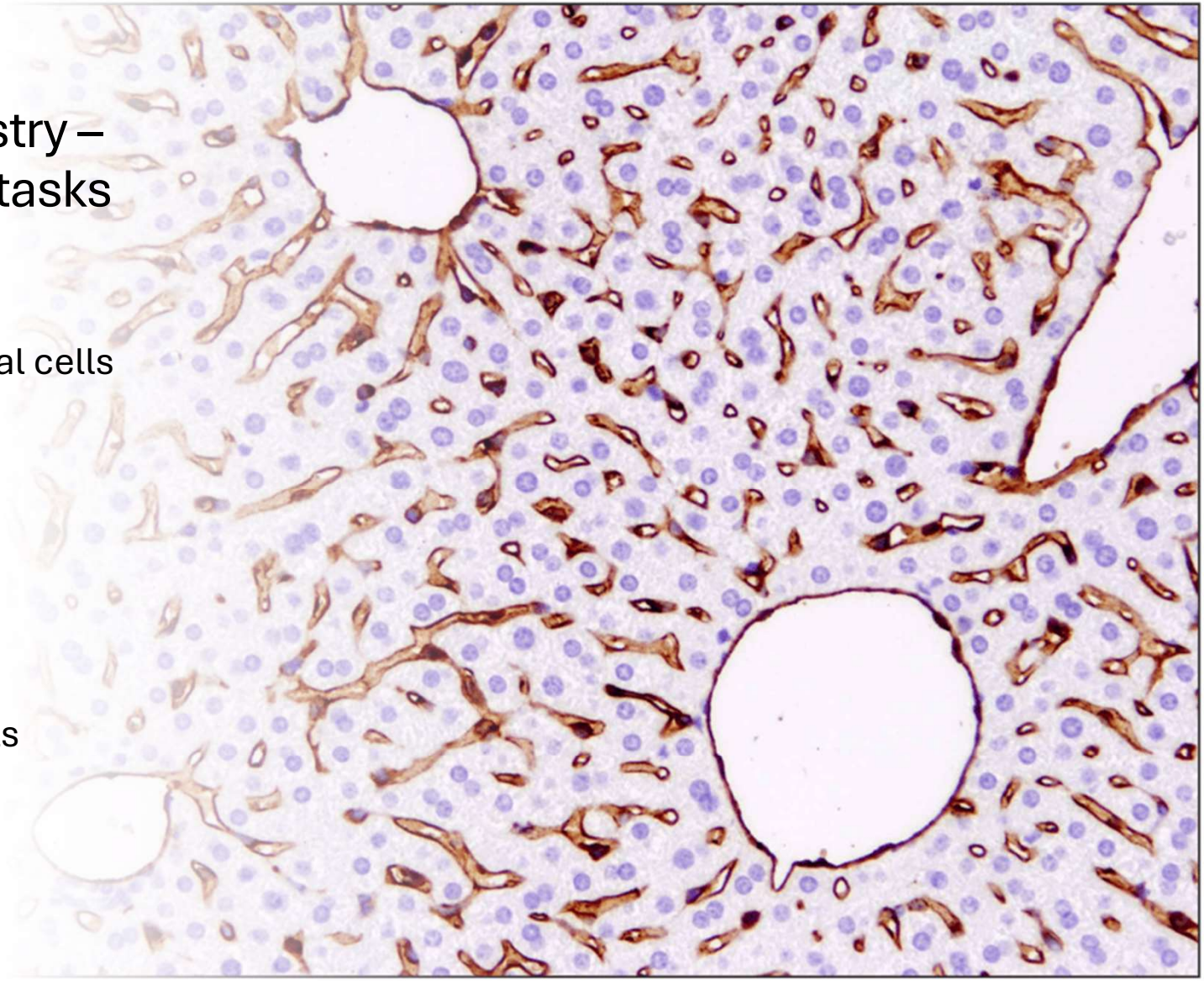
Immunohistochemistry – examples of simple tasks

- Pan cytokeratin: Epithelial cells
- CD31: vessels
- Ki67: proliferation
- CD3/CD20: T- and B-cells
- CD34: Stem cells



Immunohistochemistry – examples of simple tasks

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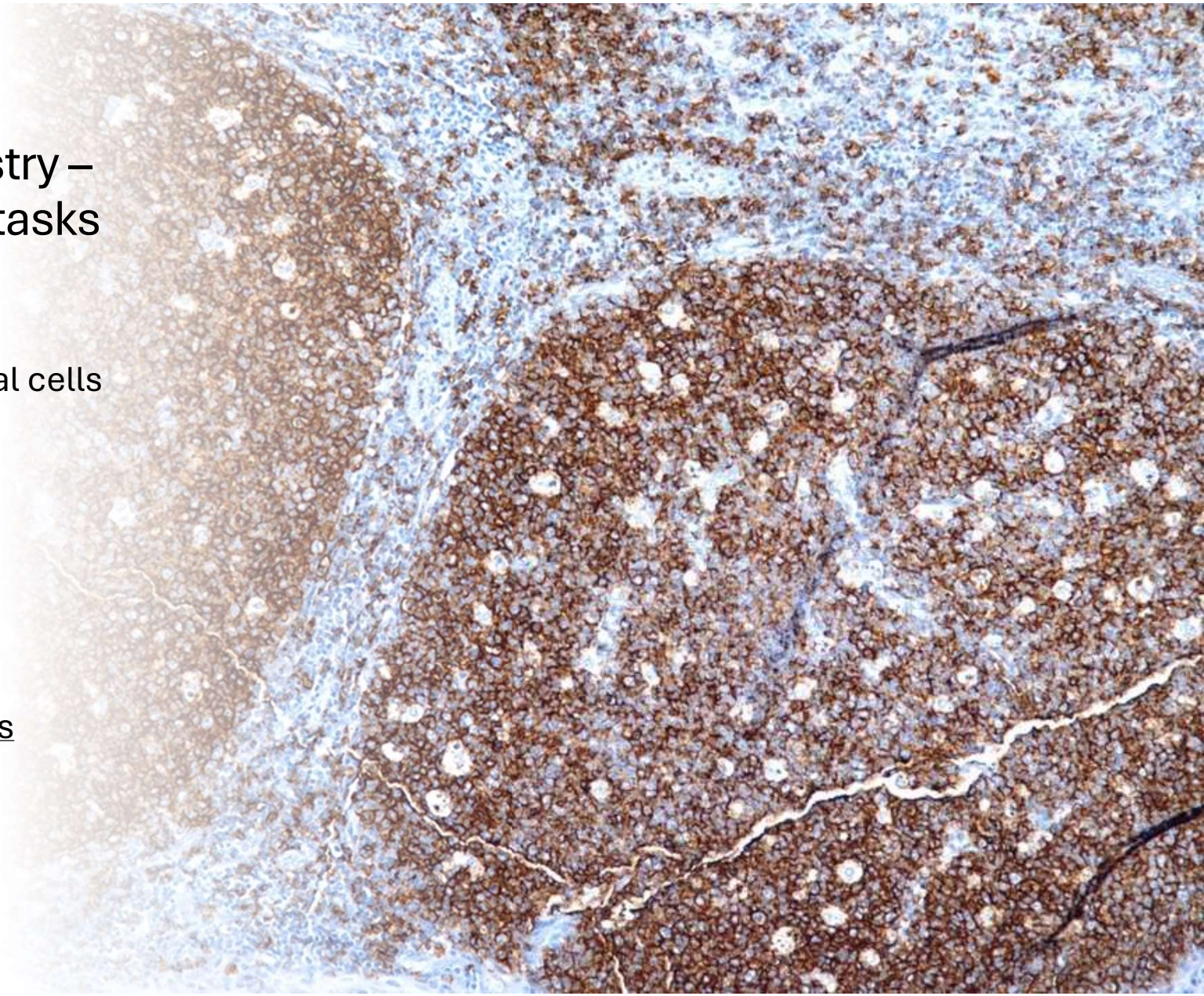
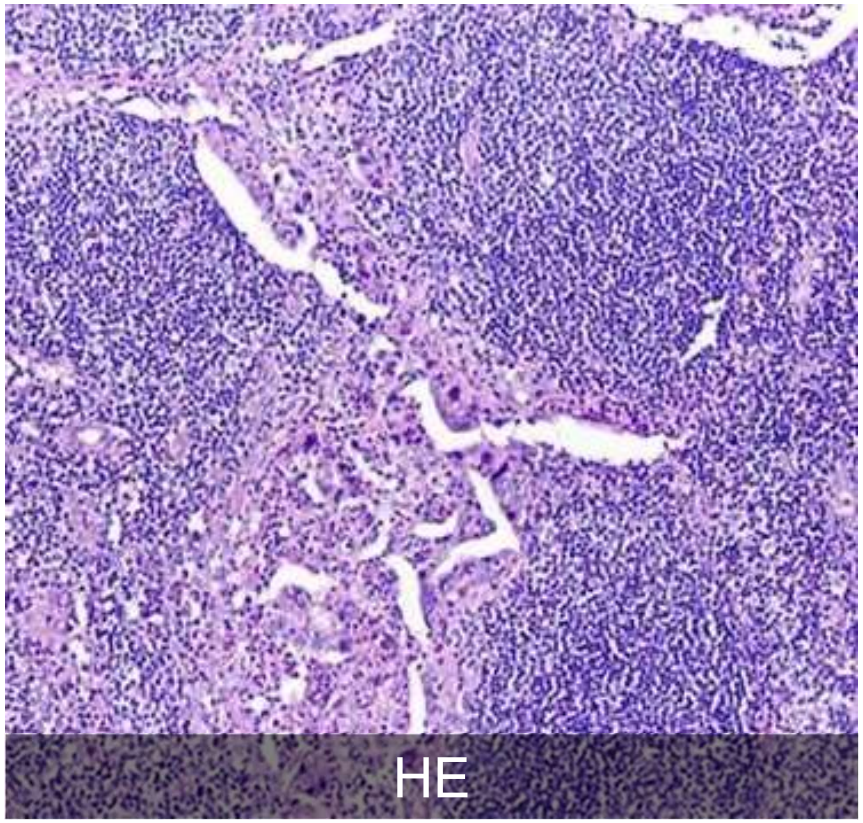
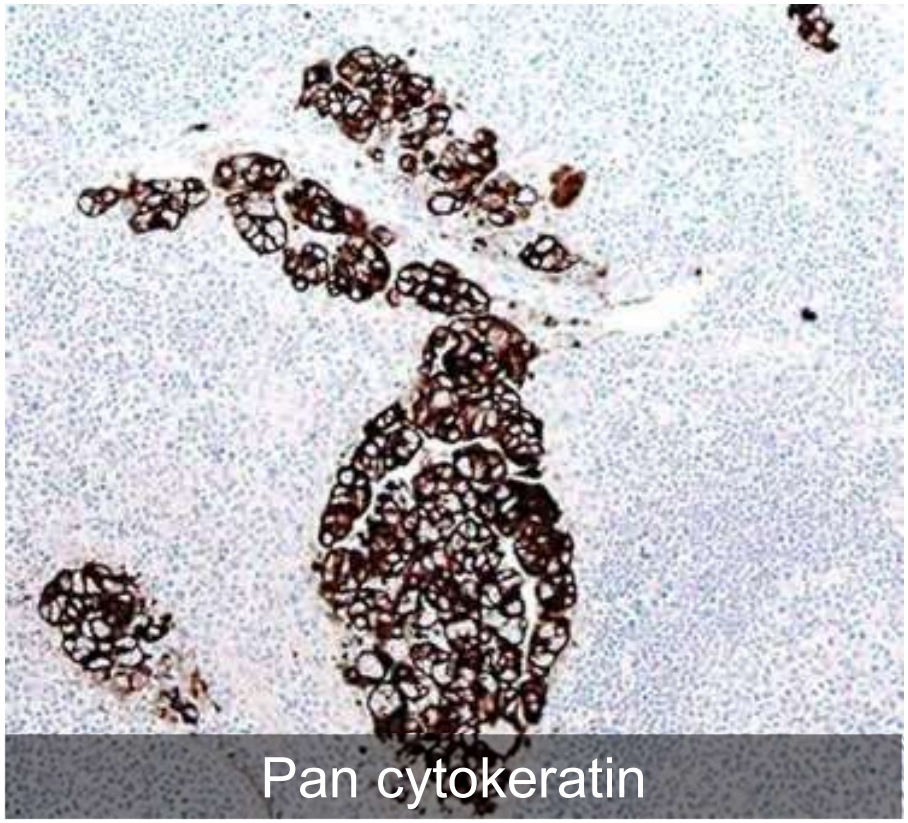


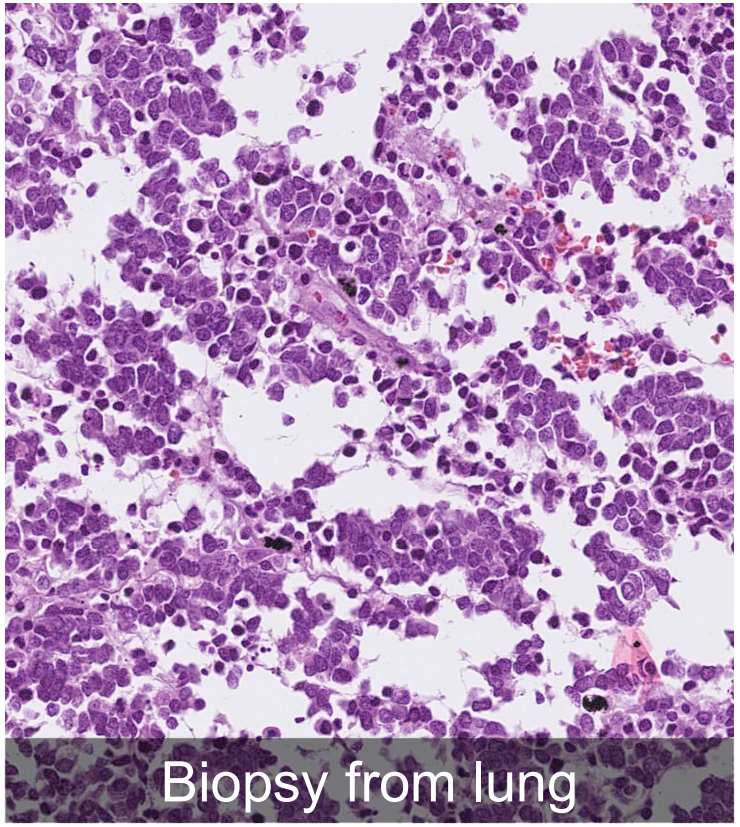
Pan cytokeratin - micrometasis





Case

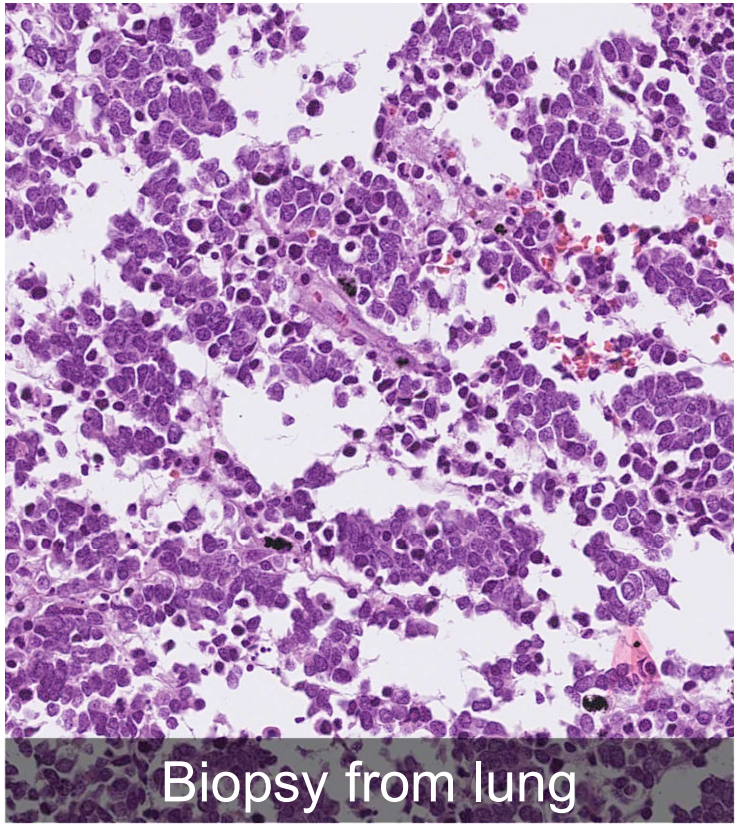
- 67 y.o. female
- Heavy smoker
- Several tumours in both lungs
- Pleural plaques and exposed to asbestos
- Previous ovarian serous carcinoma


Biopsy from lung

Case

- Carcinoma
- Lung cancer ?
  - Adeno? Squamous? Small cell (neuroendocrine)?
- Relapse ovarian serous carcinoma ?
- Mesothelioma ?

CD45	Pan cytokeratin	S100	Vimentin
Negative	Positive	Negative	Negative



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Selected panels

Lung:
TTF
Napsin

Neuroendocrine:
SYP
CGA
INSM1

Female genitals:
PAX8
WT1
ER

Mesothelioma:
Calretinin
Podoplanin

Lung	
TTF	Positive
Napsin	Negative

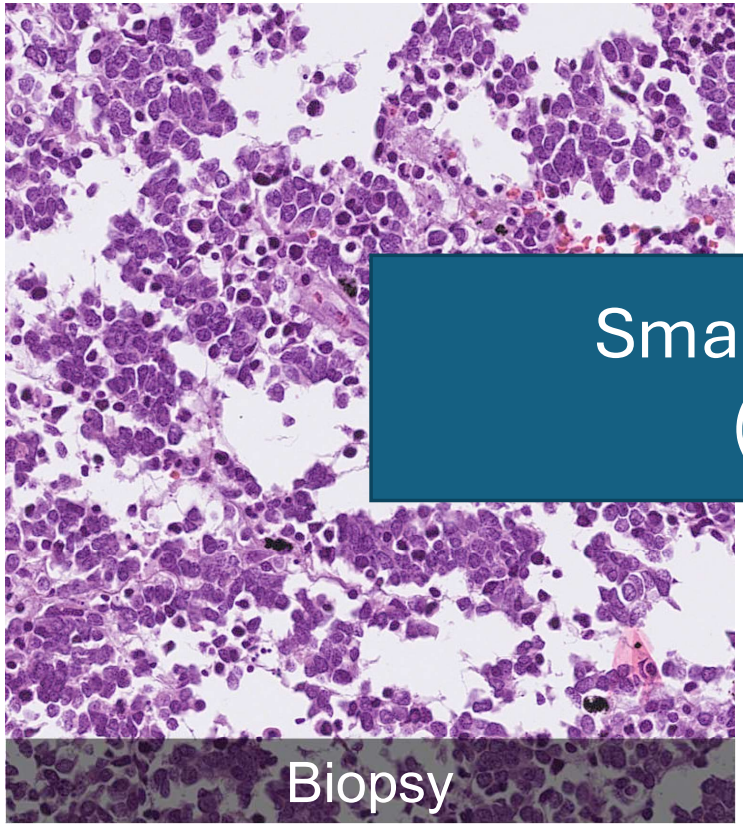
Neuroendocrine	
SYP	Positive
CGA	Positive
INSM1	Positive

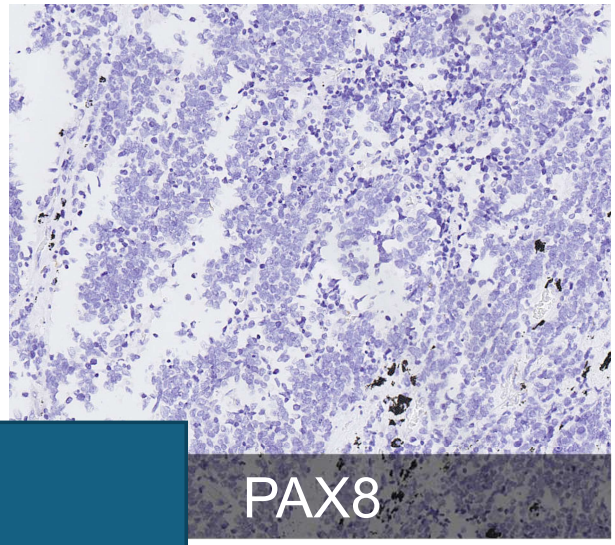
Female genitals	
PAX8	Negative
WT1	Negative
ER	Negative

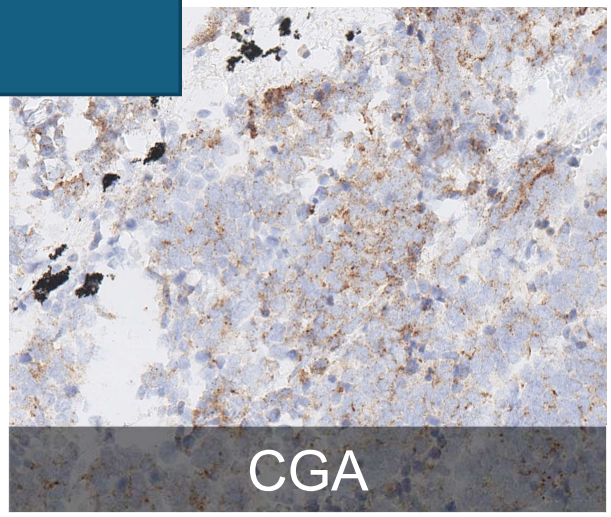
Mesothelioma	
Calretinin	Negative
Podoplanin	Negative

NordiQC

Case

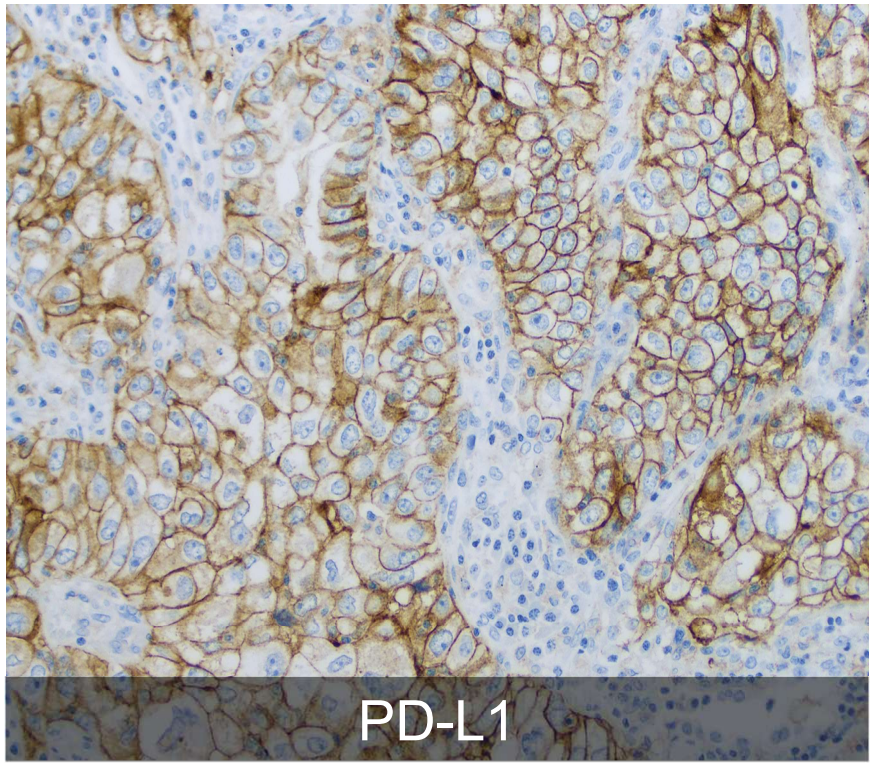




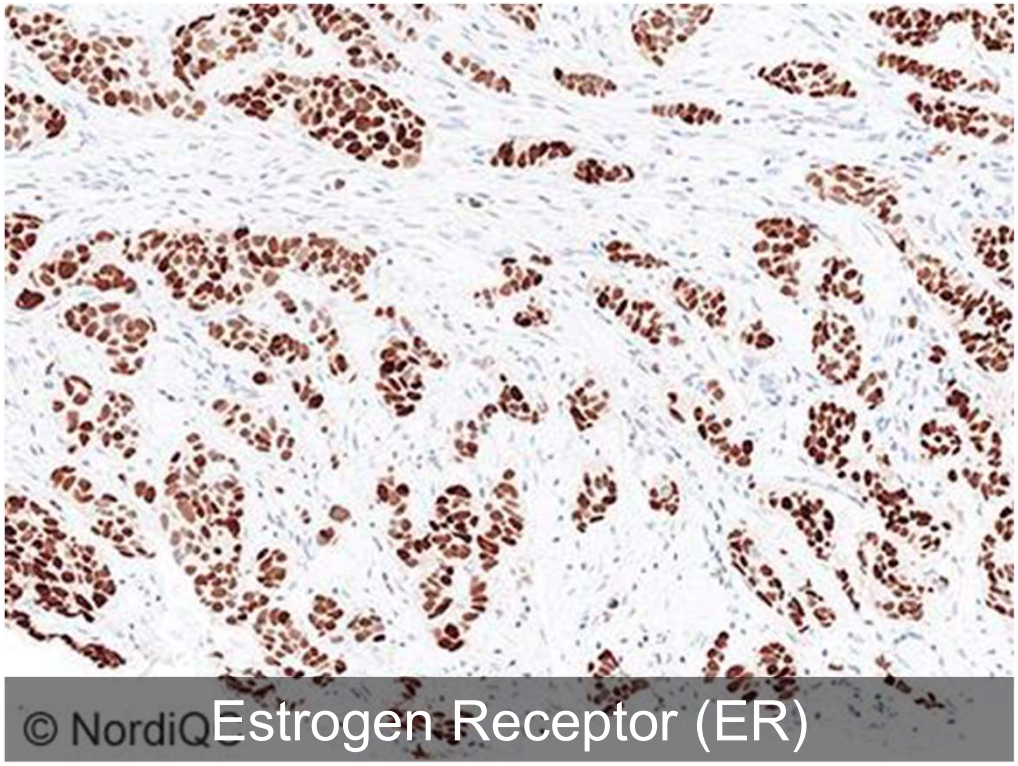


Small cell lung carcinoma
(neuroendocrine)

Predictive biomarkers



PD-L1



Estrogen Receptor (ER)

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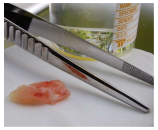
FDA approved IHC assays

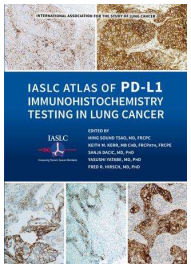
Biomarker	Indication	Drug	Test	Product
HER2	Breast cancer, Gastric ad carc.	Trastuzumab, Pertuzumab,...	IHC	HercepTest™, PATHWAY®
ALK	NSCLC	Crizotinib, Ceritinib, ...	IHC	VENTANA ALK D5F3 assay
CD117	Gastrointestinal stromal tum.	Gleevec	IHC	Dako c-KIT pharmDx
MMR	Endometrial carcinoma	Dostarlimab-gxly	IHC	VENTANA MMR RxDx panel
PD-L1	NSCLC, Urothelial carc. HNSCC, TNBC, ESCC, Cervical cancer.	Pembrolizumab	IHC	Dako 22C3 IHC pharmDX
PD-L1	NSCLC, (Melanoma optional)	Nivolumab	IHC	Dako 28-8 IHC pharmDX
PD-L1	NSCLC, Urothelial carc.	Atezolizumab	IHC	VENTANA PD-L1 SP142
PD-L1	Urothelial carc., (NSCLC)	Durvalumab	IHC	VENTANA PD-L1 SP263

"Immunohistochemistry is technically complex, and no aspect of this complexity can be ignored, from the moment of collecting the specimen to issuance of the final report "
Taylor CR. Arch Pathol Lab Med 2000; 124:945

Pre-Analytical	Analytical	Post-Analytical
Ischemia	IHC platform	Usage of controls
Fixation process	Epitope retrival	Positive controls
(Decalcification)	Primary antibody	Negative controls
Tissue processing	Detection system	"Critical controls"
Paraffin embedding	Chromogen	Scoring / read-out
Sectioning	Counterstaining	Interpretation
Storage	Mounting	Reporting







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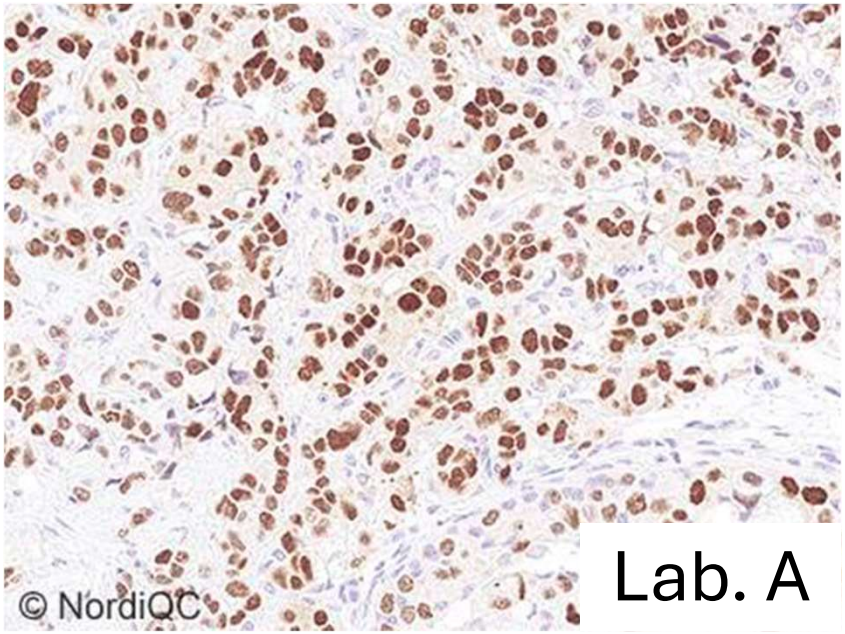
Nordic immunohistochemical Quality Control (NordiQC)

- External Quality Assessment scheme focusing on the analytical phase of IHC

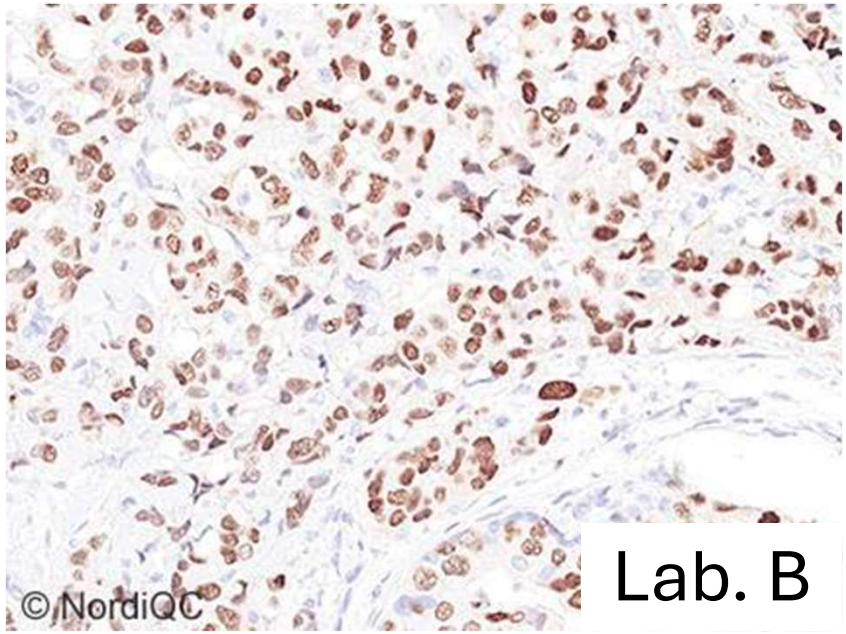
- Assessing the IHC assay quality in international pathology labs
  - Based on “standard” processed circulated tissues
- Identifying optimal and insufficient results
  - Correlated to antibodies, protocols and stainer platforms
- Giving directions for improvement
  - Individually tailored recommendations

NordiQC

Serial sections (breast carcinoma) stained for Estrogen Receptor (ER)



Lab. A



Lab. B

Run B33 - 2022

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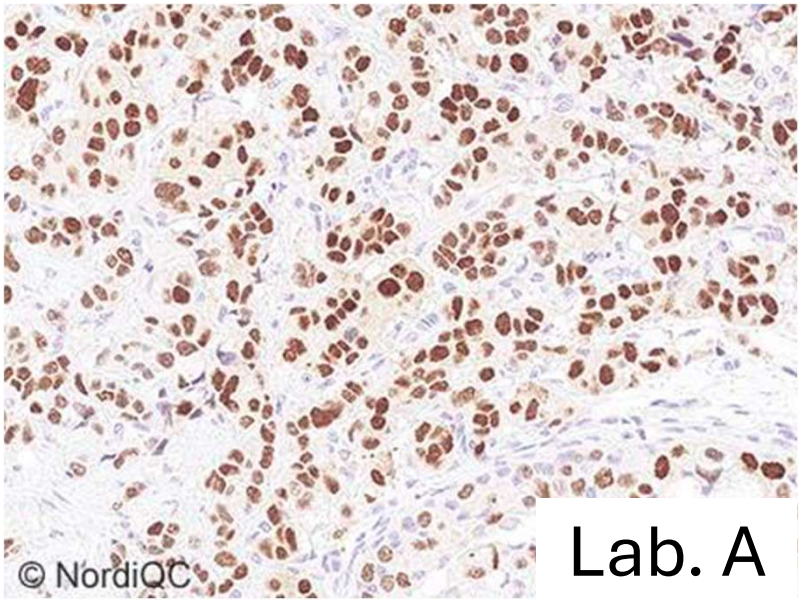
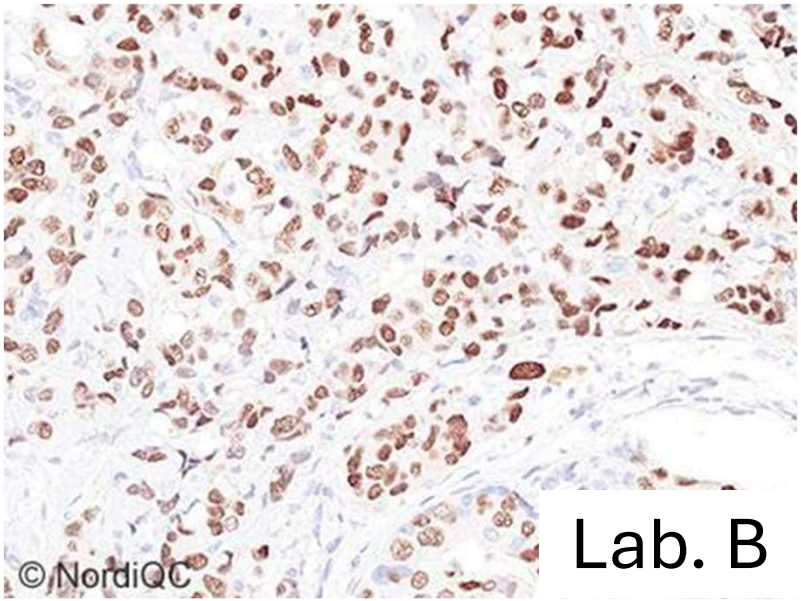
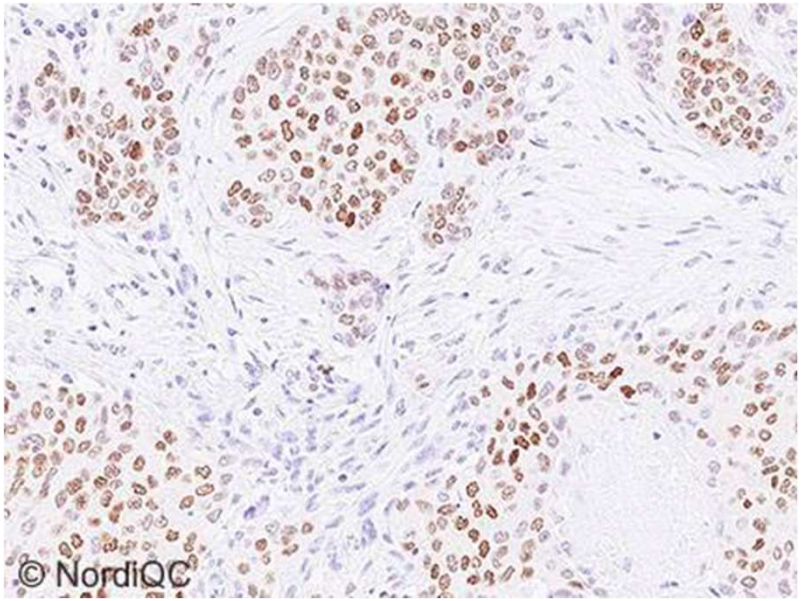
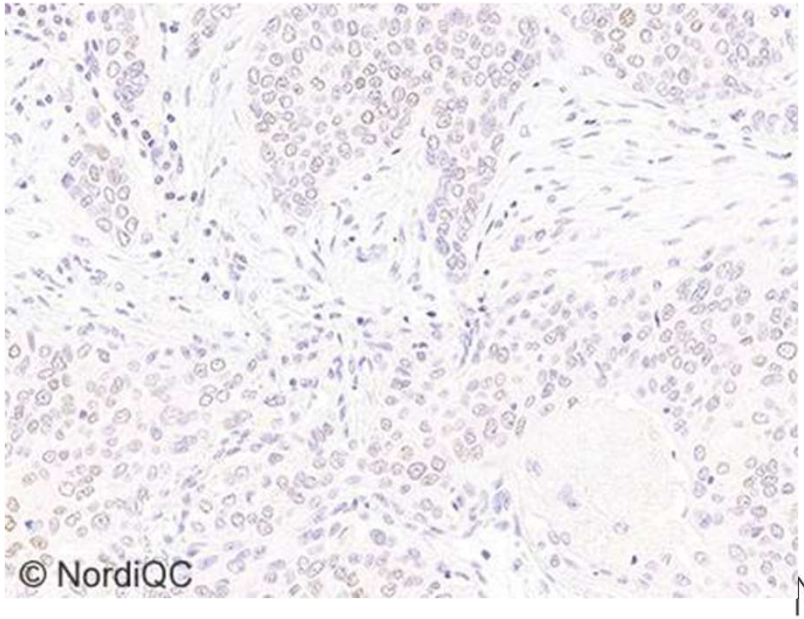
High Expressor Tumor





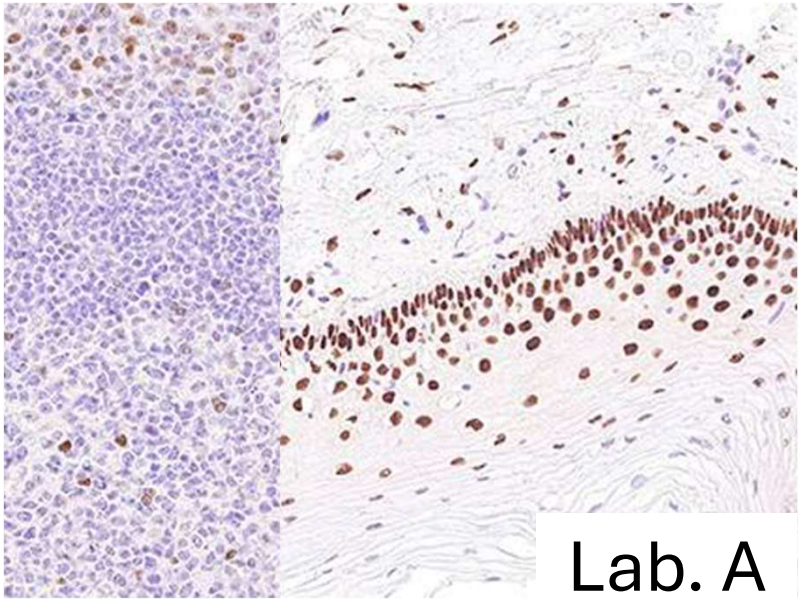
Low Expressor Tumor

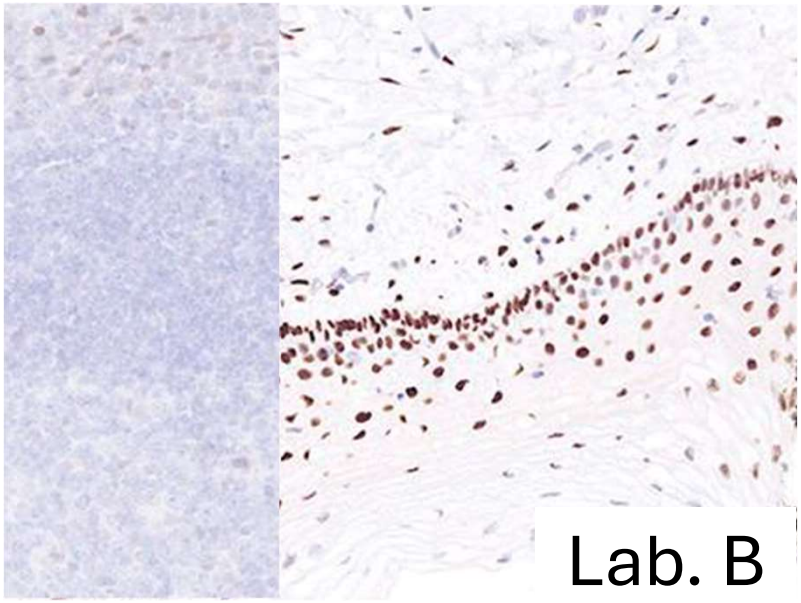




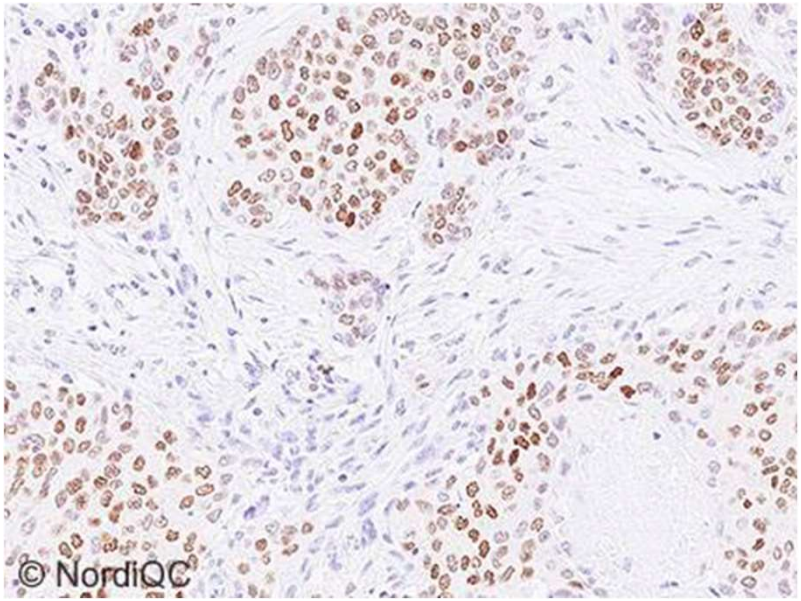
False negative

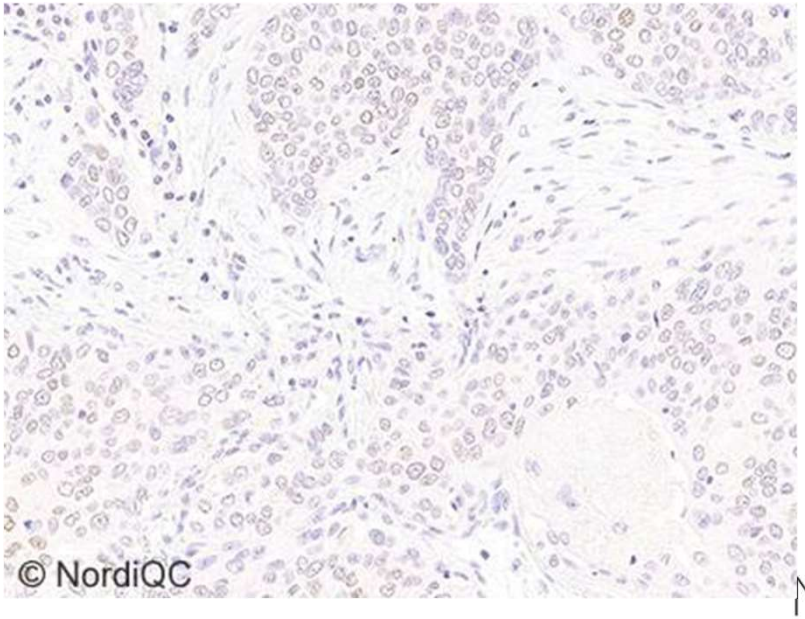
NordiQC





Low expressor



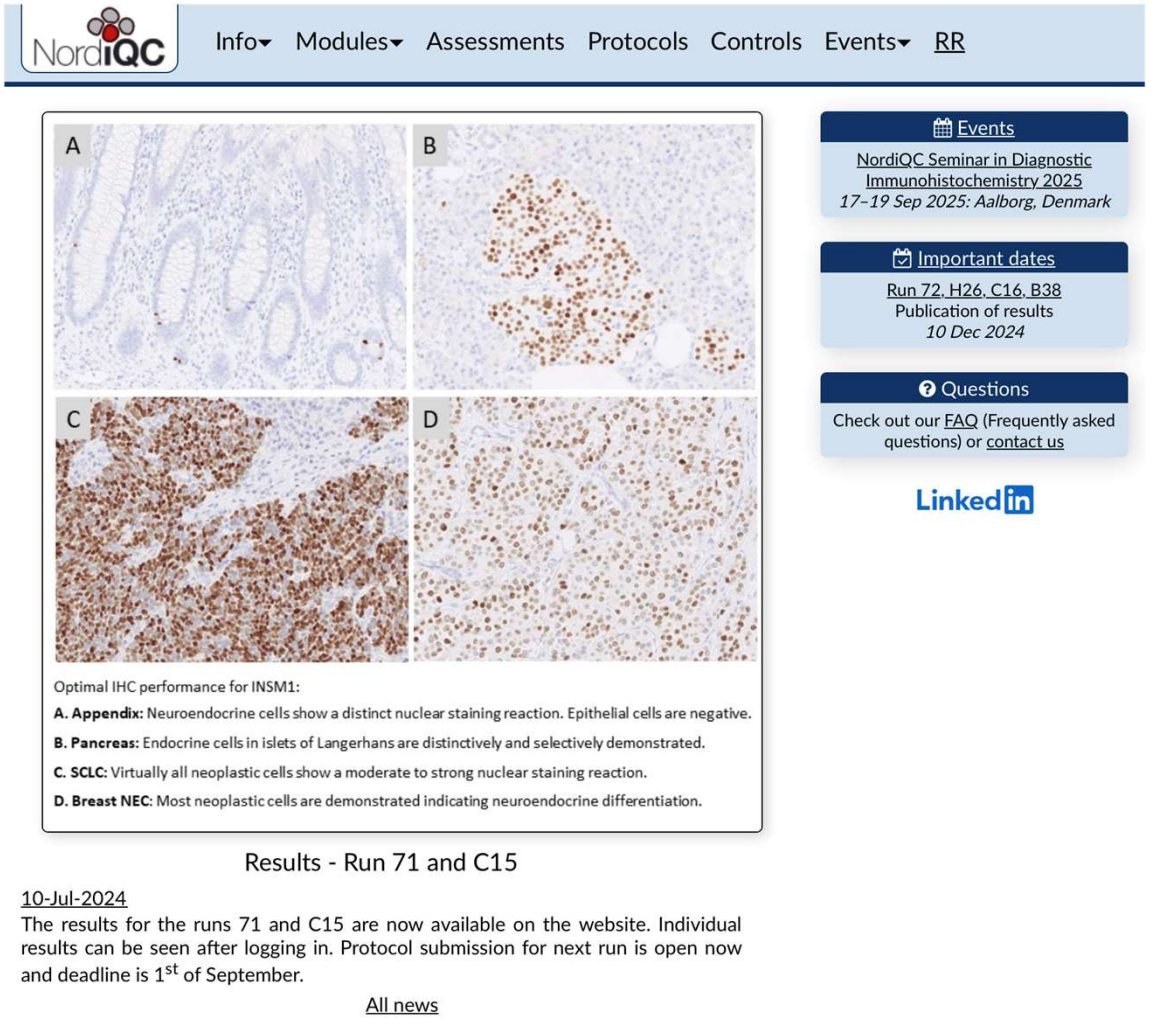


False negative

NordiQC

Homepage

www.nordiqc.org



NordiQC

NordiQC

Number of active labs: 662
from 57 different countries.

Participants by module

Module	n	Countries
General Module	509	47
Breast Cancer Module	519	52
HER2-ISH Module	293	42
Companion Diagnostic Module	327	40

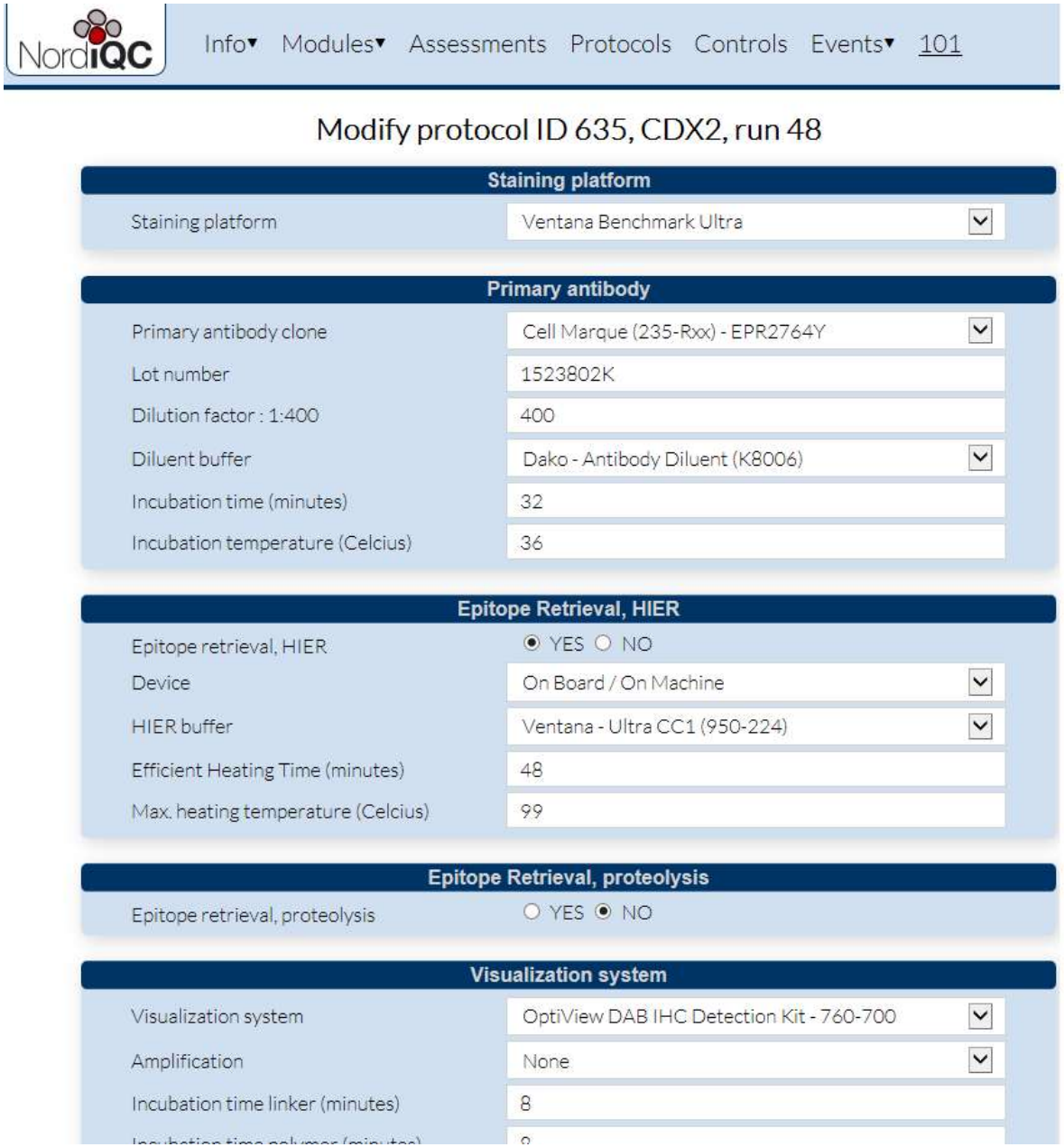
NordiQC assessment scheme 2023

Module	Winter	Spring	Autum
General	**Run 67** CD4 p40 MLH1 CGA p53	**Run 68** URO II/III MSH2 TTF1 CD10 PRAME PAX8	**Run 69** PSA EpCAM CK8/18 CD5 CD138
Breast	**Run B35** PR HER2 IHC ER		**Run B36** ER HER2 IHC
HER2 ISH	**Run H23** HER2 ISH		**Run H24** HER2 ISH
Companion		**Run C13** PD-L1 (TPS/CPS) PD-L1 (IC)	**Run C14** PD-L1 (TPS/CPS) PD-L1 (IC)

NordiQC method

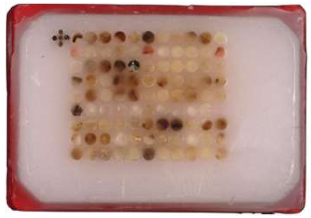
- Construction of Tissue Micro Array block containing critical tissue for a specific epitope
- Participants submit their staining protocol on the NordiQC homepage (www.nordiqc.org).
- Sections are cut from the block(s) and circulated to the participants
- Participants stain the slides using the submitted protocols and return the slides to NordiQC
- A group of pathologists and expert technicians meet for assessment of the slides
- General reports describing optimal and insufficient staining protocol parameters published on www.nordiqc.org
- Individual assessment marks and tailored protocol recommendations sent to participants

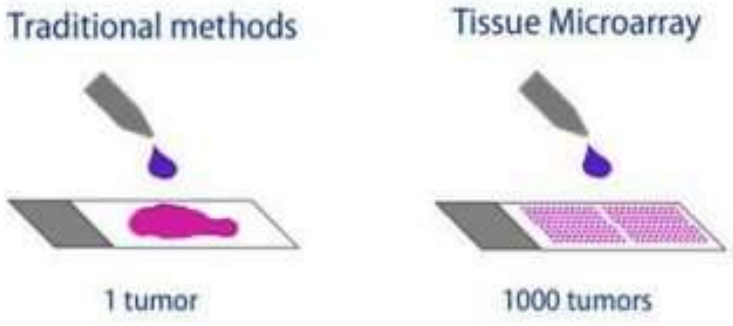
NordiQC


NordiQC
Info▾ Modules▾ Assessments Protocols Controls Events▾ 101
Modify protocol ID 635, CDX2, run 48
Staining platform
Staining platform
Ventana Benchmark Ultra
Primary antibody
Primary antibody clone
Cell Marque (235-Rxx) - EPR2764Y
Lot number
1523802K
Dilution factor : 1:400
400
Diluent buffer
Dako - Antibody Diluent (K8006)
Incubation time (minutes)
32
Incubation temperature (Celcius)
36
Epitope Retrieval, HIER
Epitope retrieval, HIER
YES NO
Device
On Board / On Machine
HIER buffer
Ventana - Ultra CC1 (950-224)
Efficient Heating Time (minutes)
48
Max. heating temperature (Celcius)
99
Epitope Retrieval, proteolysis
Epitope retrieval, proteolysis
YES NO
Visualization system
Visualization system
OptiView DAB IHC Detection Kit - 760-700
Amplification
None
Incubation time linker (minutes)
8

NordiQC – test materials

- Multi-tissue FFPE blocks
- 10% NBF 24-48 (ASCO-CAP guidelines)
  - Normal and clinically relevant tumour tissues
  - Different levels of antigen expression (High, moderate, low, negative)





NordiQC

Assessment Run 70 2024

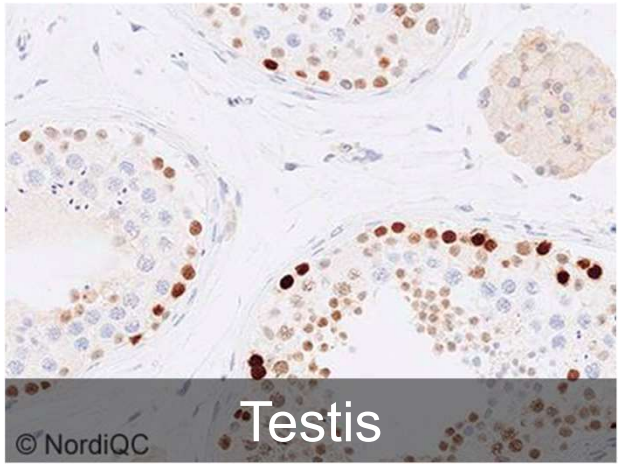
PReferentially expressed Antigen in Melanoma (PRAME)

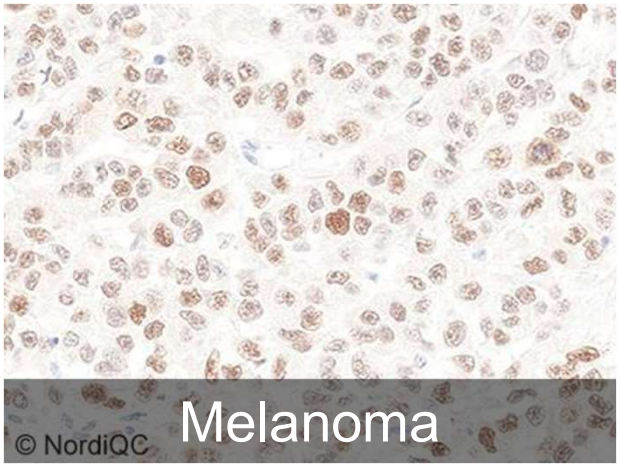
Material
The slide to be stained for PRAME comprised of:

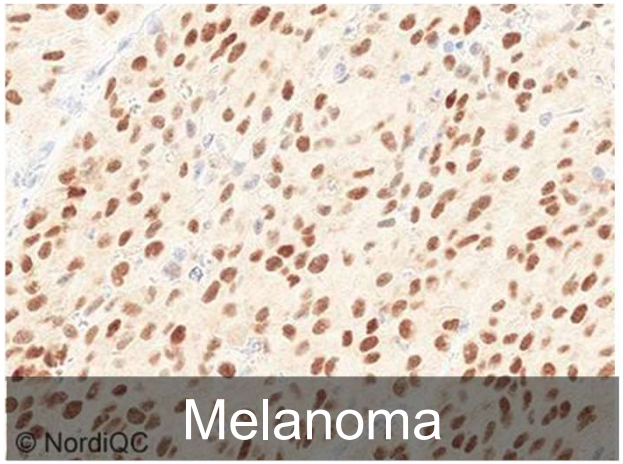
1. Compound nevus, 2. Testis, 3.-4. Melanoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PRAME staining as optimal included:







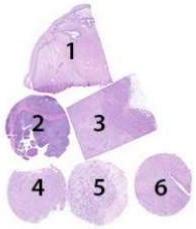
Assessment Run B27 2019

Estrogen receptor (ER)

Material

The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80- 90%	Moderate to strong
2.	Tonsil	1-5%	Weak to moderate
3.	Breast carcinoma	70-90%	Weak to moderate
4.	Breast carcinoma	80-100%	Weak to moderate
5.	Breast carcinoma	100%	Moderate to strong
6.	Breast carcinoma	Negative	-

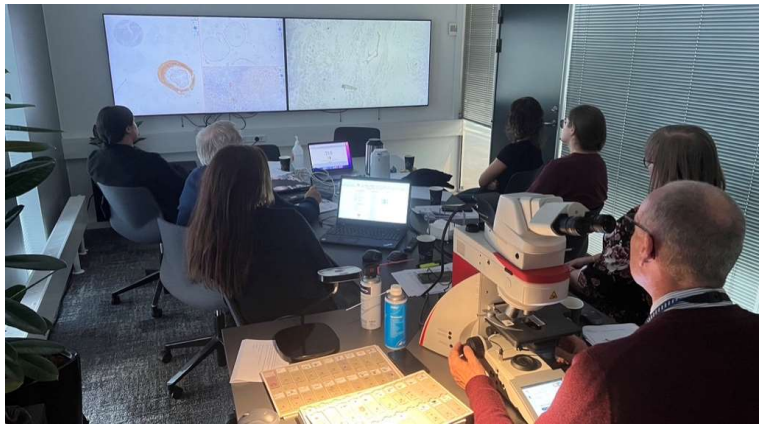


*ER-status and staining pattern as characterized by the NordiQC reference laboratories using the rmAb clones EP1 and SP1.

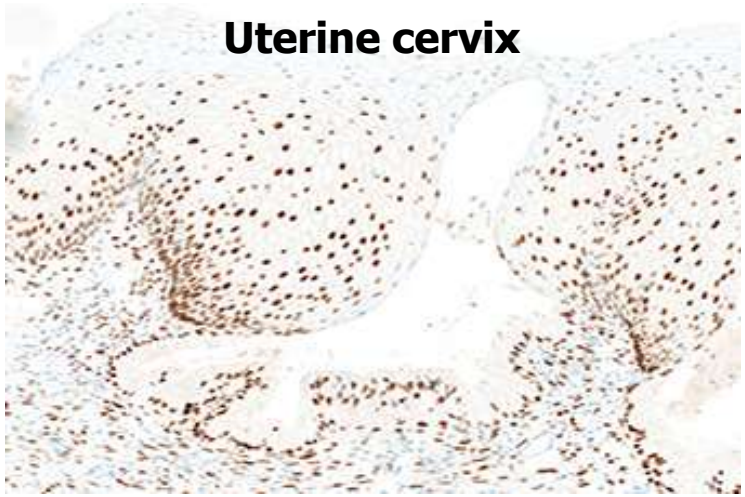
Main focus of assessment:

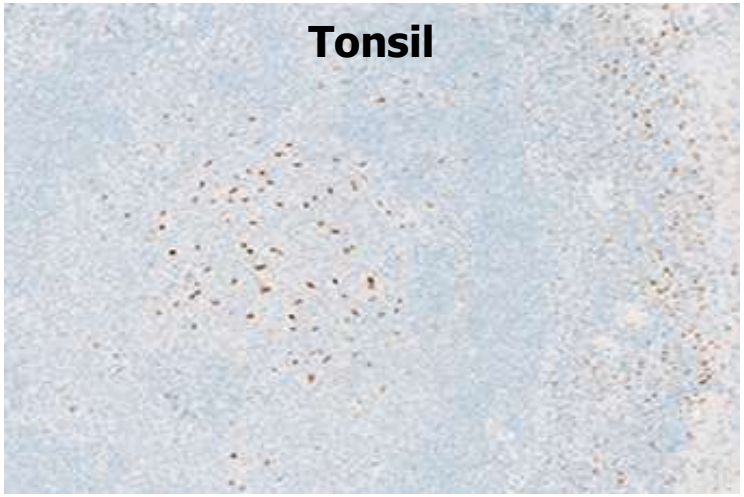
- Appropriate technical quality (signal-to-noise, good morphology etc.)
- Appropriate analytical sensitivity and specificity – indicated by concordance of ER status and proportion of positive cells in the included tumours to references

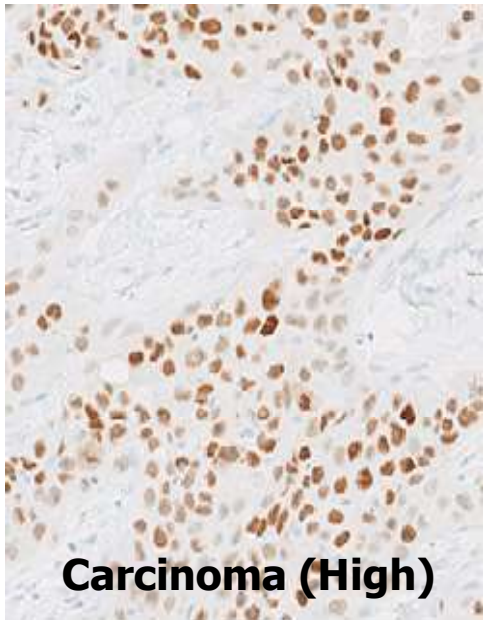
Score	Criteria: Staining reaction considered …
Optimal	… Perfect or close to perfect in all of the included tissue cores
Good	… Fully acceptable in all of the included tissue cores. However,v the protocol may be optimized to ensure the best staining intensity and signal-to-noise ratio
Borderline	… Insufficient because of, e.g., a generally too weak staining or a false negative staining of one of the included tissues, or a minor false positive staining reaction
Poor	… Very insufficient because of, e.g., false negative staining of several of the included tissues, or a major false positive staining reaction

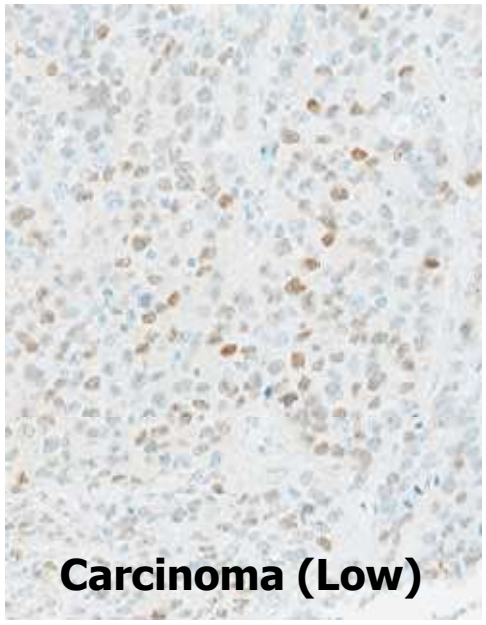


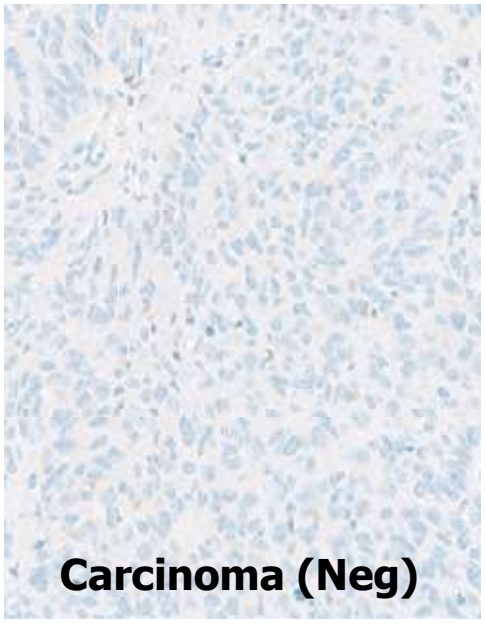
NordiQC


Uterine cervix


Tonsil


Carcinoma (High)

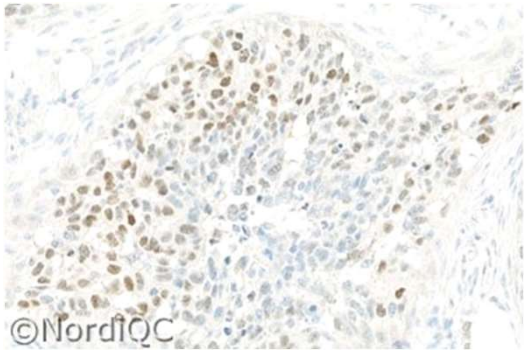

Carcinoma (Low)

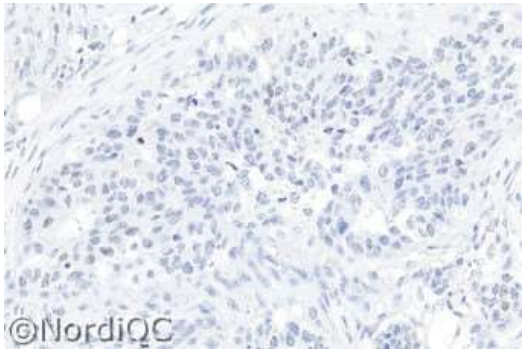

Carcinoma (Neg)

NordiQC

ER: Typical challenges

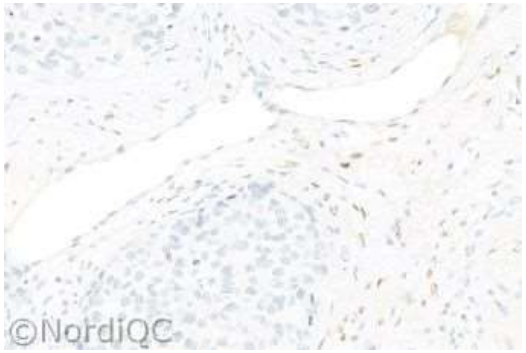
85% Weak / False negative

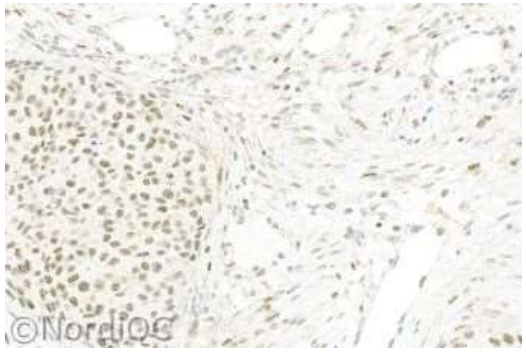




Too low titre (EP1, SP1 conc.)
Insufficient HIER,
Clone 1D5

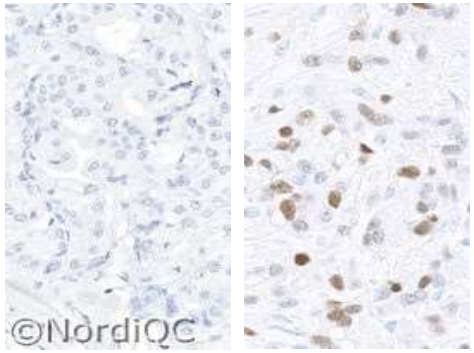
10% False positive

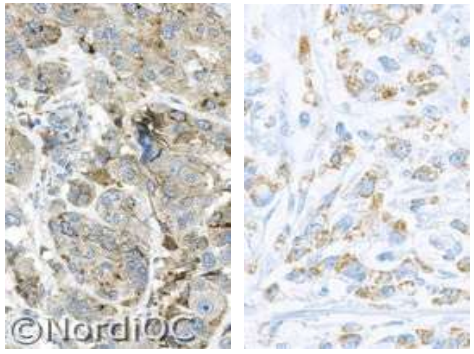




Clone 6F11 by HIER at high pH, 3-step pol.
(not observed on VMS)

5% Impaired morphology, etc





Clone 1D5 at high titre,
Biotin-based kits,
HIER in pressure cooker

Sufficient

Insufficient

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ER: Selection of primary Ab and format

Table 1. **Antibodies and assessment marks for ER, B27**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **6F11**	15	Leica/Novocastra	6	6	1	2	80%	100%
mAb clone **C6H7**	1	Celnovte	-	1	-	-	-	-
rmAb clone **EP1**	16 1	Dako/Agilent Cell Marque	8	6	3	-	82%	91%
rmAb clone **SP1**	20 7 1 1 1 1	Thermo Scientific Cell Marque Spring Bioscience Abcam Diagnostic Biosystems Zytomed Systems	19	7	4	1	84%	100%
Ready-To-Use antibodies								
mAb clone **1D5 IR/IS657**	1	Dako/Agilent	1	-	-	-	-	-
mAb clones **1D5 + ER-2-123 SK310**	1	Dako/Agilent	-	1	-	-	-	-
mAb clone **6F11 PA0009/PA0151**	13	Leica	4	4	3	2	62%	83%
rmAb **EP1 IR/IS084**	27	Dako/Agilent	10	13	4	-	85%	84%
rmAb **EP1 IR/IS084**[3]	8	Dako/Agilent	3	3	1	1	-	-
rmAb **EP1 GA084**	32	Dako/Agilent	14	15	3	-	91%	91%
rmAb **EP1 GA084**[3]	3	Dako/Agilent	3	-	-	-	-	-
rmAb clone **SP1 790-4324/5**	187	Ventana/Roche	113	65	6	3	95%	95%
rmAb clone **SP1 790-4324/5**[3]	1	Ventana/Roche	1	-	-	-	-	-
rmAb clone **SP1 249R-1**	4	Cell Marque	1	3	-	-	-	-
rmAb clone **SP1 KIT-0012**	1	Maixin	1	-	-	-	-	-
rmAb **SP1 M3011**	1	Spring Biosystems	-	1	-	-	-	-
rmAb clone **SP1 MAD-000306QD**	1	Master Diagnostica	-	-	1	-	-	-
rmAb clone **EP1 8361-C010**	1	Sakura Finetek	-	1	-	-	-	-
rmAb clone **SP1 RMPD001**	2	Diagnostics Biosystem	2	-	-	-	-	-
r/mAb clones **6F11 + SP1 PM308**	1	Biocare Medical	1	-	-	-	-	-
Total	348		187	126	26	9	-	
Proportion			54%	36%	7%	3%	90%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system used on a different platform than it was developed for.

Concentrated format:
Overall protocol parameters

HIER alk. pH
2- & 3-step kits

Carefully calibration of primary Ab

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Causes of insufficient results

- Less successful antibodies
  - Poor antibodies
  - Less robust antibodies
  - Poorly calibrated RTUs
  - Stainer platform dependent antibodies
- Insufficiently calibrated antibody dilutions
- Insufficient or erroneous epitope retrieval
- Less sensitive visualization systems
- Other
  - Impaired morphology
  - Technical issues
  - Excessive counterstaining impairing interpretation

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Individual feedback

Nordic Immunohistochemical Quality Control

Institute of Pathology, Aalborg University Hospital, Ladegaardsgade 3, P.O.Box 561, DK-9100 Aalborg, Denmark

Assessment of run 54 - individual results

Klinik XXX

Epitope	Podop	GATA3	CK-PAN	CEA
Assessment	Poor	Poor	Good	Good

Comments

Podop - Poor

Comment: False negative.

Advice: Increase primary Ab conc. Consider change of HIER buffer to Tris-EDTA pH 9 or equivalent - cave biotin.

GATA3 - Poor

Comment: False negative. False positive.

Advice: Use Hier in alkaline buffer and/or change to a more sensitive detection system and recalibrate portocol settings .

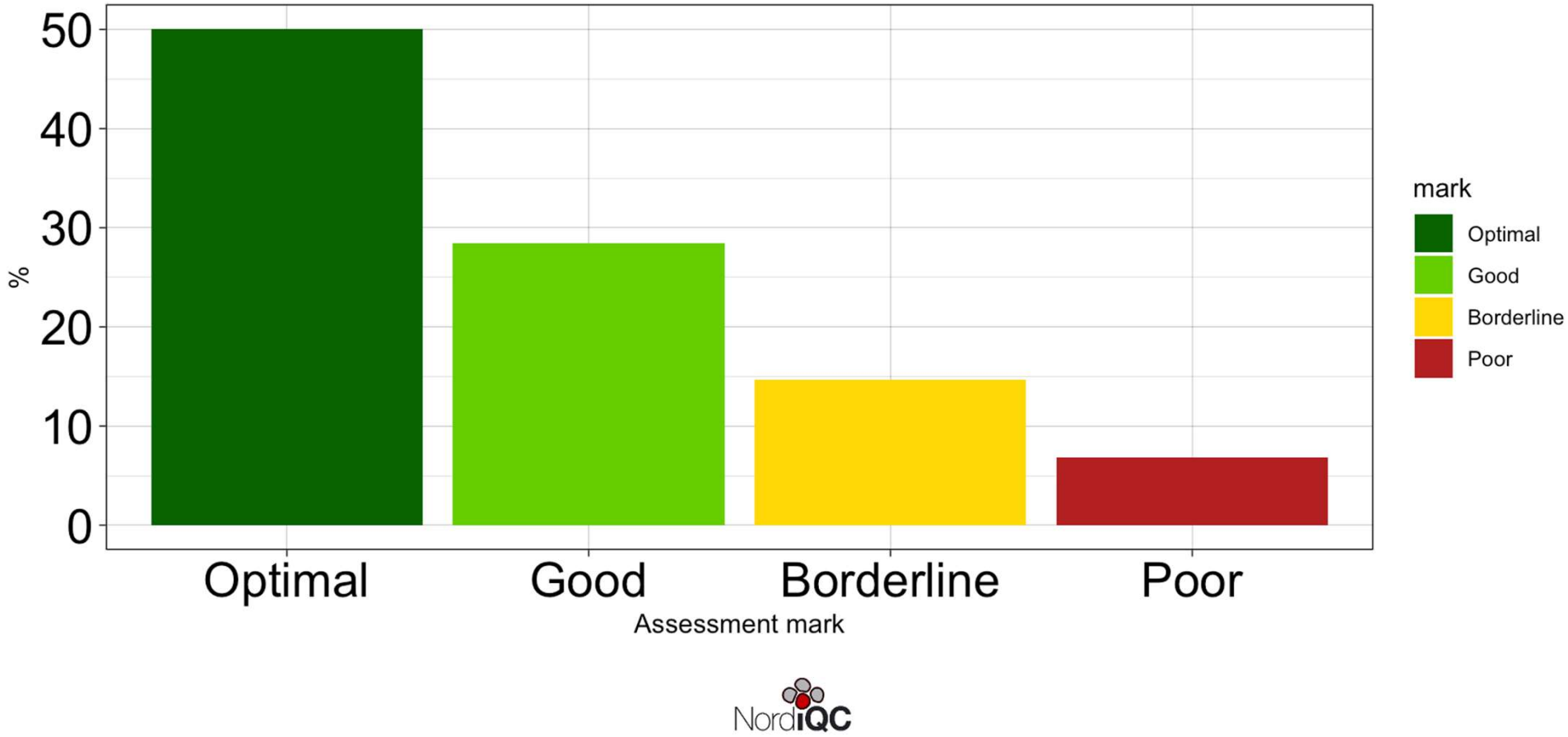
CK-PAN - Good

Comment: Weak.

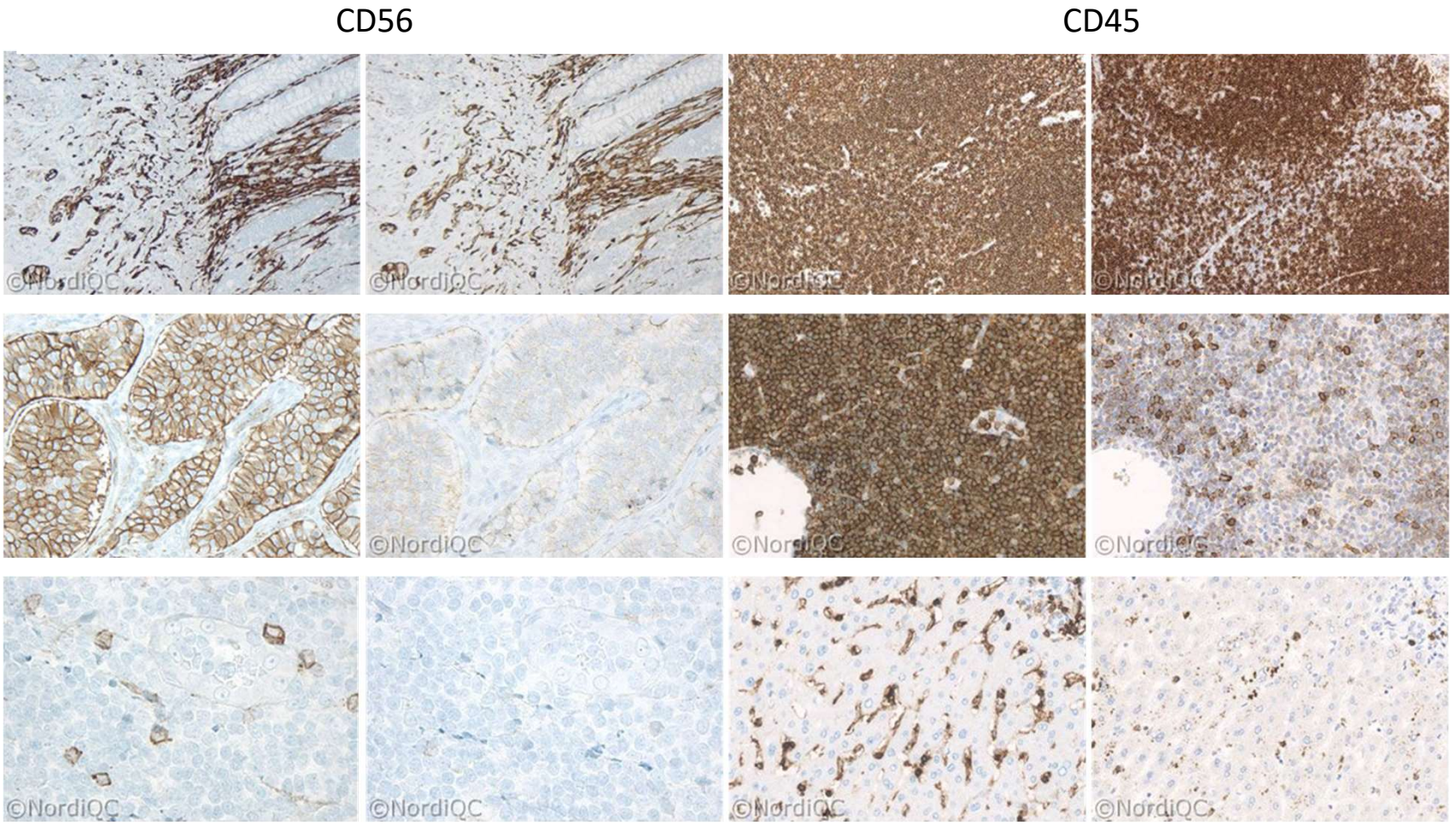
CEA - Good

Comment: Weak. Excessive background.

Results – assessment marks 2016-24



Selection of controls is imperative for IHC quality

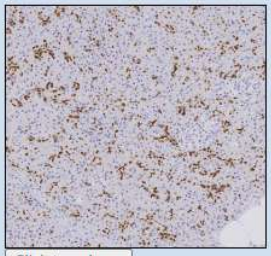
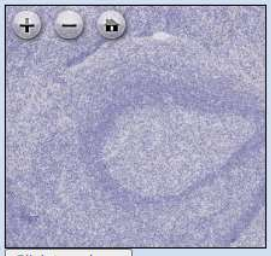


Courtesy of HL Kristoffersen

NordiQC IHC control atlas

NordiQC Info ▾ Modules ▾ Assessments Protocols Controls Events ▾ SN

CDX2 - CDX2

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Appendix/colon	Pancreas	Tonsil
Description	All epithelial cells must show a strong nuclear staining reaction. *Note, a weak cytoplasmic staining reaction in CDX2 positive cells can be seen and should be accepted if signal-to-noise ratio otherwise is acceptable.*	The vast majority of epithelial cells of intercalated ducts must show a weak to moderate nuclear staining reaction.	No staining reaction should be seen. *Note, dispersed lymphocytes can show a faint nuclear staining reaction.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Available for NordiQC participants

Tissues

Purpose

Reaction patterns

Online scans accessible

Conclusions

- EQA
  - Provides objective evidence of lab performance
  - Helps identify methodological errors
- Around 20-25% of slides submitted to NordiQC are still insufficient!
- Labs not participating in EQA ?
- How many scientific publications are based on insufficient IHC stains?
- What are the consequences for patients?

Thank you for your attention!

Collaborators in NordiQC

Søren Nielsen
Heidi Lykke Kristoffersen
Birgit Truumees
Lise Emanuelsen
Louise Rønnow Holler
Michael Bzorek
Tanya Julio
Kristi Bøgh Andersson

And the whole assessor team!