International Symposium on Immunohistochemistry
January 4th - 7th, 2018
Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India
In collaboration with NordiQC, Alborg, Denmark and ISIMM, California, USA

The impact of proficiency testing on lab immunoassays

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Aalborg, Denmark

Nordic Immunohistochemical Quality Control

Denmark


N
Skagerrak
NORTH SEA
Kattegat
SWEDEN
GERMANY
BALTIC SEA
DENMARK
Gothenburg
Göteborg
Skagen
Hjørring
Frederikshavn
VENDSYSSEL-THY
LÆSØ
Aalborg
Thisted
Nykøbing Mors
Skive
Hobro
Struer
Viborg
Randers
Grenaa
Holstebro
Herning
Silkeborg
Århus
Ringkøbing
JUTLAND
Horsens
SAMSØ
Grindsted
Vejle
Kalundborg
Fredericia
Kolding
Middelfart
Odense
Esbjerg
FANØ
Ribe
FUNEN
Nyborg
Korsør
Slagelse
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Køge
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Aabenraa
Svendborg
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LANGE-
LAND
ÆRØ
ALS
Sønderborg
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Nakskov
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Falster
Helsingør
Helsingborg
Frederiksværk
Hillerød
Jægerspris
Holbæk
Roskilde
Copenhagen
Malmø
SEALAND
Sjælland
Ystad
BORNHOLM
Rønne
Halmstad
DENMARK
National capital
Regional capital
Town
Airport
International boundary
Motorway
Planned motorway
Main road
Railroad
Ferry
0 25 50 75 km
0 25 50 mi

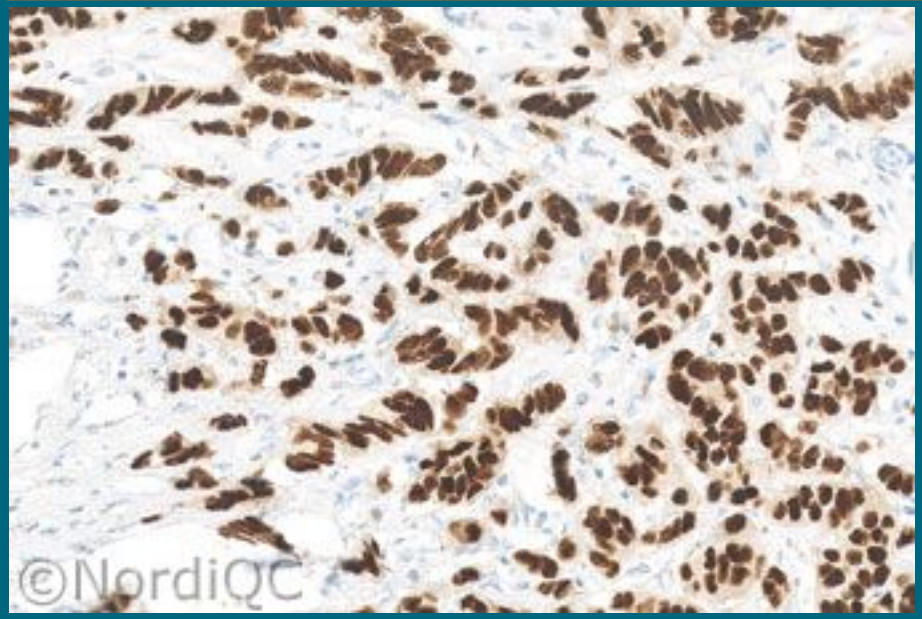
AALBORG

Budolfi Church

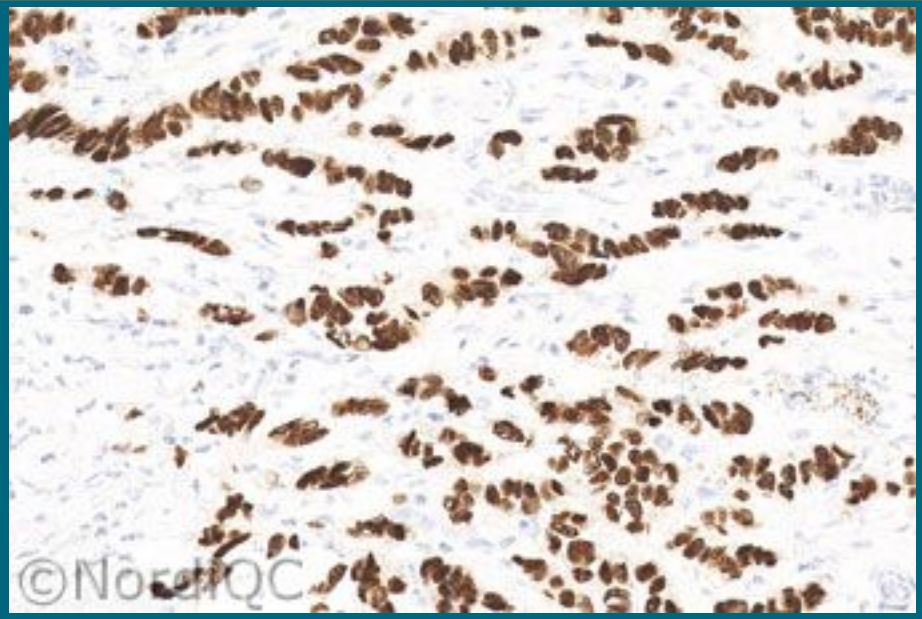
Aalborg Harbour Front

Aalborg House of Music

Serial sections stained for Estrogen receptor



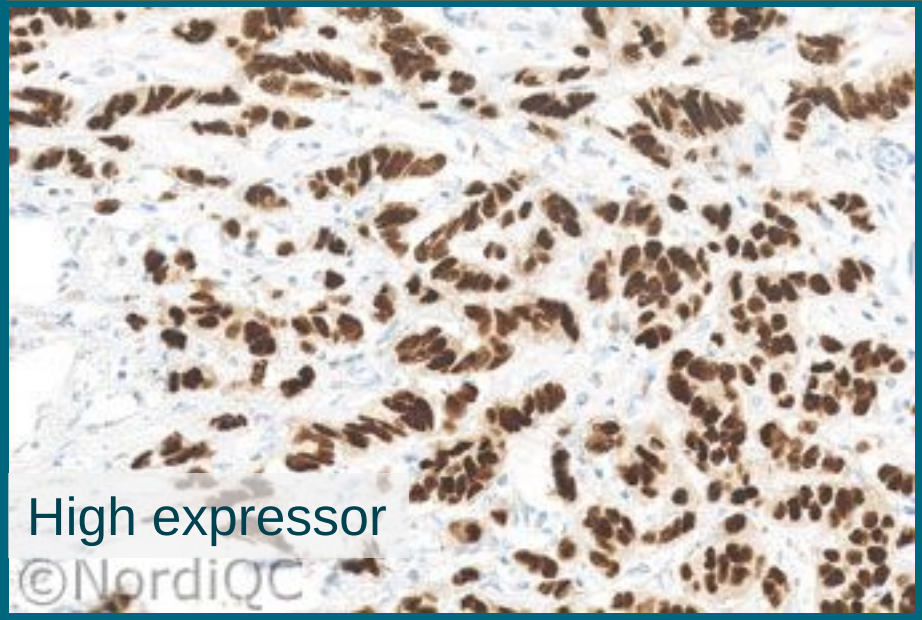
Lab. A

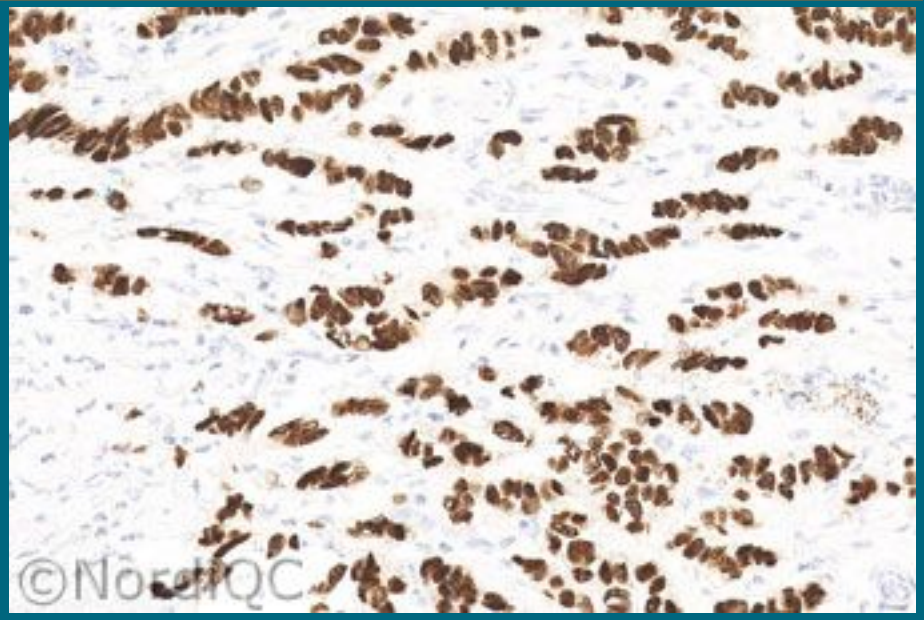


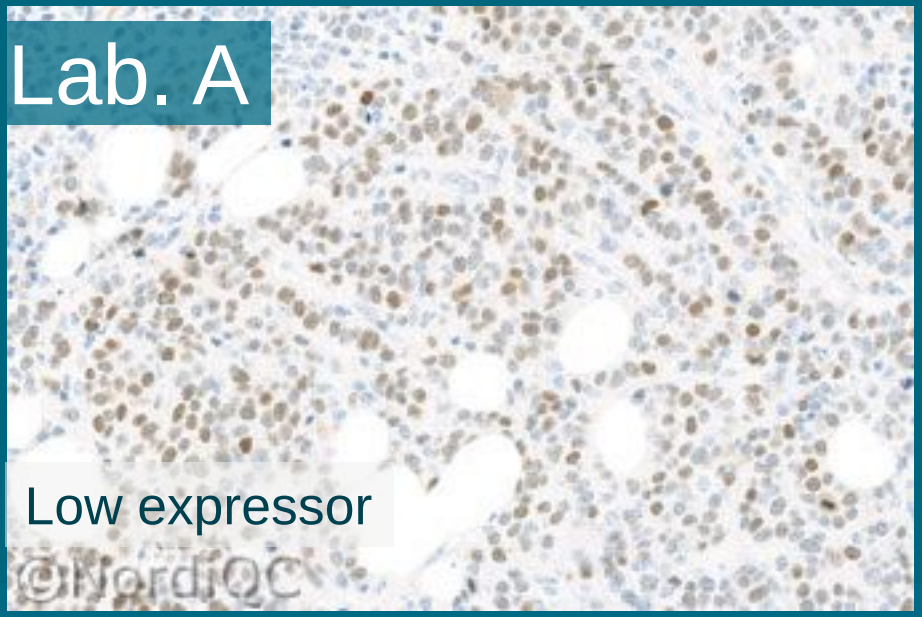
Lab. B

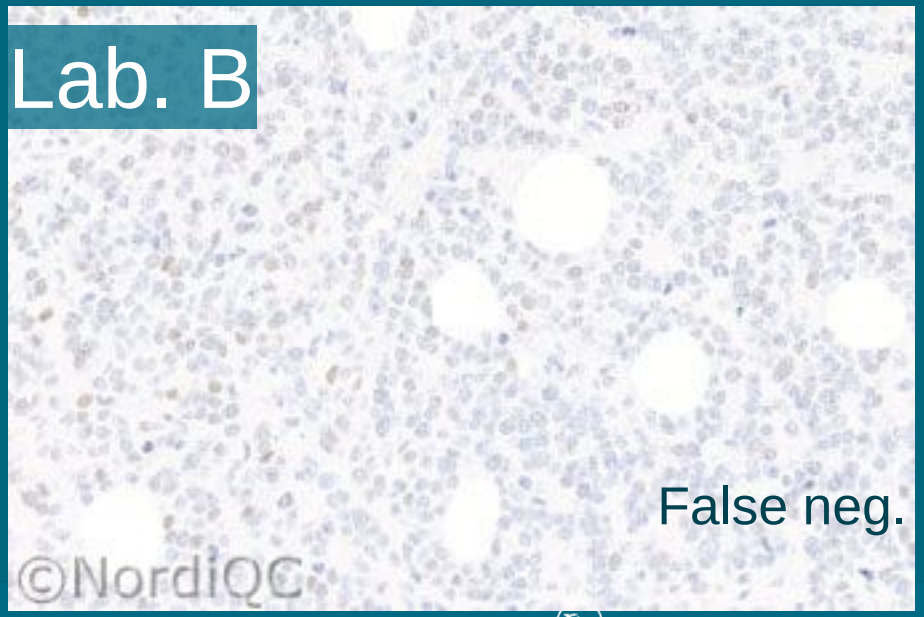
Optimally processed ductal breast carcinoma tissue

Serial sections stained for Estrogen receptor

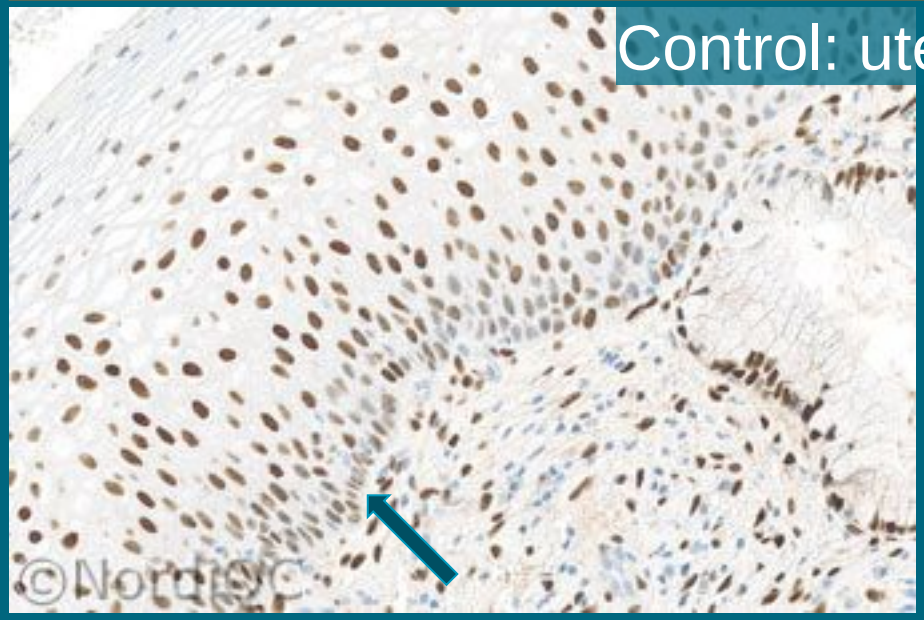


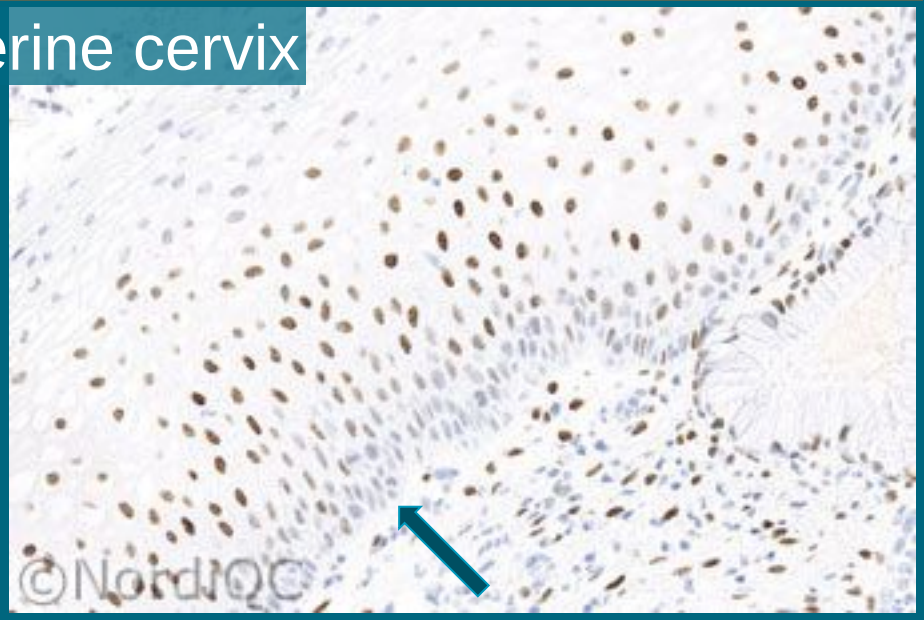


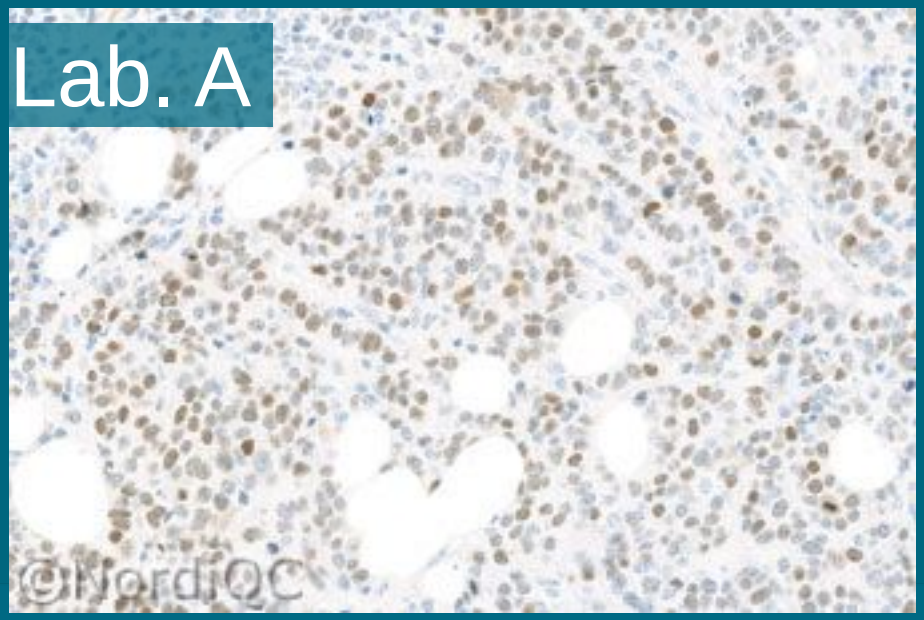


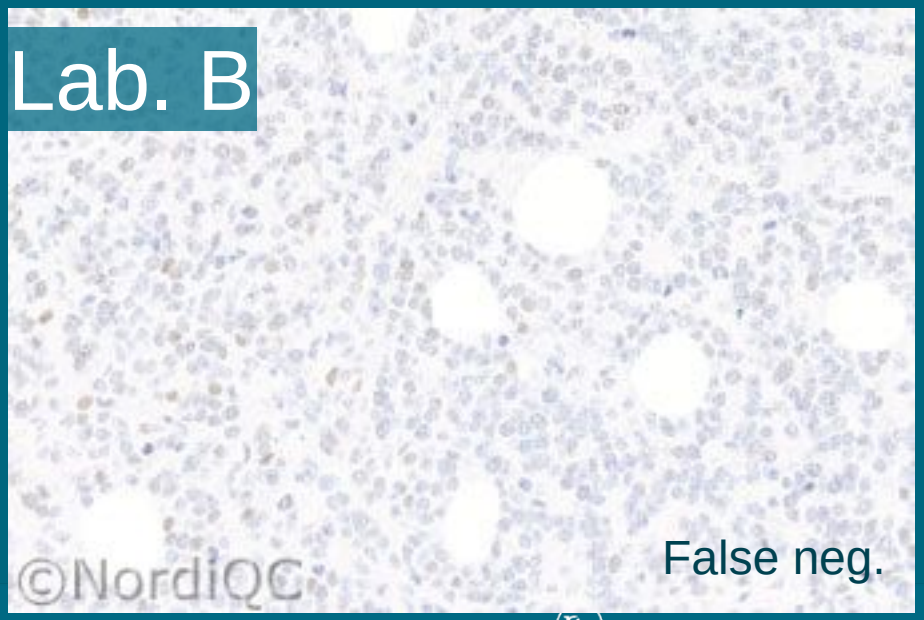


Serial sections stained for Estrogen receptor

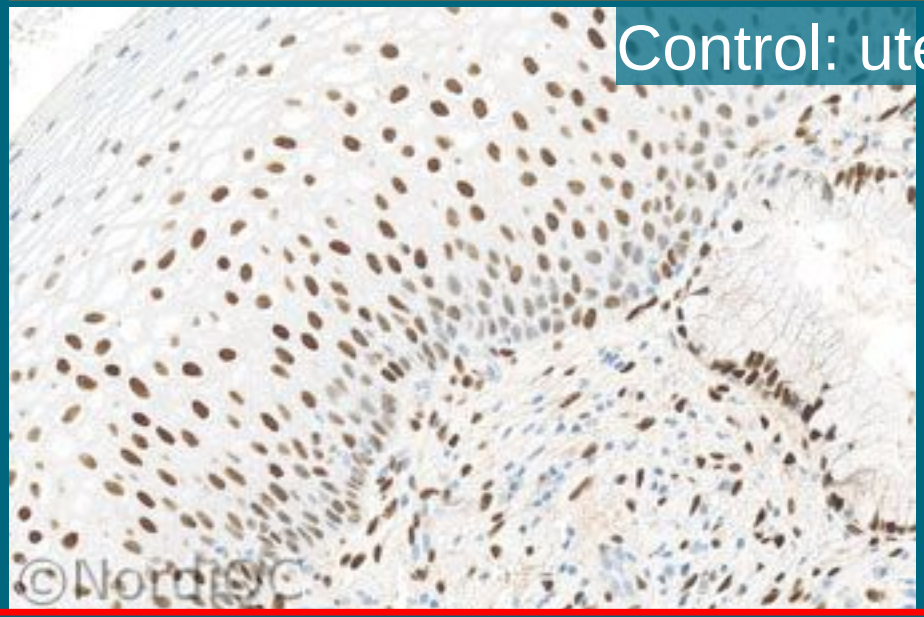


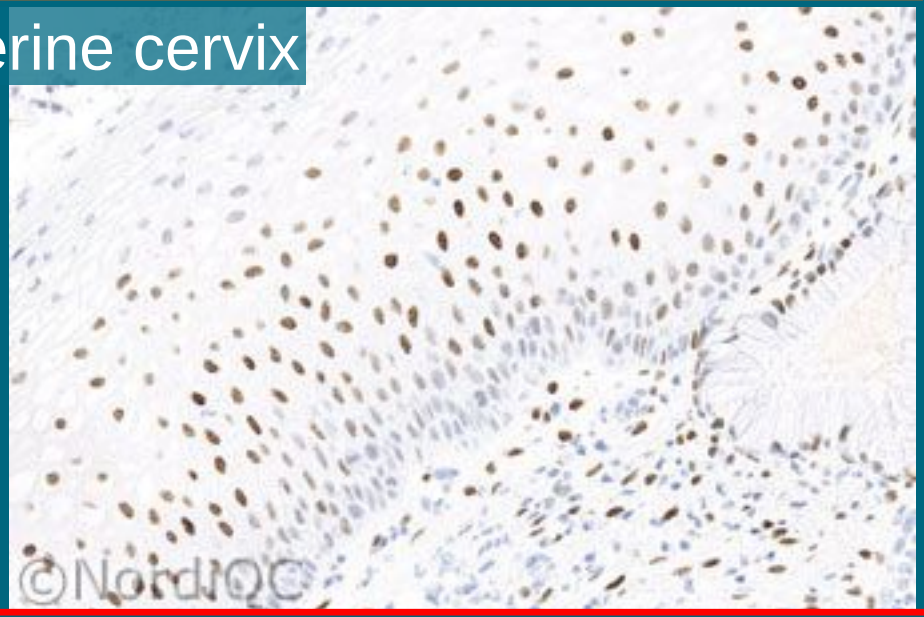


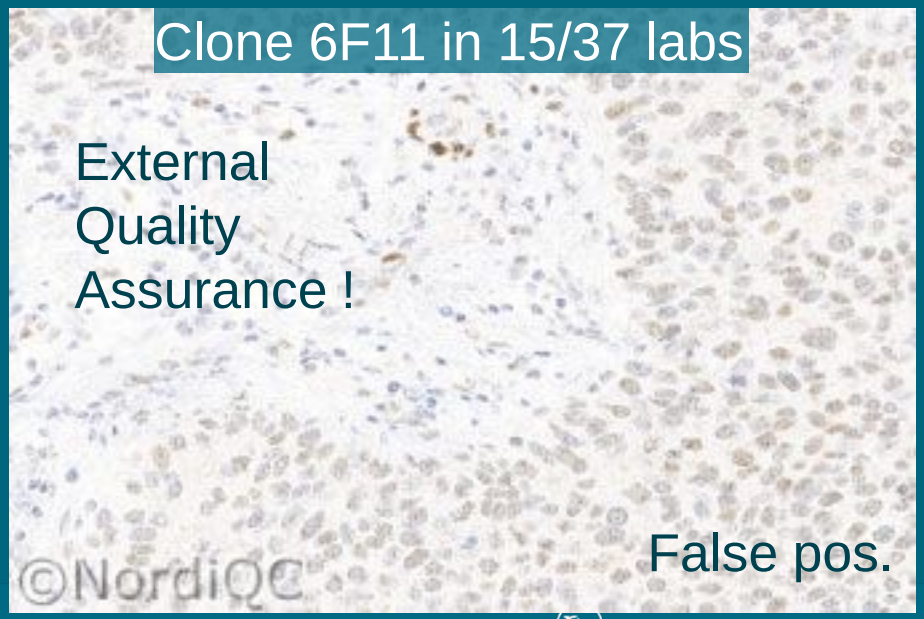




Serial sections stained for Estrogen receptor







IHC – Biomarker controls

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

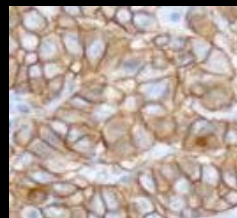
With 3 choices for 5 variables in each phase = > 4 million protocols….

Controlment

Quantification
Reporting

Post-analytic

Interpretation
Localization
Positive/Negative - cut-off level



The challenge of IHC

Suboptimal IHC assays may be due to:

- Preanalytical issues
  - Fixation too short, too late, decalcification too soon…
- Analytical issues:
  - Less successful or too dilute antibody clones/RTUs
  - Insufficient epitope retrieval
  - Insensitive visualization systems
  - Platform problems
- Post-analytical issues
  - Interpretation criteria, interobserver variation …

Should be identified with proper external on-slide controls


AaS 16-400001
21.01.21 R
VIM
010116-5JM9
AaS 16-400001
21.01.22 R
VIM
010116-5JM9
AaS 16-400001
21.01.23 R
VIM
010116-5JM9
AaS 16-400001
21.01.24 R
VIM
010116-5JM9
AaS 16-400001
21.01.25 R
VIM
010116-5JM9
AaS 16-400001
21.01.26 R
VIM
010116-5JM9
AaS 16-400001
21.01.27 R
VIM
010116-5JM9
AaS 16-400001
21.01.28 R
VIM
010116-5JM9
AaS 16-400001
21.01.29 R
VIM
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AaS 16-400001
21.01.30 R
VIM
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VIM
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AaS 16-400001
21.01.19 R
VIM
010116-5JM9
AaS 16-400001
21.01.20 R
VIM
010116-5JM9


”Cold spots” – a problem with ~30% of all slides
AaS 16-400001
21.01.13 R
VIM
010116-5JM9
AaS 16-400001
21.01.14 R
VIM
010116-5JM9
AaS 16-400001
21.01.15 R
VIM
010116-5JM9
AaS 16-400001
21.01.16 R
VIM
010116-5JM9
AaS 16-400001
21.01.17 R
VIM
010116-5JM9
AaS 16-400001
21.01.18 R
VIM
010116-5JM9

Nordic immunohistochemical Quality Control

- International organization for **proficiency testing** of IHC
- Founded 2003 by Nordic pathologists
- Independent, scientific, not-for-profit organisation
- Institute of Pathology, Aalborg University Hospital, DK

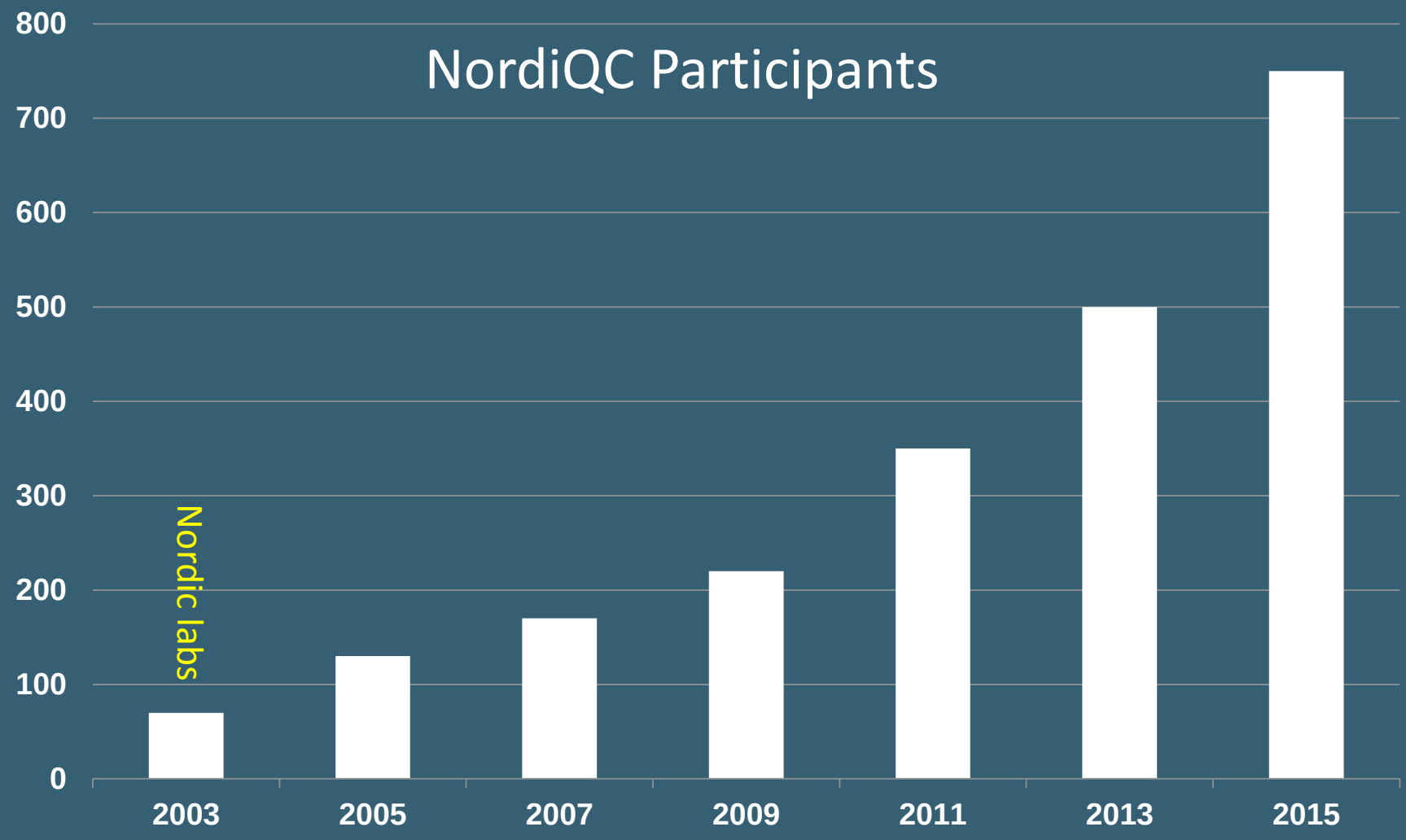
- General module: 3 runs/year
  - 15-18 different marker challenges
- Breast cancer IHC module: 2 runs/y
  - HER-2, ER/PR, Ki67/E-Cad ...
- HER-2 ISH module: 2 runs/year
  - BRISH, FISH
- Companion module: 2 runs/year
  - PD-L1 ...

www.nordiqc.org

~ 90 IHC markers in NordiQC Runs Tested 1-15 times

Alpha-methylacyl-CoA racemase	CyclinD1	MLH1
Alpha-smooth muscle actin	Cytokeratin 5	MSH2
Anaplastic lymphoma kinase	Cytokeratin 7	MSH6
B-cell specific activator protein	Cytokeratin 19	Multiple myeloma oncogene 1
bcl-2protein	Cytokeratin 20	Myosin, smooth muscle heavy chain
bcl-6protein	Cytokeratin, high molecular weight	Napsin A
Calretinin	Cytokeratin, low molecular weight	Neurofilament protein
Cancer antigen 125	Cytokeratin, pan-	Octamer transcription factor-3/4
Carcinoembryonic antigen	Desmin	$p16^{ink4a}$
CD3	Detected on GIST-1	p40
CD4	E-cadherin	p53
CD5	Epithelial cell adhesion molecule	p57
CD8	Epithelial membrane antigen	p63
CD10	Estrogen receptor alpha	Paired box gene-2 protein
CD14	Factor VIII related antigen	Paired box gene-8 protein
CD15	GATA3	Placental alkaline phosphatase
CD19	Glial fibrillary acidic protein	PMS2
CD20	Glypican 3	Podoplanin
CD23	Gross cystic disease fluid protein-15	Prostate specific acid phosphatase
CD30	HER-2	Prostate specific antigen
CD31	Hepatocyte antigen	Prostein
CD34	Human chorionic gonadotropin	Progesterone receptor
CD45	Immunoglobulin kappa	S-100 protein beta
CD56	Immunoglobulin lambda	Sal-like protein 4
CD68	Immunoglobubin M	SOX10
CD79a	Ki-67	Synaptophysin
CD99	Mammaglobin	Terminal deoxynucl. transferase
CD117	Melan-A	Vimentin
Chromogranin	Melanosoma specific antigen	Wilm's tumour-1 protein

Nordic immunohistochemical Quality Control



Nordic immunohistochemical Quality Control

Virchows Arch (2016) 468:19–29
DOI 10.1007/s00428-015-1829-1

ANNUAL REVIEW ISSUE

Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordiQC)

Mogens Vyberg[1,2] · Søren Nielsen[1]

Free PMC Article

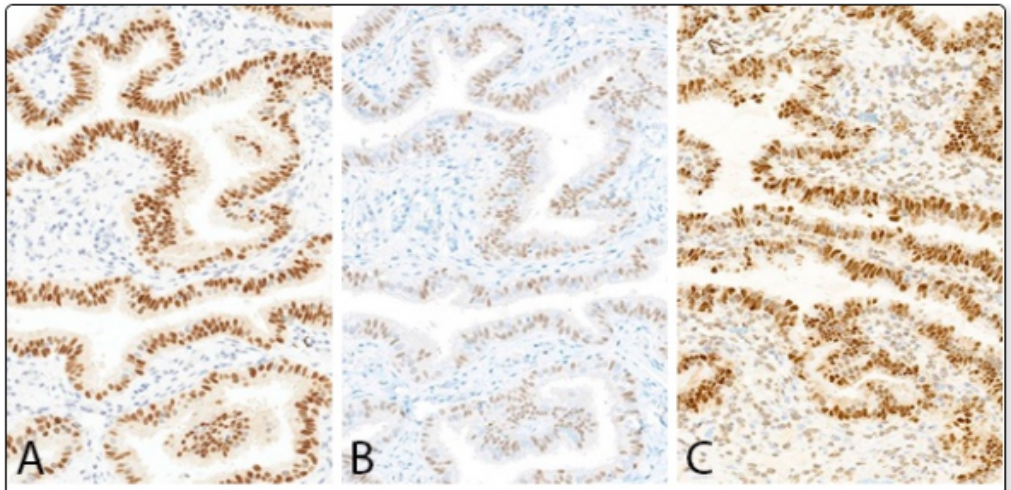
Vyberg et al. BMC Health Services Research (2015) 15:352
DOI 10.1186/s12913-015-1018-6

BMC Health Services Research

RESEARCH ARTICLE Open Access

Immunohistochemical expression of HER2 in breast cancer: socioeconomic impact of inaccurate tests

Mogens Vyberg[1*], Søren Nielsen[1], Rasmus Røge[1], Beth Sheppard[2], Jim Ranger-Moore[2], Eric Walk[2], Juliane Gartemann[3], Ulrich-Peter Rohr[3] and Volker Teichgräber[3]



Serial sections of Fallopian tube stained for PAX8 in three laboratories. Lab A gets an optimal result, lab B a too weak staining with false negative reaction in ciliated cells, and lab C a too strong staining with false positive reaction in stromal cells. See the details in the PAX8 assessment, run 51.

Results - module 51, B24, H12, C2

15-Dec-2017

Individual results for the runs 51, B24 and H12 are now available (after logging in). Results from C2 will be available on the 14th January 2018. Click to see an overview of the results.

Events

International Symposium on Immunohistochemistry
4–7 Jan 2018: Tata Medical Center, Kolkata, India

QuIP/NordiQC Workshop in Applied Immunohistochemistry
13–15 Jun 2018: Brugge, Belgium

NordiQC Workshop in Diagnostic Immunohistochemistry
18–20 Sep 2018: Aalborg, DK

Important dates

Run 52, H13, C3, B25
Protocol submission deadline
3 Jan 2018
Slide circulation
9 Jan 2018
Slide return deadline
13 Feb 2018
Publication of results
20 Apr 2018

Questions

Check out our FAQ (Frequently asked questions) or contact us

Google Custom S Search

WWW.NORDIQC.ORG

NordiQC assessment scheme 2018

Module	Winter	Spring	Autum
General	Run 52 CR MSH6 SYP TdT VIM	Run 53 BSAP CGA ECAD OCT3/4 PMS2	Run 54 CD8 CEA CK-PAN GATA3 Podop
Breast	Run B25 ER HER2 IHC		Run B26 ER HER2 IHC PR
HER2	Run H13 HER2 ISH		Run H14 HER2 ISH
Companion	Run C3 PD-L1		Run C4 PD-L1

Dates	Winter	Spring	Autum
Protocol submission opens	1 Dec 2017	12 Feb	1 Aug
Protocol submission closes	3 Jan	13 Mar	4 Sep
Shipping of slides	9 Jan	21 Mar	12 Sep
Deadline for slide return	13 Feb	1 May	11 Oct
Assessment General	6 Mar - 8 Mar	23 May - 25 May	24 Oct - 26 Oct
Assessment Breast	15 Mar - 16 Mar		8 Nov - 9 Nov
Assessment HER2	23 Mar		16 Nov
Assessment Companion	5 Apr		20 Nov
Publication of results	20 Apr	9 Jul	7 Dec

CD31

Characteristics

CD31 is a transmembrane glycoprotein, 130-140 kDa, also designated platelet-endothelium cell adhesion molecule = PECAM-1, belonging to the immunoglobulin super family. CD31 is ligand for CD38 and plays a role in thrombosis and angiogenesis. CD31 is strongly expressed in endothelial cells and weakly expressed in megakaryocytes, platelets, occasional plasma cells, lymphocytes (espc. marginal zone B-cells, peripheral T-cells) and neutrophils.

Protocols

Reommended protocols

Assessments

Run 46
Run 38
Run 32
Run 26
Run 11

Neoplasms

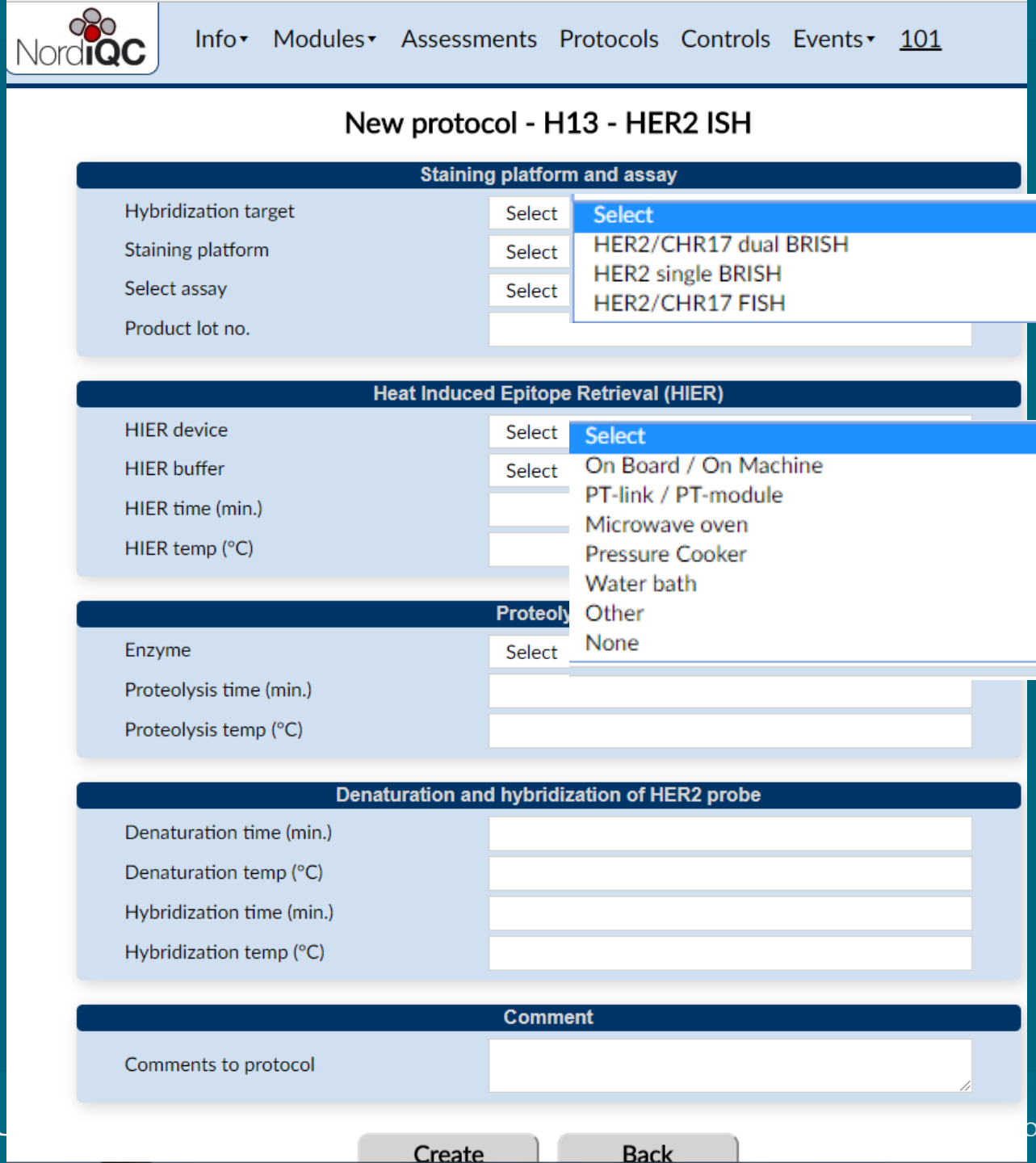
CD31 is expressed in the vast majority of all types of vascular neoplasms, such as hemangioendothelioma, angiofibroma, hemangioma, and angiosarcoma. CD 31 is also expressed in most cases of Kaposi sarcoma and epithelioid hemangioendothelioma.
CD 31 may be expressed in a haematolymphoid neoplasms like chronic lymphatic leukaemia, plasmacytoma, histicytosis and juvenile xantogranuloma. When it comes to carcinomas, very few cases have been reported to express CD31. However, there are reports where singular cases of mucoepidermoid carcinoma, papillary thyroid carcinoma, sweat gland tumours, and metaplastic breast carcinoma with spindle cells have been reported to stain for CD31. There are also reports on CD 31-positivity in a minority of malignant fibrous histiocytoma of giant cell type and malignant mesothelioma. Finally CD31 has been detected in malignant gliomas, not only in the vesssels but also the neoplastic cells.

Application

CD31 is most used in the panel for recognizing endothelial cell differentiation in tumours and should be considered a reliable marker for all types of vascular neoplasms.

Controls

Liver and tonsil are recommendable positive tissue controls for CD31. In liver an at least weak to moderate, distinct staining reaction in virtually all hepatic sinusoidal endothelial cells must be seen.
Endothelial cells of the portal tract vessels must show a moderate to strong staining reaction. In tonsil the majority of activated mantle zone B-cells must display an at least weak to moderate, distinct embranous staining reaction, whereas plasma cells and endothelial cells must show a strong staining reaction.
Appendix can be used as negative tissue control, as no staining reaction of the epithelial cells should be seen (only intraepithelial lymphocytes should be demonstrated).


NordiQC
Info
Modules
Assessments
Protocols
Controls
Events
101
New protocol - H13 - HER2 ISH
Staining platform and assay
Hybridization target
Staining platform
Select assay
Product lot no.
Select
Select
HER2/CHR17 dual BRISH
HER2 single BRISH
HER2/CHR17 FISH
Heat Induced Epitope Retrieval (HIER)
HIER device
HIER buffer
HIER time (min.)
HIER temp (°C)
Select
On Board / On Machine
PT-link / PT-module
Microwave oven
Pressure Cooker
Water bath
Other
None
Proteoly
Enzyme
Proteolysis time (min.)
Proteolysis temp (°C)
Denaturation and hybridization of HER2 probe
Denaturation time (min.)
Denaturation temp (°C)
Hybridization time (min.)
Hybridization temp (°C)
Comment
Comments to protocol
Create
Back

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

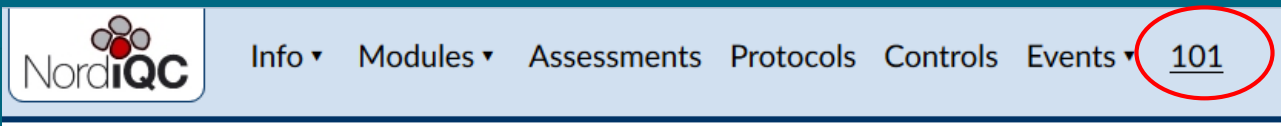
Epitope retrieval, HIER	(•) YES ( ) NO
Device	On Board / On Machine
HIER buffer	Ventana - Ultra CC1 (950-224)
Efficient Heating Time (minutes)	48
Max. heating temperature (Celcius)	99

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
Incubation time polymer (minutes)	8
Incubation temperature (Celcius)	36



Participant site

Protocol submission

Run	Module	Epitope	Protocol status	Slide received by NordiQC	Action
49	General Module	CD5	✔	2017-02-13	
49	General Module	CK-LMW	✔	2017-02-13	
49	General Module	MLA	✔	2017-02-13	
49	General Module	MLH1	✔	2017-02-13	
49	General Module	NKX3.1	✔	2017-02-13	
49	General Module	PSA	✔	2017-02-13	
B23	Breast Cancer Module	ER	✔	2017-02-13	
B23	Breast Cancer Module	HER2 IHC	✔	2017-02-13	
C1	Companion Diagnostic Module	PD-L1	✔		

Module status

49	General Module	**Slides sent**
B23	Breast Cancer Module	**Slides sent**
H11	HER2-ISH Module	**Slides sent**
C1	Companion Diagnostic Module	**Slides sent**

Open	Homepage open for protocol submission. New protocols can be created, edited and deleted.
Closed	Homepage closed for new protocol submission. Protocols already submitted can be edited. NordiQC are preparing to send slides.
Slides sent	Slides for the submitted protocols have been sent to participants. Only protocol corrections are allowed.

Test material

Multi-tissue FFPE blocks

10% NBF 24-48 h (ASCO/CAP guidelines …)

- Normal and clinically relevant tumour tissues
- Different levels of antigen expression
  - high, moderate, low, none

2 unstained slides for each marker send to the participants

1 stained slide returned for central assessment

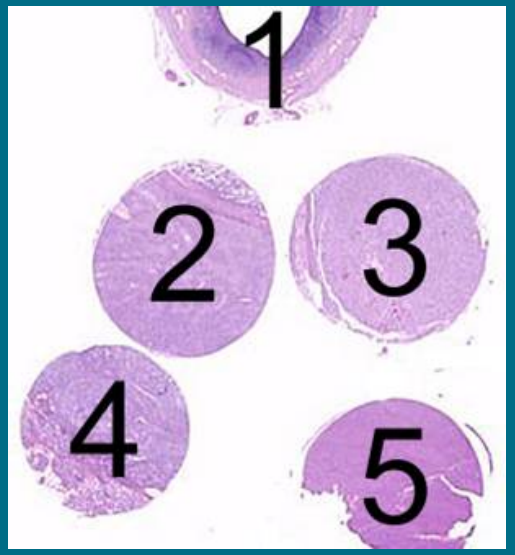
Test material

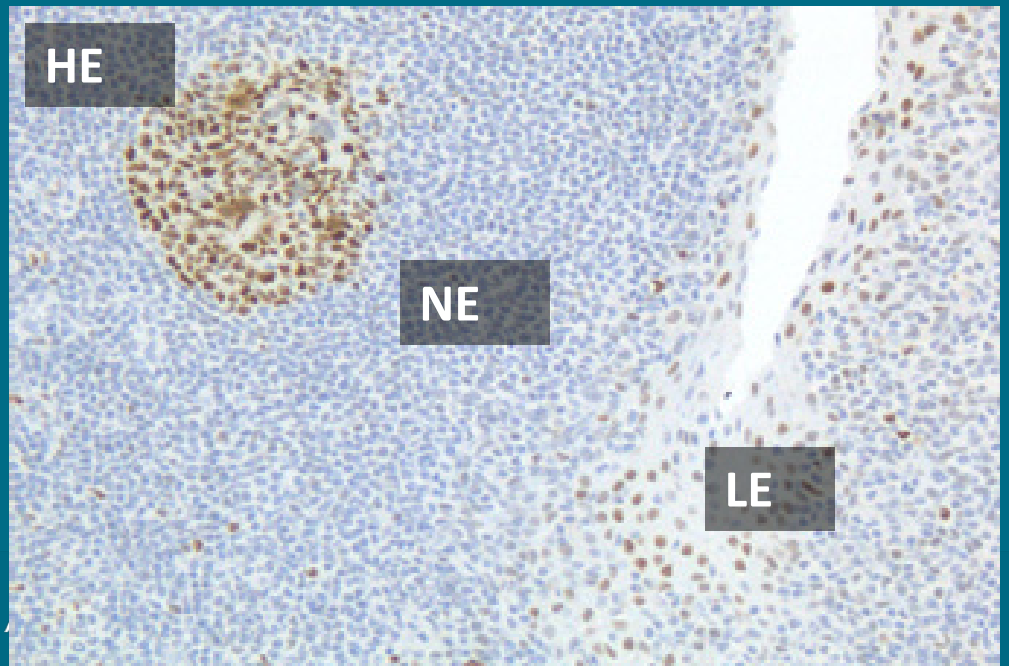
The slide to be stained for **Bcl-6** comprised:
1-2. Tonsils, 24 h/48 h

3. Follicular lymphoma, grade I

4. Follicular lymphoma, grade II

5. Diffuse large B-cell lymphoma





Tissue selection:

- **H**igh **E**xpressor
  - to confirm antibody
- **L**ow **E**xpressor
  - to ensure sensitivity
- **N**o-**E**xpressor
  - to ensure specificity

Open website

NordiQC

Assessment Run 42 2014

Bcl-6 protein (Bcl-6)

Recommended Bcl-6 protocols Recommended Bcl-6 control tissue

Material
The slide to be stained for Bcl-6 comprised:

1. Tonsil, 24h fixation, 2. Tonsil, 48h fixation*, 3. Follicular lymphoma grade I, 4. Follicular lymphoma grade II, 5. Diffuse large B-cell lymphoma, non-Germinal Centre B-cell type (DLBCL non-GCB), 6. DLBCL, GCB.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Bcl-6 staining as optimal included:

- A moderate to strong distinct nuclear staining reaction of virtually all normal germinal centre B-cells in the tonsil fixed 24h.
- An at least weak to moderate distinct nuclear staining reaction of the majority of the squamous epithelial cells in the tonsil fixed 24h.
- A moderate to strong distinct nuclear staining reaction of the neoplastic cells in the two follicular lymphomas.
- An at least weak to moderate nuclear staining reaction of the majority of the neoplastic cells in th DLBCL, GCB subtype, tissue core no. 6.
- No or only a nuclear staining reaction in dispersed neoplastic cells of the DLBCL, non-GCB subtyp tissue core no. 5

* The tonsil fixed for 48h (tissue core no 2) was excluded from the assessment due to an aberrant inconsistent staining reaction in the circulated material.

Participation

Number of laboratories registered for Bcl-6, run 42	244
Number of laboratories returning slides	228 (93%)

Results
228 laboratories participated in this assessment. Of these, 168 (74%) achieved a sufficient mark (optima or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- too low concentration of the primary antibody
- less successful performance of the mAb clone PG-B6p
- use of low sensitivity detection systems

Performance history
This was the third NordiQC assessment of Bcl-6. An increased pass rate was seen compared to the two previous runs 17, 2006 and 28, 2010 (see table 2).

Table 2. **Proportion of sufficient results for Bcl-6 in the three NordiQC runs performed**

	Run 17 2006	Run 28 2010	Run 42 2014
Participants, n=	69	132	228
Sufficient results	42%	48%	74%

Conclusion
The mAbs clones **GI191E/A8**, **LN22** and **PG-B6p** could all be used to produce optimal staining results for Bcl-6. Irrespective of the clone applied, efficient HIER in alkaline buffer, use of a high sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an

Table 1. **Antibodies and assessment marks for Bcl-6, run 42**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **GI191E/A8**	13 1 1	Cell Marque Immunologic Zytomed	6	8	0	1	93%	100%
mAb clone **LN22**	38 2 1 1 1	Leica/Novocastra DBS Biocare BioGenex Zeta Corporation	20	16	4	3	84%	100%
mAb clone **PG-B6p**	43 1 1	Dako DBS Thermo/Neomarkers	9	22	11	3	69%	86%
Ready-To-Use antibodies								
mAb clone **GI191E/A8 760-4241**	59	Ventana/Cell Marque	24	25	9	1	83%	84%
mAb clone **GI191E/A8 227M-9x**	1	Cell Marque	0	0	1	0	-	-
mAb clone **LN22 PA0204**	10	Leica/Novocastra	3	7	0	0	100%	100%
mAb clone **LN22 PM410**	1	Biocare	1	0	0	0	-	-
mAb clone **LN22 MAD-00638QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **PG-B6p IR/IS625**	44	Dako	4	17	21	2	48%	75%
mAb clone **PG-B6p GA625**	7	Dako	2	2	3	0	57%	75%
mAb **PG-B6p MAD-004023QD**	2	Master Diagnostica	0	1	1	0	-	-
Total	228		69	99	50	10	-	
Proportion			30%	44%	22%	4%	74%	

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

Detailed analysis of Bcl-6, Run 42
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **GI191E/A8:** Protocols with optimal results were all based on HIER using Cell Conditi (CC1; Ventana) (6/14)* as retrieval buffer. The mAb was typically diluted in the range of 1:50-1: depending on the total sensitivity of the protocol employed. Using these protocol settings 9 of 9 (laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **LN22:** Protocols with optimal results were all based on HIER using Target Retrieval So (TRS) pH 9 (3-in-1) (Dako) (2/2), CC1 (Ventana) (9/18) or Epitope Retrieval Solution 2 (BERS2; (9/11) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on sensitivity of the protocol employed. Using these protocol settings 27 of 27 (100%) laboratories p a sufficient staining result.

PDF file e-mailed to participants with assessment marks and – when needed – explanations and recommendations

Nordic Immunohistochemical Quality Control

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

Assessment of ER, B24 - individual results

Aalborg Sygehus (101)

Epitope	ER
Assessment	Optimal

NordiQC has assessed the submitted slides. In general, the assessment is based on staining intensity and distribution in cells expected to be demonstrated, background staining, cross-reactivity, quality of counter-staining and preservation of tissue morphology. Specific criteria for each epitope are described on http://www.nordiqc.org/epitope.php.
Each slide was marked as optimal, good, borderline or poor based on the following criteria:

Optimal: The staining reaction is considered perfect or close to perfect in all of the included tissues.
Good: The staining reaction is considered acceptable in all of the included tissues. However, the protocol settings may be optimized to ensure improved sensitivity or higher signal-to-noise ratio.
Borderline: The staining reaction is considered insufficient because of a generally too weak staining reaction, false negative or false positive staining reaction of one of the included tissues. The protocol should be optimized.
Poor: The staining reaction is considered insufficient because of, e.g., false negative or false positive staining reactions of several of the included tissues. An optimization of the protocol is urgently needed.

Moderate or strong cross reaction (due to the character of the primary antibody) or other false positive staining reaction (e.g. due to endogenous biotin) is not compatible with an optimal result and will usually cause downgrading.
For stains assessed as borderline or poor, comments and recommendations are given to the protocols. Good stains may also be accompanied by comments if specific problems are identified.
Recommended protocols from each staining platform are available at the NordiQC homepage (http://www.nordiqc.org/recommended.php) for comparison. Implementation of NordiQC recommended protocols as well as changes suggested in this letter must be tested carefully in your own laboratory before implementation into diagnostic work. NordiQC do not take any responsibility for consequences of changes in protocols or methods in your laboratory.

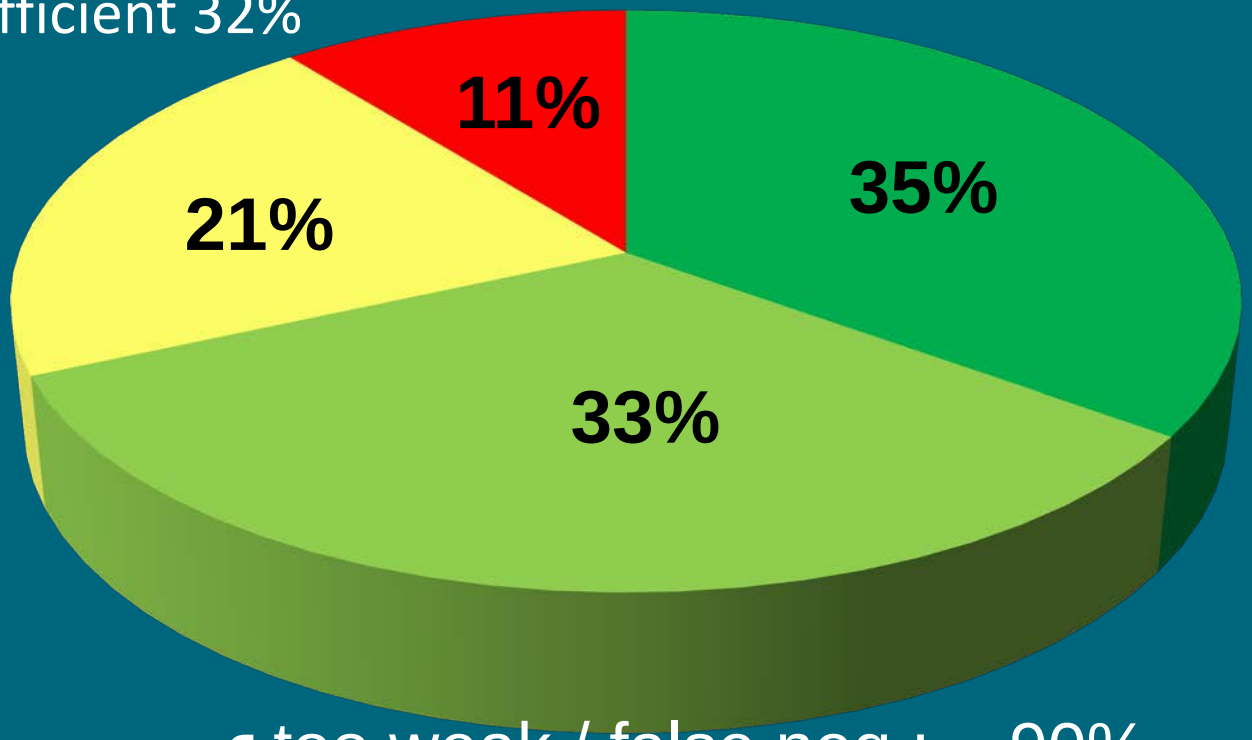
Marker	CD23	CR	CyD1	Ki67	Podop	TTF1
Assessment:	Poor	Optimal	Optimal	Good	Good	Borderline
Comments to the protocol:	False negative	-	-	Excessive counterstain	Weak	Weak*
Suggestions for improvement:	Consider change of primary Ab and recalibrate	-	-	-	-	Increase primary Ab conc. and/or prolong HIER

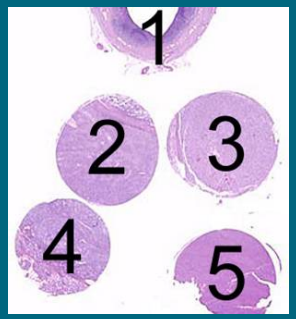
* Please read the epitope description and assessment summary carefully, as the choice of the Ab clone will influence the sensitivity and specificity.

NordiQC assessment results 2006 – 2015

General module ~ 20,000 slides (~100.000 core sections)

Insufficient 32%



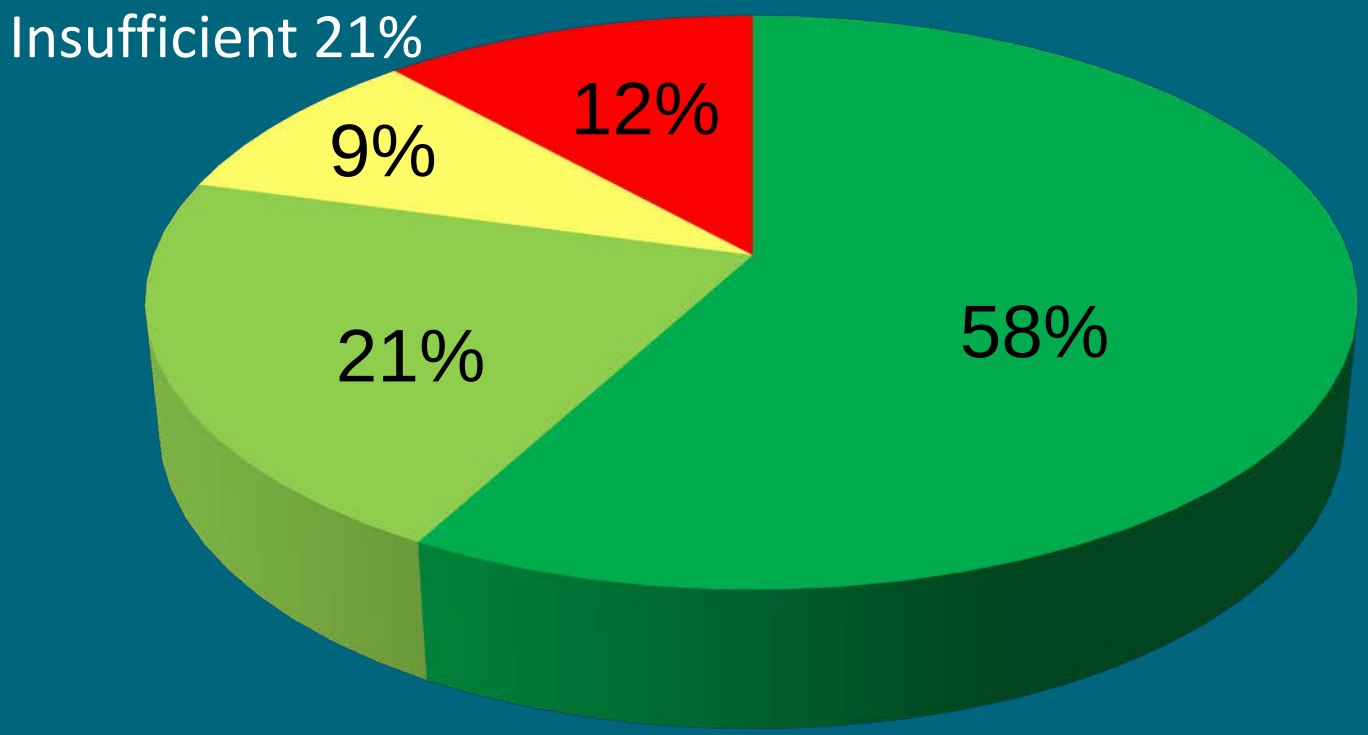


- Optimal
- Good
- Borderline
- Poor

Insuff. { too weak / false neg.: ~ 90%
over-stained / false pos.: ~ 10%

NordiQC assessment results 2006 – 2015

Breast cancer module ~ 9,000 slides (~35,000 core sections)



Insuff. { too weak / false neg.: ~ 90%
over-stained / false pos.: ~ 10%

Major causes of **insufficient** stains in ~ 9,000 slides

Less successful antibodies/RTUs	17 %
Inappropriate antibody dilution	20 %
Inappropriate epitope retrieval	27 %
Inappropriate detection kit	19 %
Other inappropriate lab. performance	17 %

- Endogenous biotin reaction
- Section drying-out after HIER
- Technical platform error
- . . . .
- Unexplained

IHC – Biomarker controls

Go for Low antigen expressors ~

Critical Stain Quality Indicators (CSQI)

- essential to evaluate sensitivity
- essential to assure consistency

Normal tissues when ever possible - easier to recognize and ensure the quality

- 90 % of insufficient staining results in EQA are caused by weak/false negative results
- often related to the use of inappropriate positive tissue controls.....

Publications

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§§‖‖*

AIMM 2014, 22:241

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‡§*

AIMM 2015, 23:1

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)

NordiQC

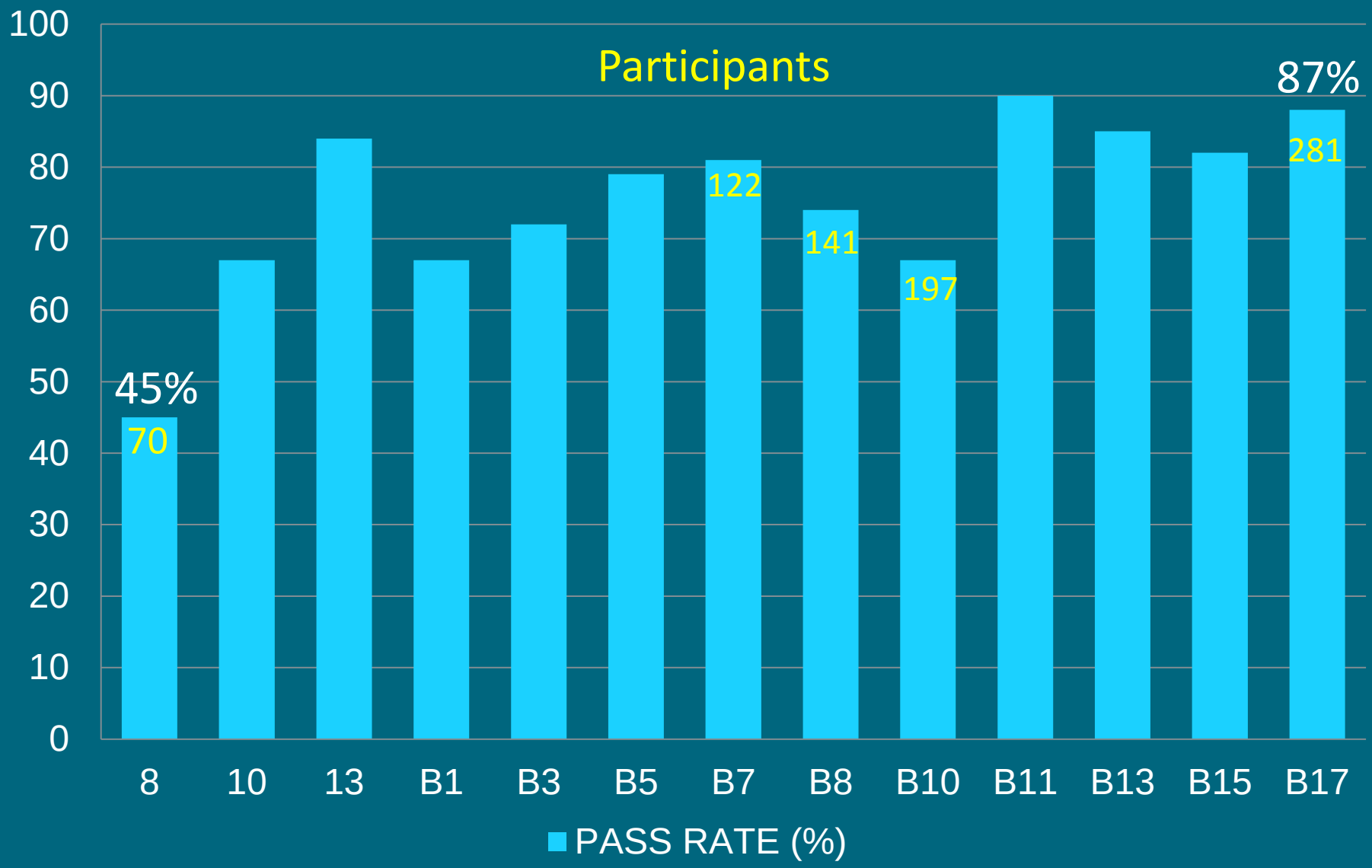
AIMM 2016-17

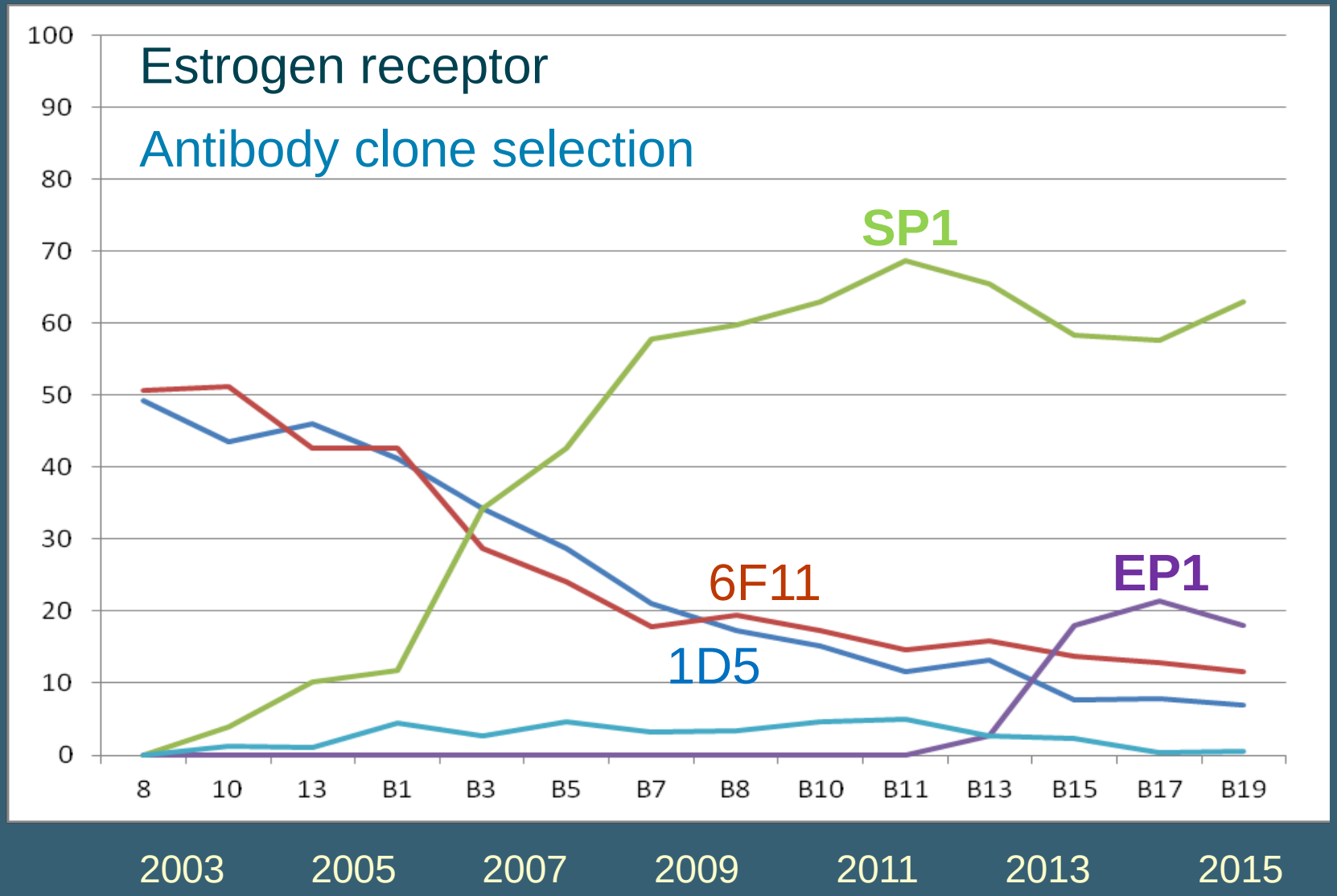
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine – Part 2: Immunohistochemistry Test Performance Characteristics

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

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

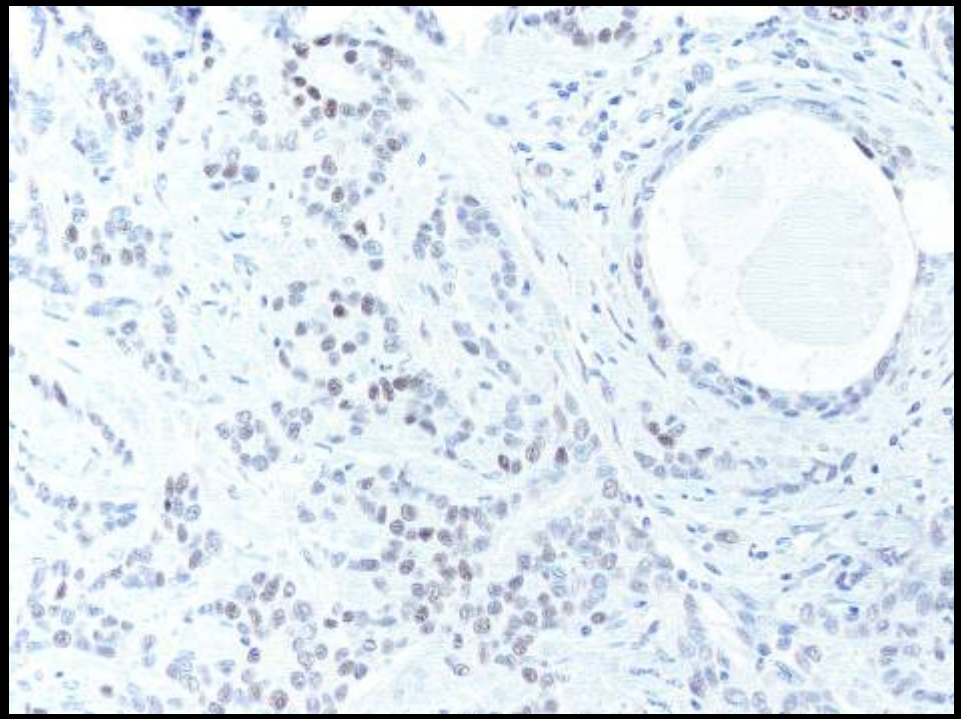
NordiQC EQA: Estrogen Receptor in 13 runs



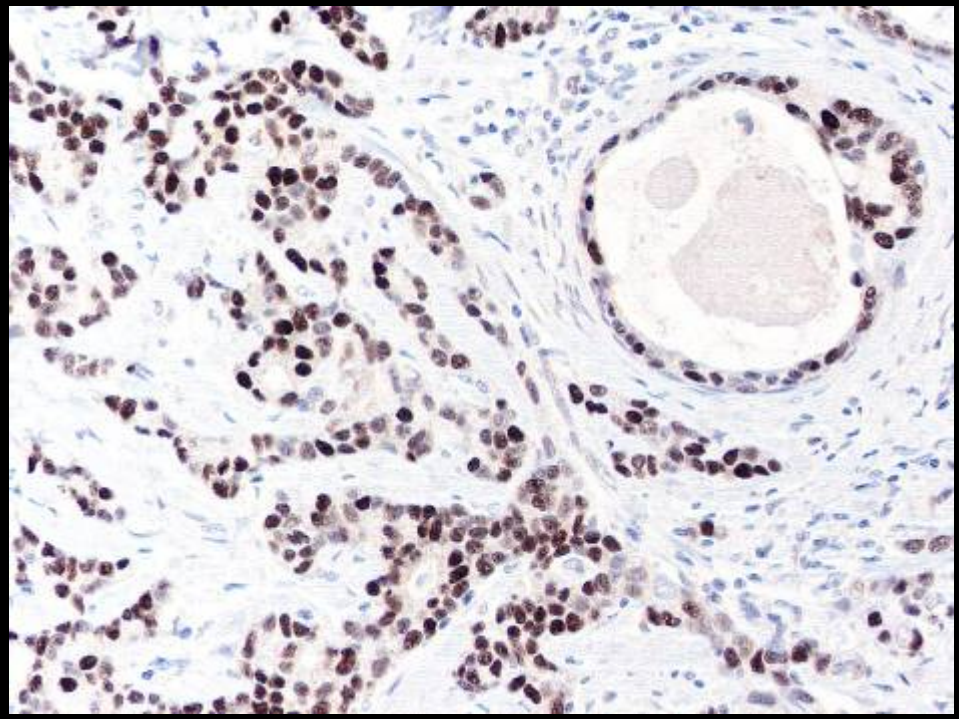

Estrogen receptor
Antibody clone selection
SP1
6F11
EP1
1D5
100
90
80
70
60
50
40
30
20
10
0
8
10
13
B1
B3
B5
B7
B8
B10
B11
B13
B15
B17
B19
2003
2005
2007
2009
2011
2013
2015

IHC – Optimal performance

ER 1D5 1:100
HIER Ci pH 6



2-step polymer



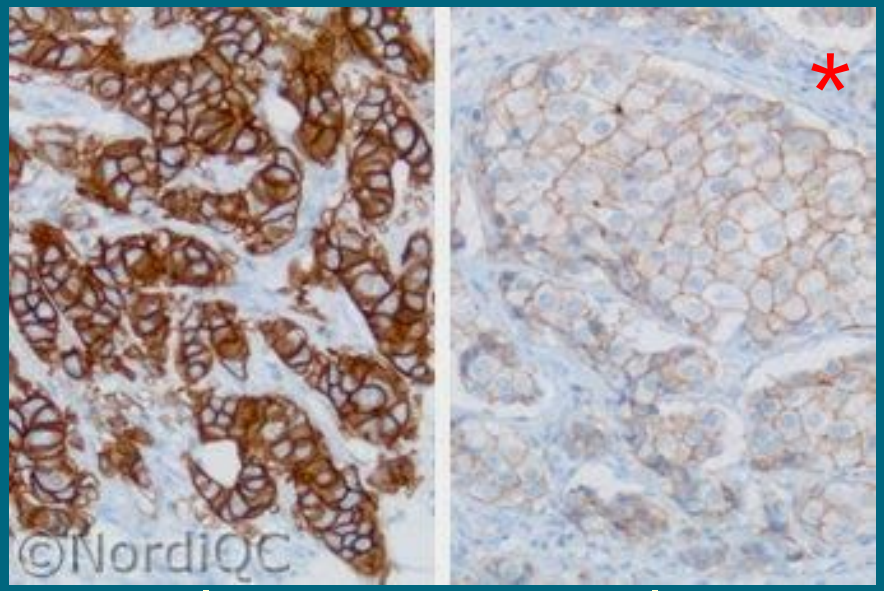
3-step polymer

Results of NordiQC recommendations

Pass rate (optimal + good) by participant status

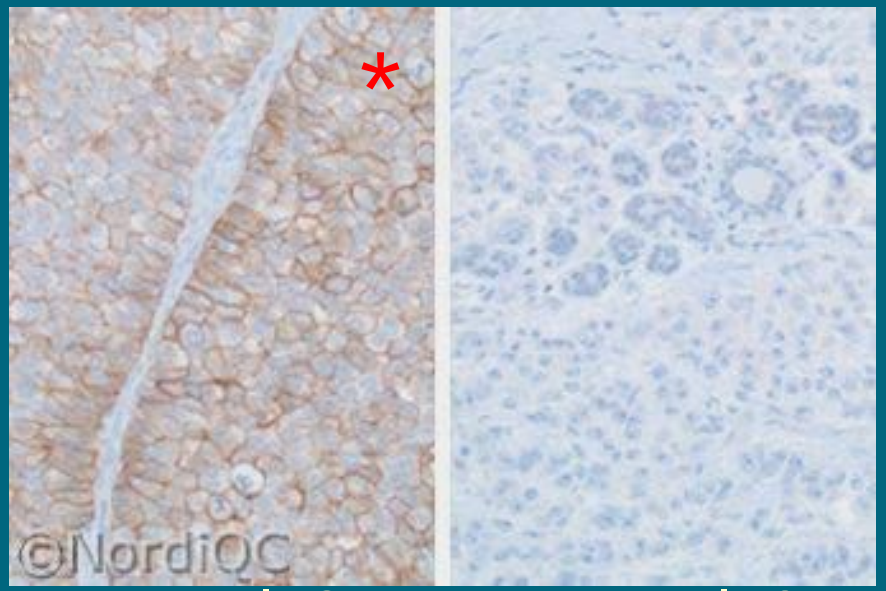
Estrogen receptor	New participants	'Old' participants
Run 10, 2004	57%	71%
Run B15, 2010	70%	86%
Run B19, 2015	51%	73%
Average	**59%**	**77%**

NordiQC runs for HER2 IHC

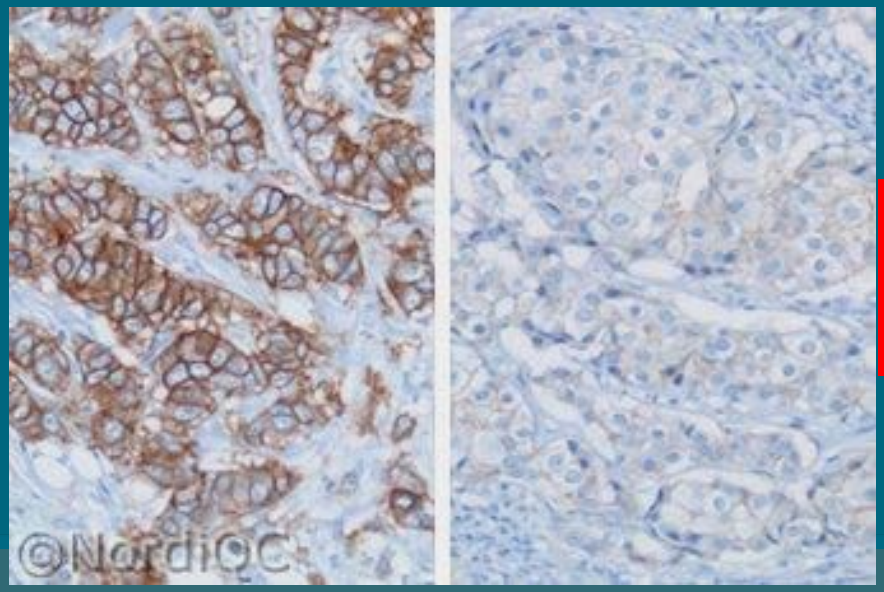


Ampl. 3+ Ampl. 2+

Optimal

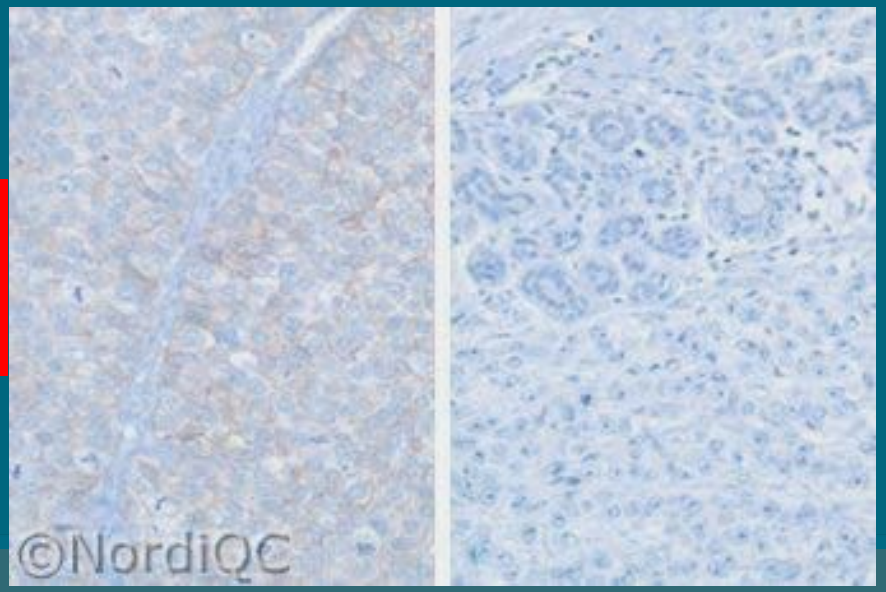


Unampl. 2+ Unampl. 0



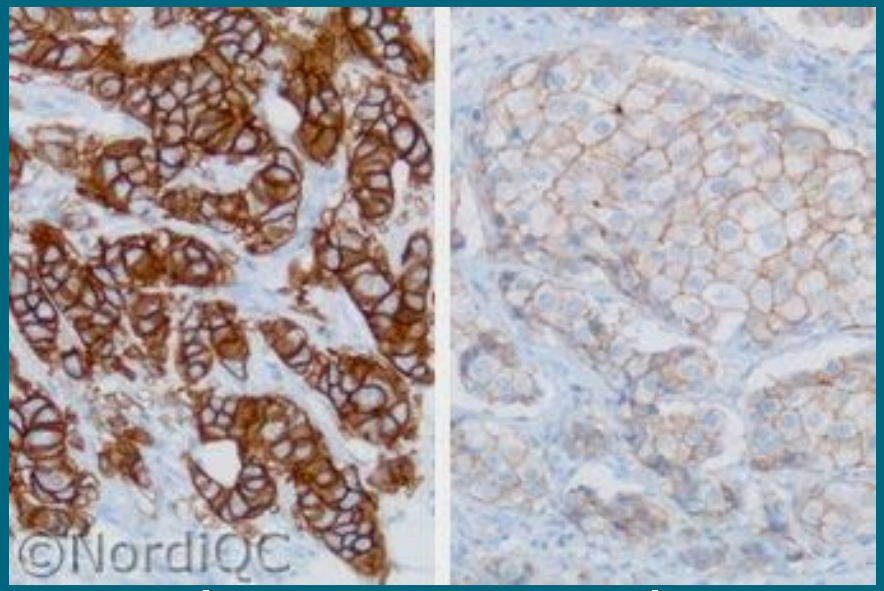
Ampl. 3+ Ampl. 1+

Poor



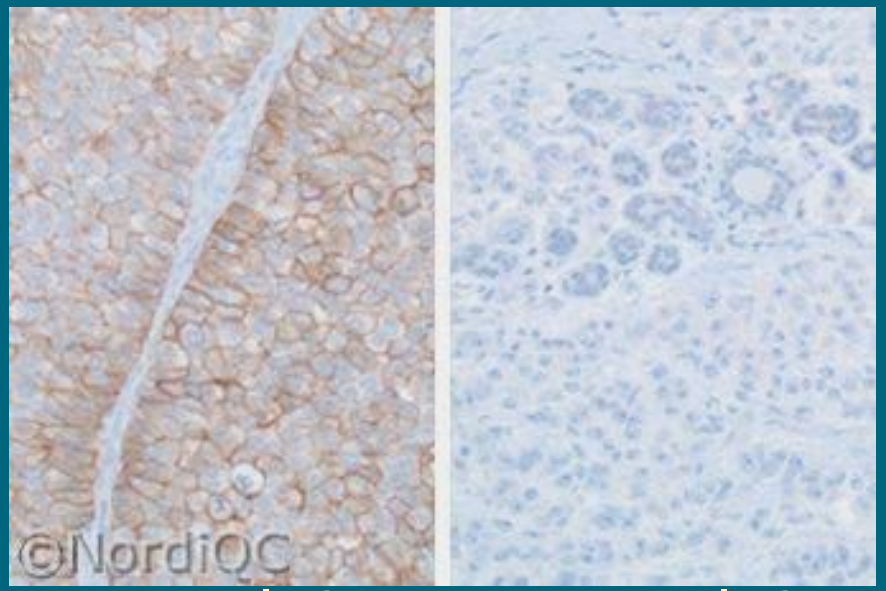
Unampl. 1+ Unampl. 0

NordiQC runs for HER2 IHC

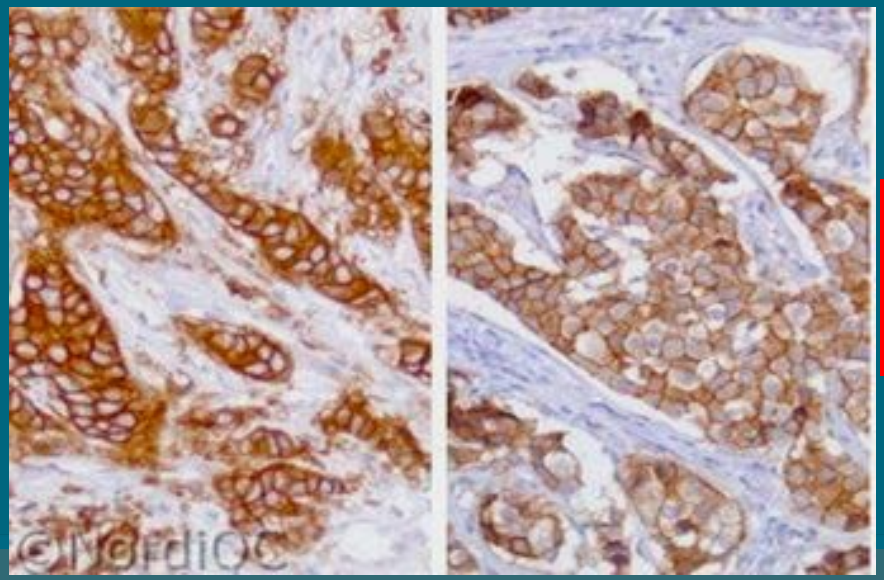


Ampl. 3+ Ampl. 2+

Optimal

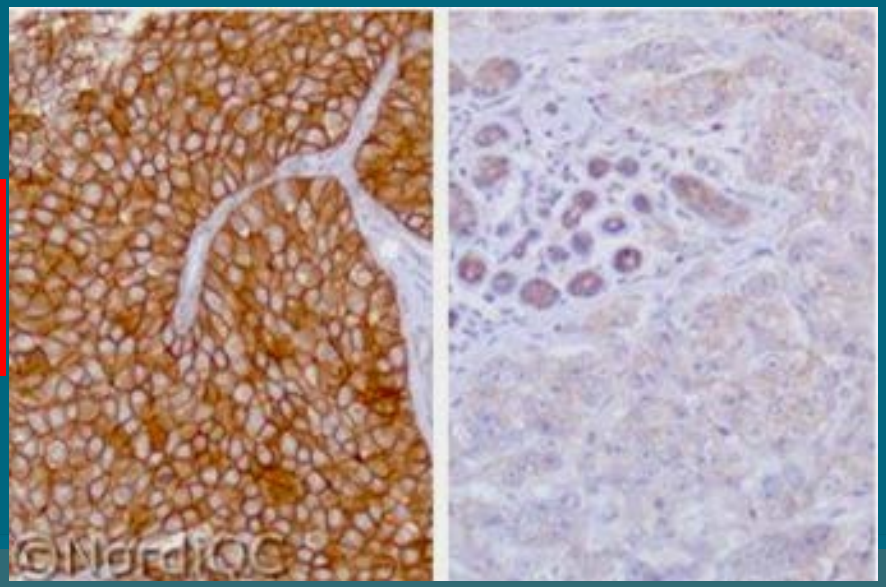


Unampl. 2+ Unampl. 0



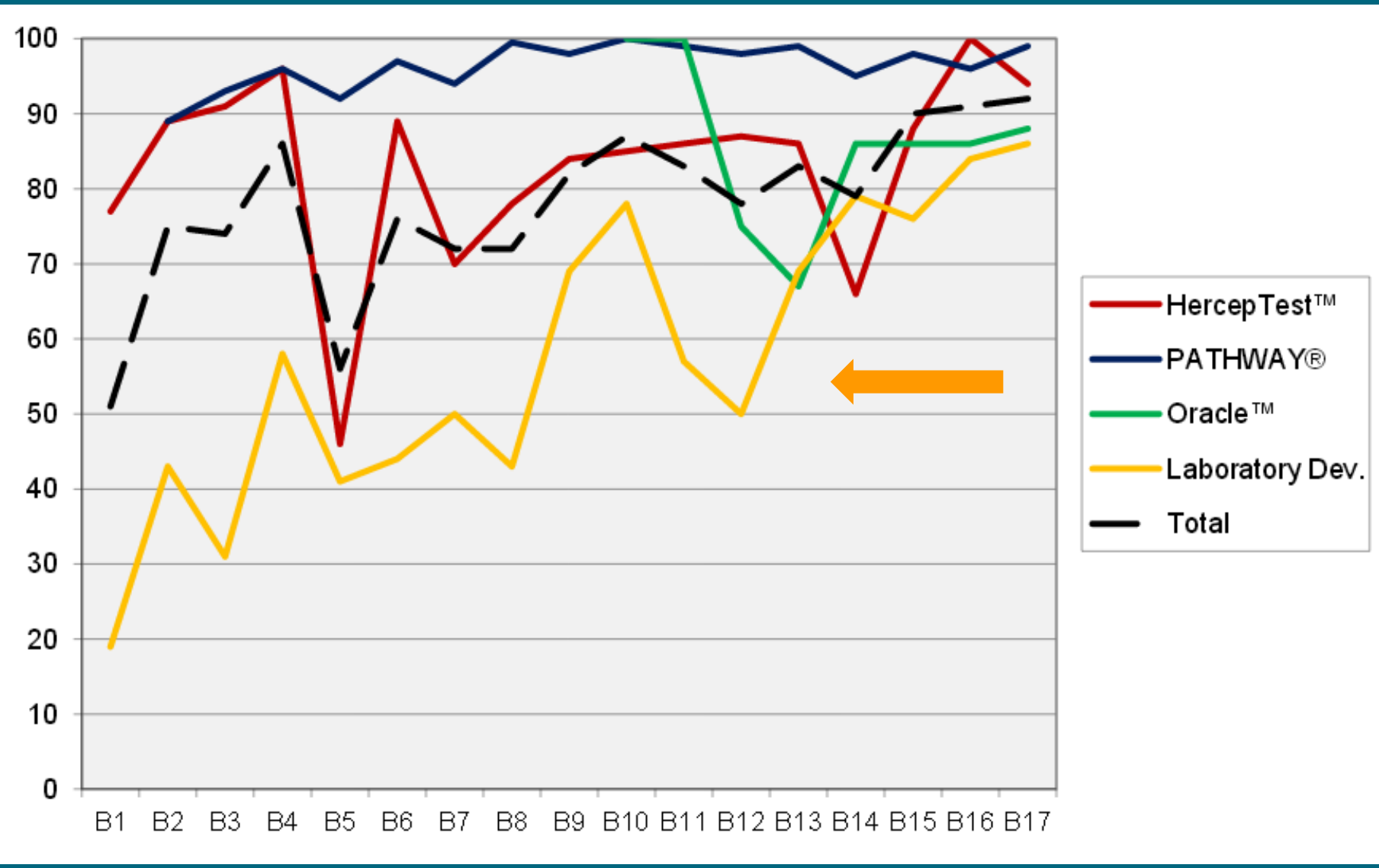
Ampl. 3+ Ampl. 2+

Poor



Unampl. 3+ Unampl. 1

HER-2 staining results in 17 runs



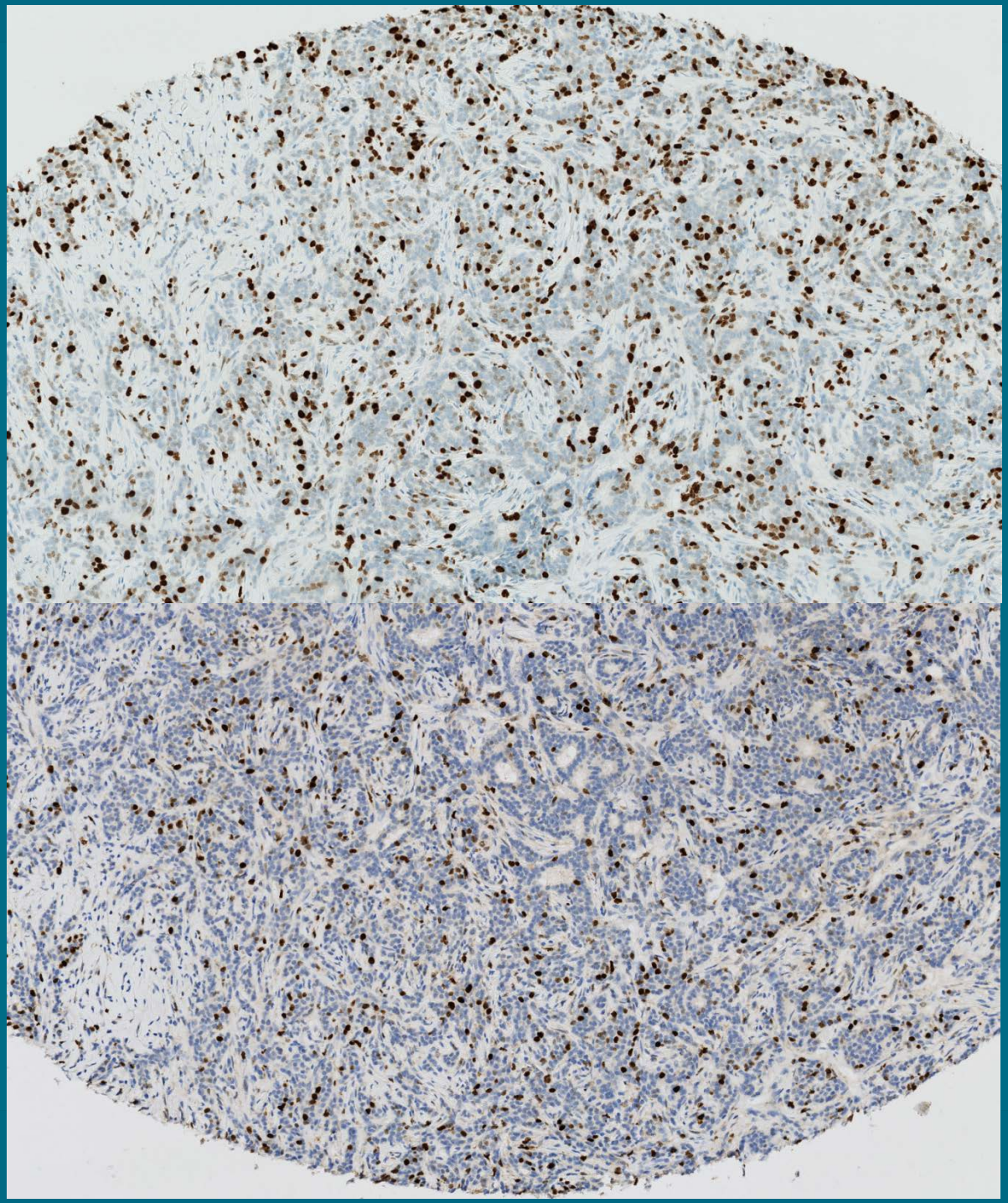
Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

J Natl Cancer Inst 2009;101: 736 – 750

Ki67 antibody clone SP6 applied at a 1:200 dilution for 32 minutes, by following the Ventana Benchmark automated immunostainer standard Cell Conditioner 1 (CC1) protocol at 98°C for 30 minutes.

The best Ki67 index cut point to distinguish luminal B from luminal A tumors was **13.25%**.



Ki67 immunoassay
Digital image analysis

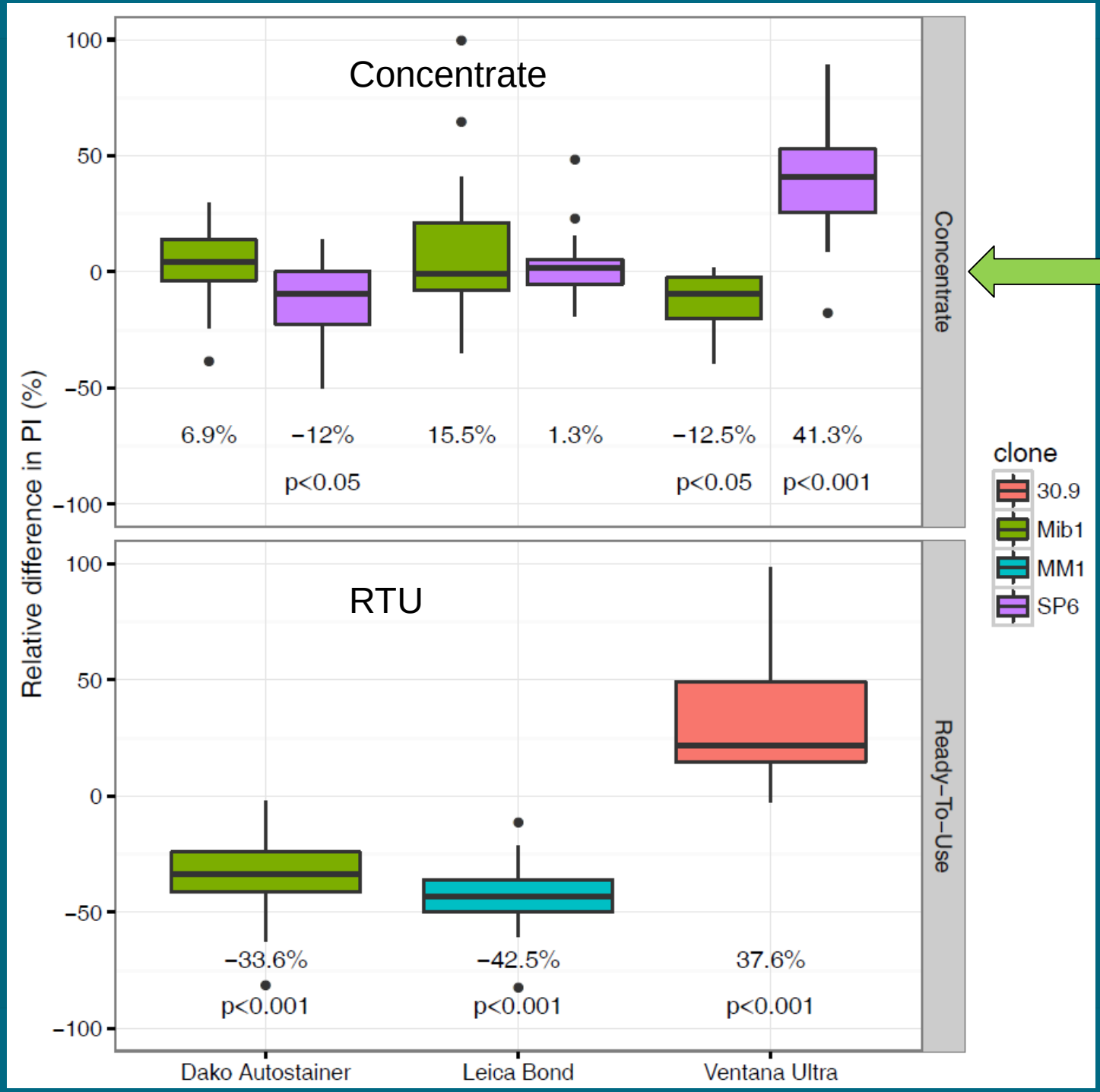
Clone SP6
concentrate
Ventana
platform

Prolif.index
38 %

Clone MM1
RTU
Leica platform

Prolif.index
12 %

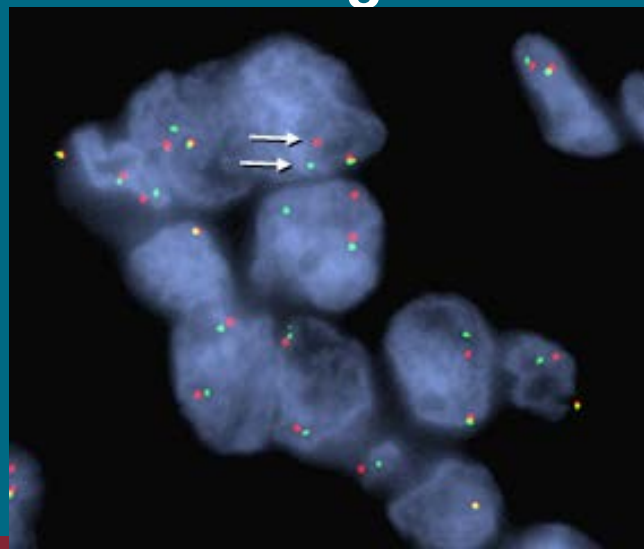
NORTH DENMARK REGION



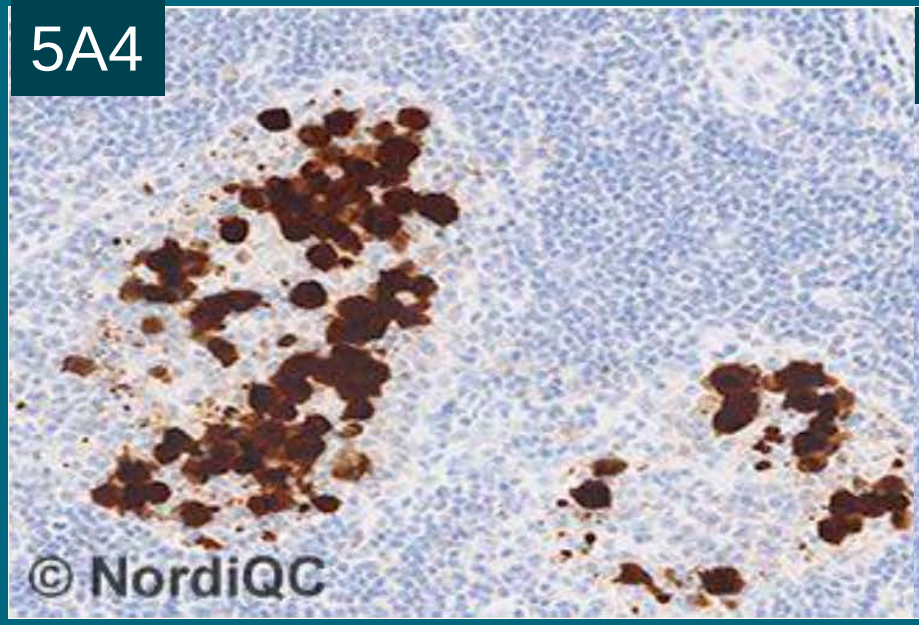
Mib1 as concentrate in optimized protocols on 3 platforms

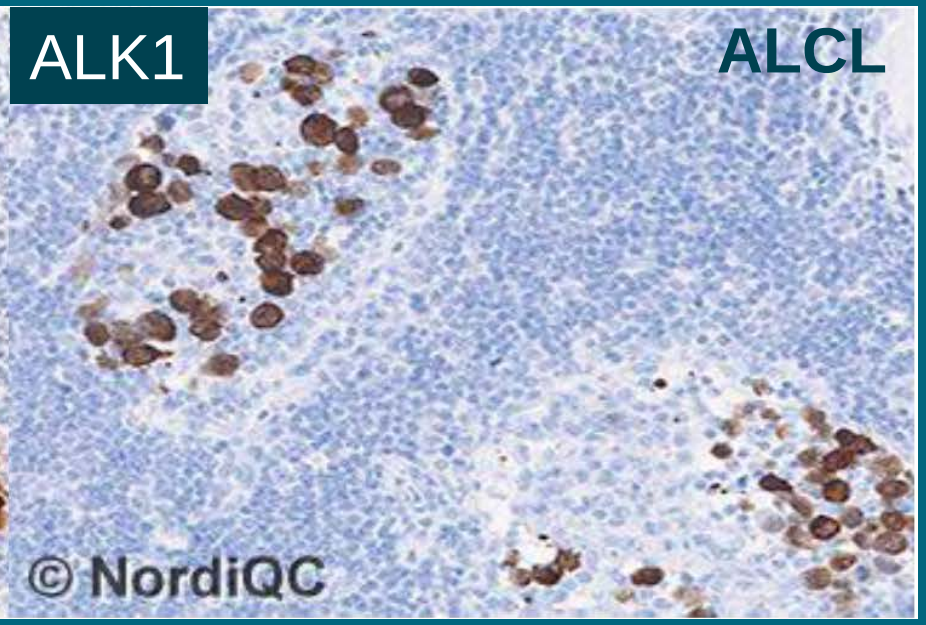
Mutations can be identified by mutation-specific proteins: **ALK** (anaplastic large cell lymphoma, lung adenocarcinoma).

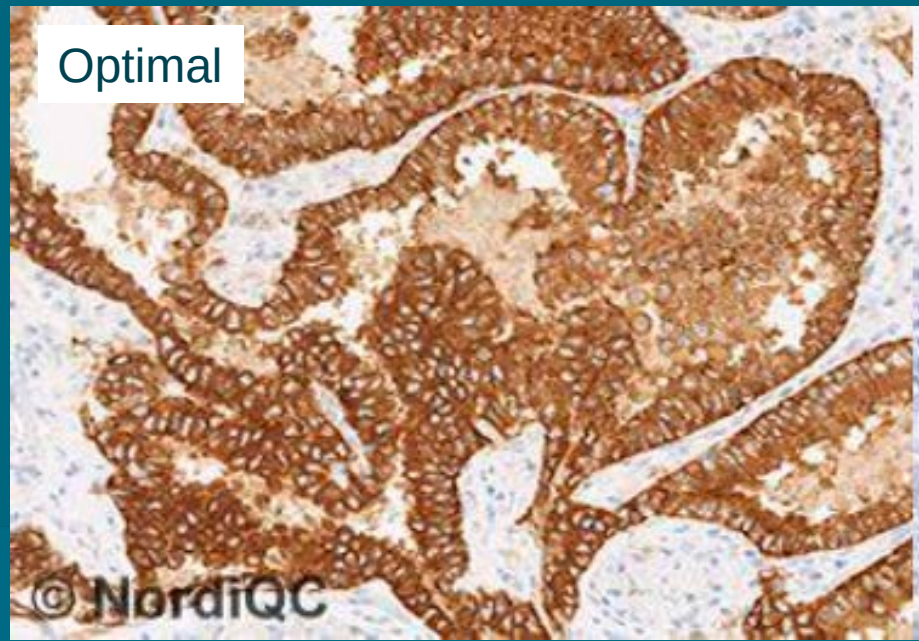
- Anaplastic lymphoma kinase (ALK): a transmembrane receptor tyrosin kinase
- CD30+ anaplastic large cell lymphomas (ALCLs) may be associated with a balanced (2;5)(p23;5q35) chromosomal translocation
- Anaplastic lymphoma kinase (ALK) rearrangements are present in about **5% of advanced non-small-cell lung cancer**
- Crizotinib was the first ALK tyrosine kinase inhibitor licensed for the treatment of metastatic ALK-positive NSCLC

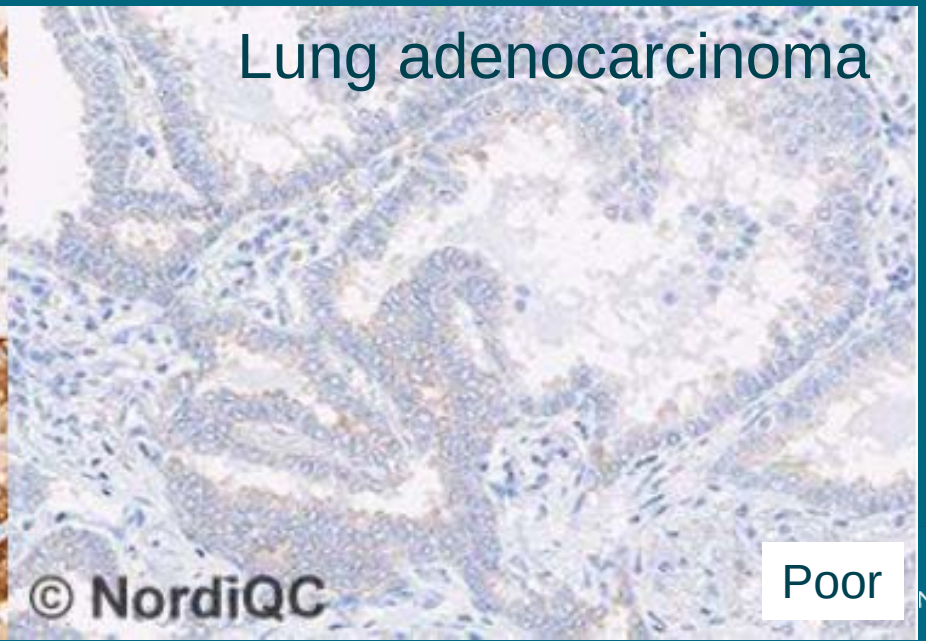


Lung ALK – run 45, 176 labs









Lung ALK – run 45, 176 labs

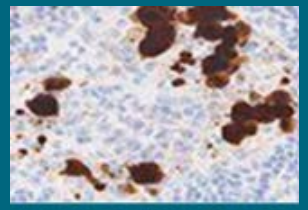
Table 1. **Antibodies and assessment marks for lu-ALK, run 45**

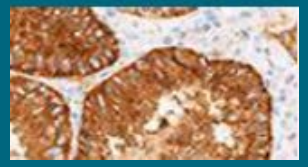
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5A4**	46 3 2 1 1 1	Leica/Novocastra Thermo/NeoMarkers Monosan Abcam Biocare Zytomed	24	16	13	1	74%	81%
mAb clone **ALK1**	8	Dako	0	0	3	5	0%	-
mAb clone **OTI1A4**	5	ORIGENE	4	1	0	0	100%	100%
rmAb clone **D5F3**	21 1	Cell Signaling PrimeBioMed	18	2	1	1	91%	95%
rmAb clone **SP8**	2	Thermo/NeoMarkers	0	0	1	1	-	-
Ready-To-Use antibodies								
mAb **ALK1 IR641**	15	Dako	0	0	4	11	0%	-
mAb clone **ALK1 790/800-2918**	10	Ventana	0	1	6	3	10%	-
mAb clone **ALK1 204M-18**	1	Cell Marque	0	0	0	1	-	-
mAb clone **ALK1 GA641**	1	Dako	0	0	0	1	-	-
rmAb clone **D5F3 790-4794**	47	Ventana	41	4	2	0	96%	96%

Lung ALK

The immunoassay must fit for the purpose:

- Identify the antibody useful for the specific task



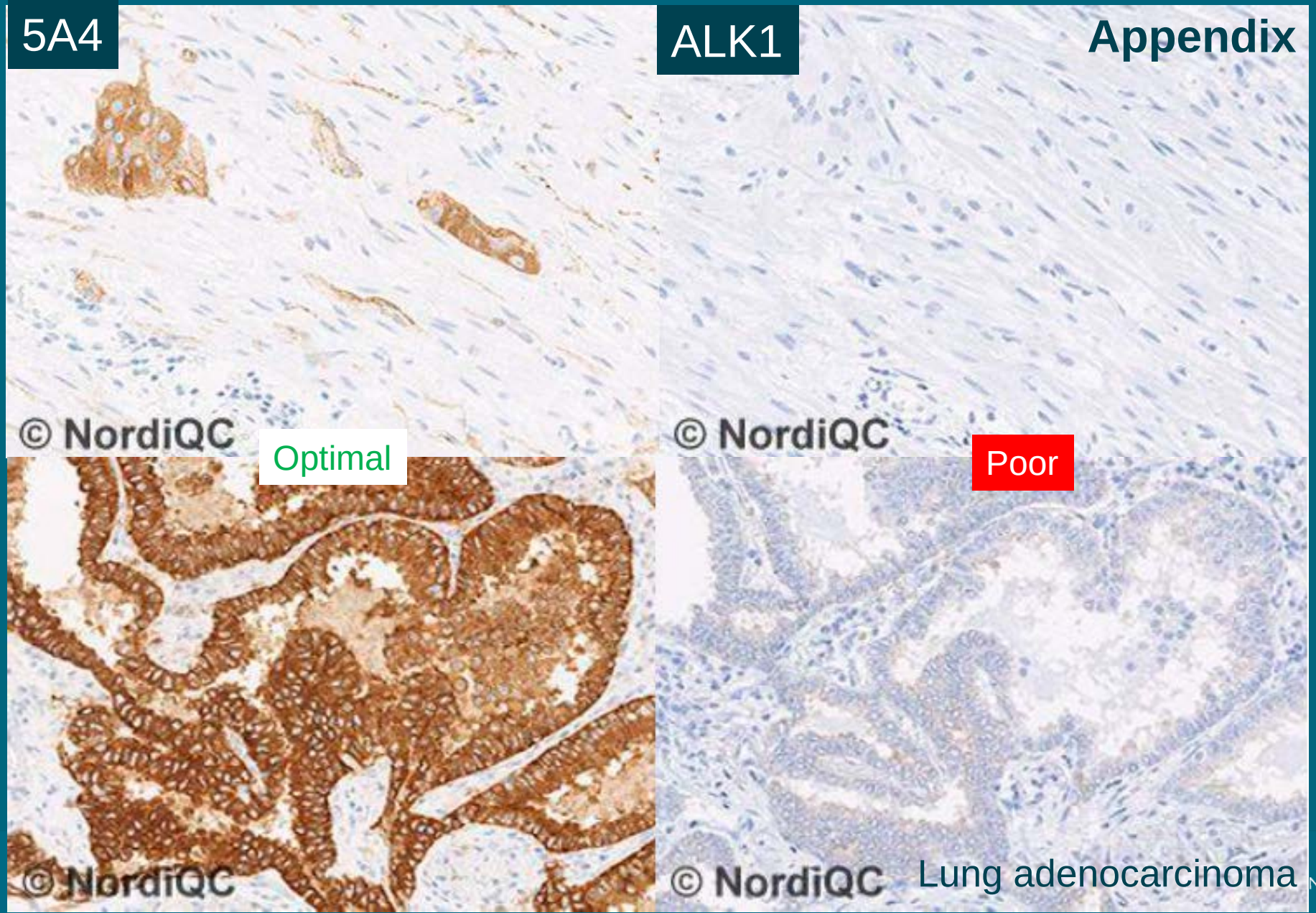


The right external controls must be used:

- Tissue with high epitope expression to identify the right antibody
  - Appendix

- Tissue with low epitope expression to assure the sensitivity:
  - ALK-positive lung adenocarcinoma
- Tissue with no epitope expression to assure the specificity
  - e.g., liver

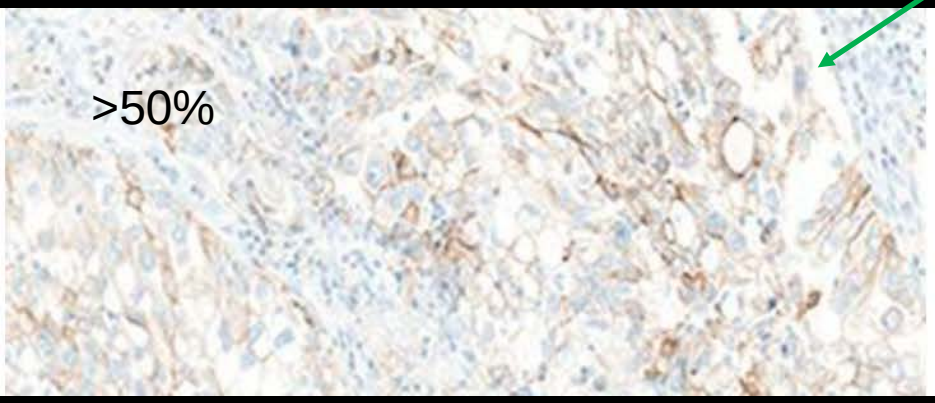
Lung ALK – run 45, 176 labs

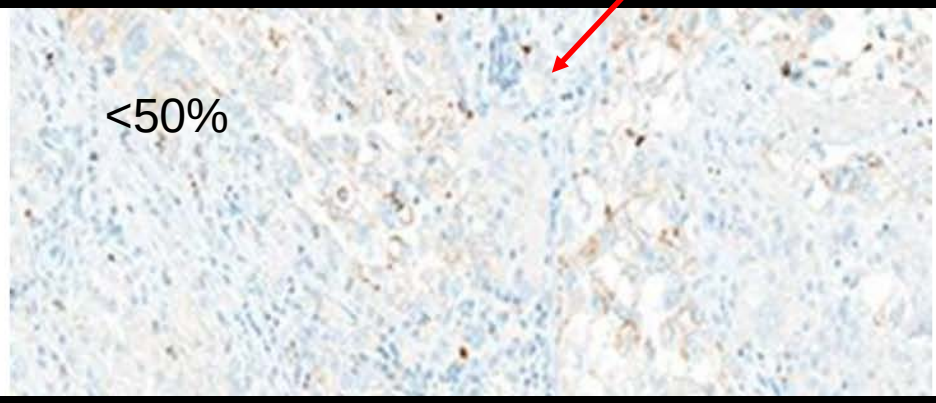


External Quality Assurance – PDL1

Table 1. **Assessment marks for IHC assays and antibodies run C1, PD-L1 IHC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
22C3 pharmDX, **SK006**	12	Dako/Agilent	10	1	0	1	92%	92%
22C3 pharmDX, **SK006**[4]	2	Dako/Agilent	0	0	1	1	-	-
28-8 pharmDX, **SK005**	7	Dako/Agilent	3	3	1	0	86%	86%
SP263, 790-4905	16	Ventana/Roche	9	2	2	3	69%	77%
SP142, 740-4859	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	13	Dako/Agilent	1	1	4	7	15%	-
mAb clone **E1L3N**	8	Cell Signaling	1	1	1	5	25%	-
mAb **CAL10**	1	Biocare	0	0	1	0	-	-
rmAb clone **28-8**	6	Abcam	0	1	1	4	17%	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-

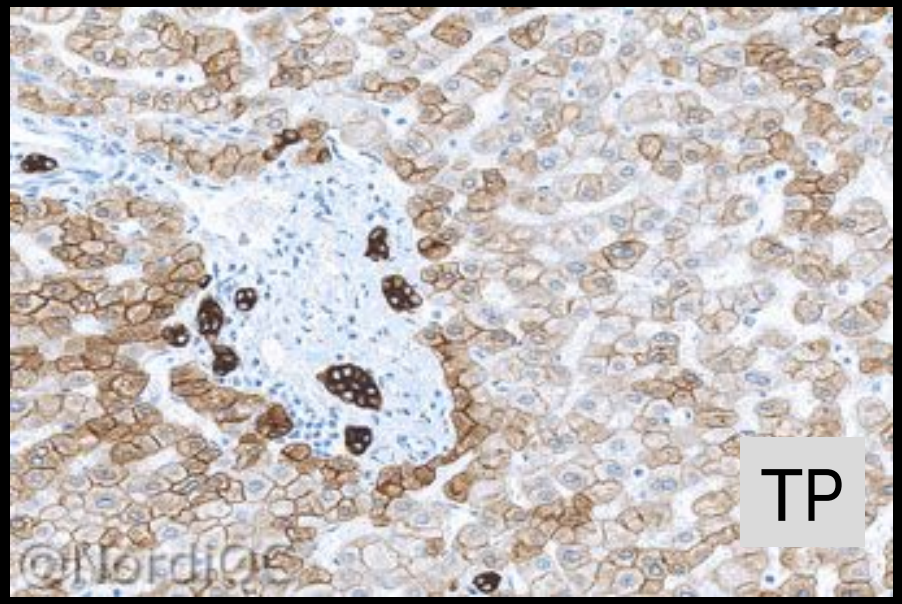




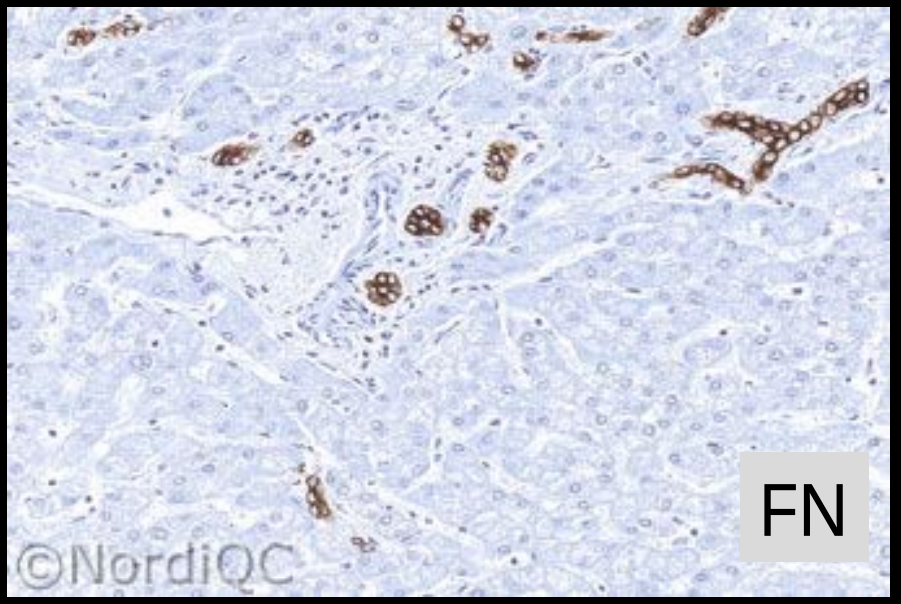
Misleading data sheets

Pan-Cytokeratin

Inappropriate retrieval (31%)

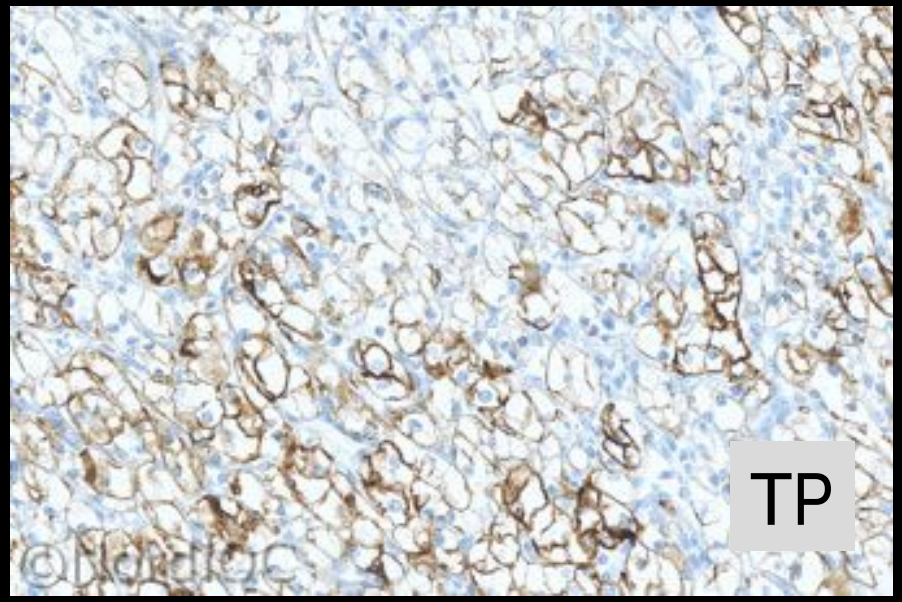


Liver

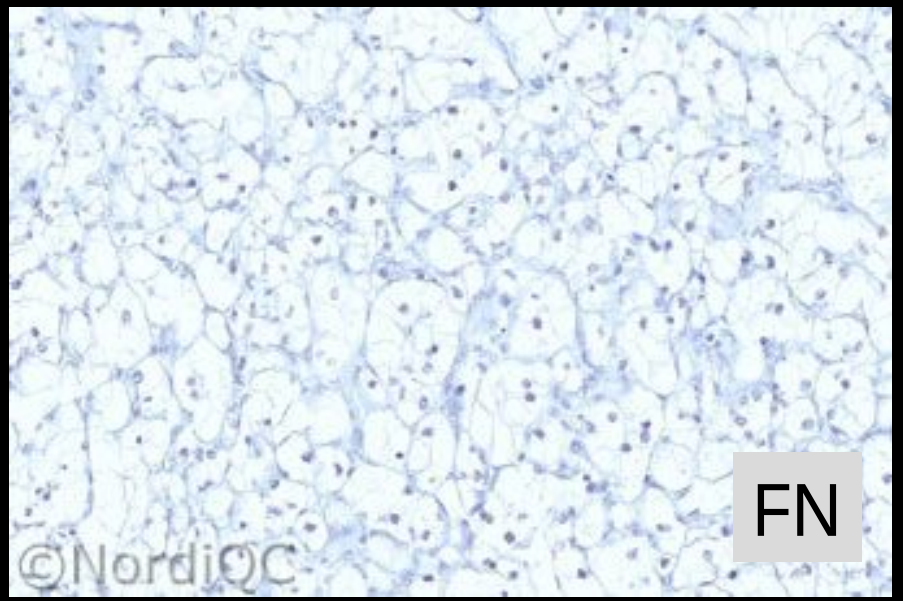


AE1/AE3 + HIER

AE1/AE3 + proteolysis



RCC



IHC - NordiQC 2014

Performance history
This was the 7th NordiQC assessment of CK-PAN. The overall pass rate has remained almost constant in the last 5 runs performed, as shown in table 2.

Table 2. **Proportion of sufficient results for CK-PAN in the seven NordiQC runs performed**

	Run 8 2003	Run 15 2005	Run 20 2008	Run 24 2008	Run 30 2010	Run 36 2012	Run 41 2014
Participants, n=	72	85	103	123	168	202	233
Sufficient results	53%	58%	62%	60%	65%	65%	67%

AE1/AE3 : Optimal results only obtained by **HIER** in NordiQC runs

Dako: RTU – HIER
Leica: RTU – Proteolysis
Thermo:
.....
VMS: RTU - Proteolysis

Conc: Proteolysis or HIER
Conc: HIER
Conc: HIER Quanto – Proteolysis UltraVision

Misleading data sheets + Wrong control material used

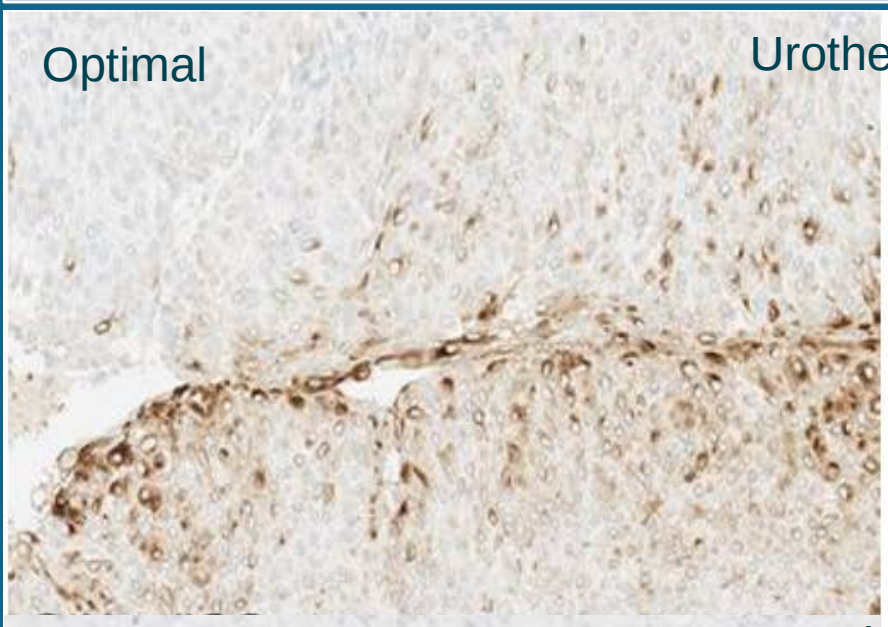
CEA – run 47, 2016, 255 labs

Table 1. **Antibodies and assessment marks for CEA, run 47**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **12-140-10**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **CEA31**	9 1	Cell Marque BioSB	6	0	3	1	67%	75%
mAb **COL-1**	6 5 5 2 1 1	Thermo/Neomarkers Invitrogen/Zymed Biocare Immunologic Zytomed GeneTex	11	7	2	0	90%	94%
mAb **II-7**	85	Dako/Agilent	2	19	60	4	25%	58%
Ready-To-Use Antibodies								
mAb clone **CEA31 760-4594**	53	Ventana/Cell Marque	22	26	5	0	91%	100%
mAb clone **II-7 IR/IS622/GA622**	47	Dako/Agilent	0	6	40	1	13%	-
mAb clone **II-7 PA0004**	12	Leica	0	5	6	1	42%	-
mAb clone **TF3H8-1 760-2507**	13	Ventana/Roche	0	0	0	13	0%	-

CEA

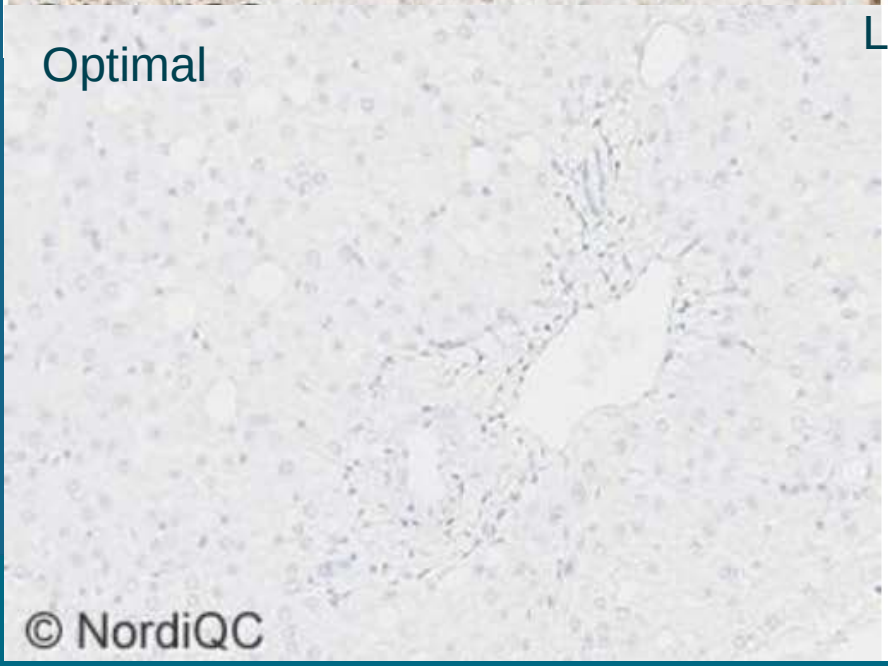
Optimal



Urothelial carcinoma

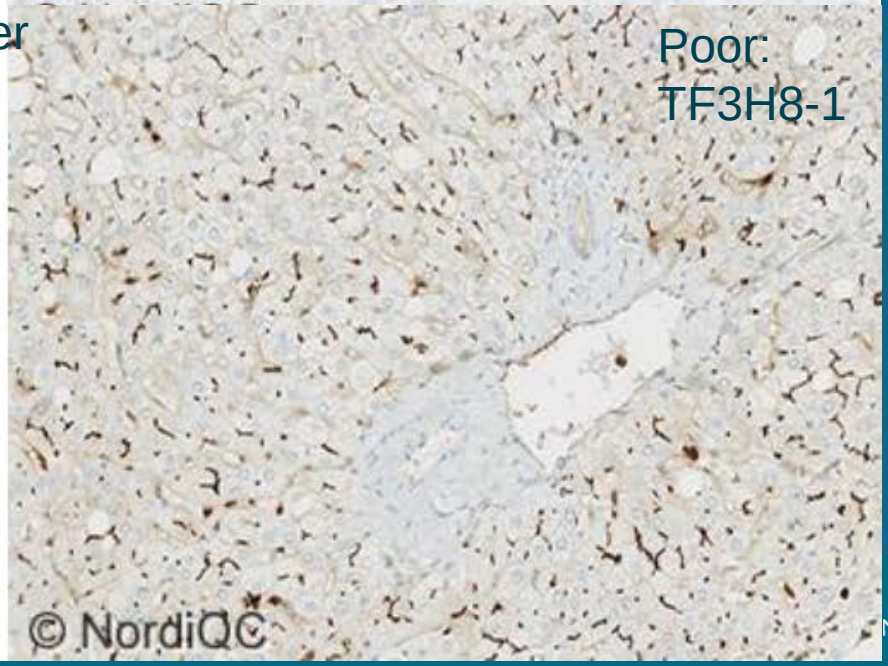
Poor:
TF3H8-1

Optimal



Liver

Poor:
TF3H8-1



CDX2 – run 48

Table 1. **Antibodies and assessment marks for CDX2, run 48**

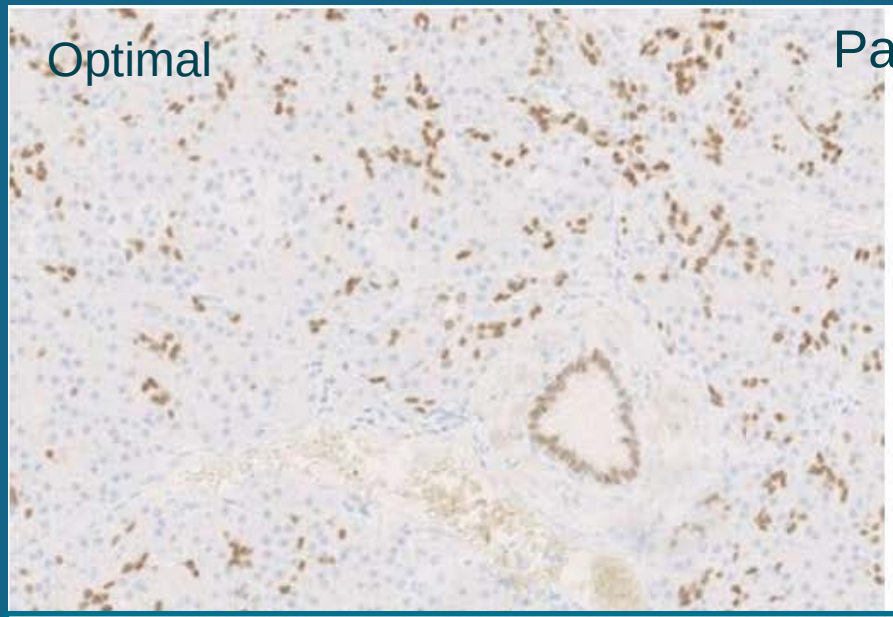
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **AMT28**	2	Leica/Novocastra	0	0	0	2	-	-
mAb clone **CDX2-88**	2 2	Biocare Biogenex	0	0	1	3	-	-
mAb clone **DAK-CDX2**	31	Agilent/Dako	6	9	7	9	48%	57%
rmAb clone **EPR2764Y**	31 5 4 4 2 2 1	Cell Marque Thermo/Neomarkers Immunologic Zytomed Monosan Zeta Corporation A. Menarini	28	14	7	3	81%	81%
Ready-To-Use antibodies								
mAb **DAK-CDX2 IR080/IS080**	34	Agilent/Dako	18	10	5	1	82%	93%
mAb **DAK-CDX2 GA080**	26	Agilent/Dako	16	4	3	3	77%	100%
rmAb clone **EPR2764Y 760-4380/ 235R***	103	Ventana/Cell Marque	81	15	5	2	93%	96%

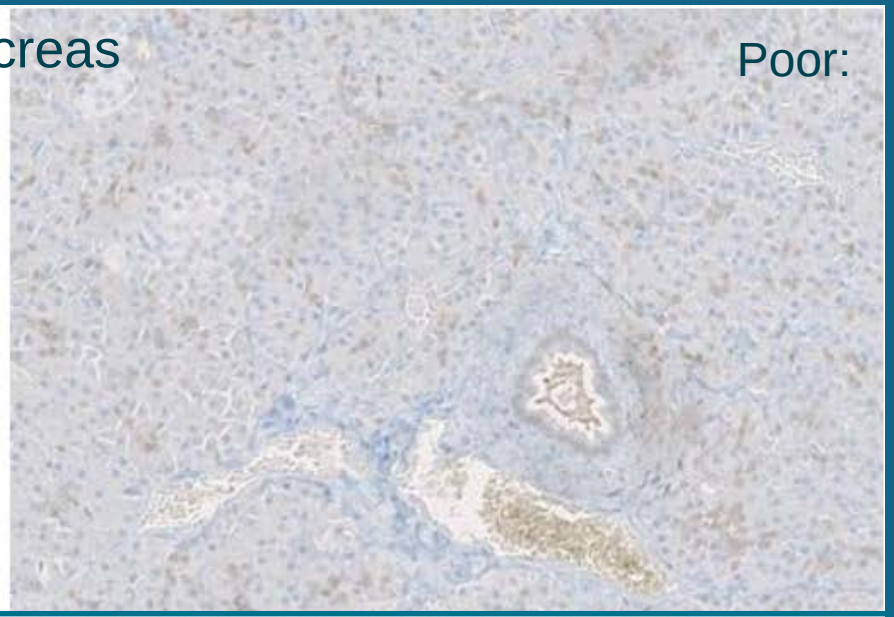
CDX2

Optimal

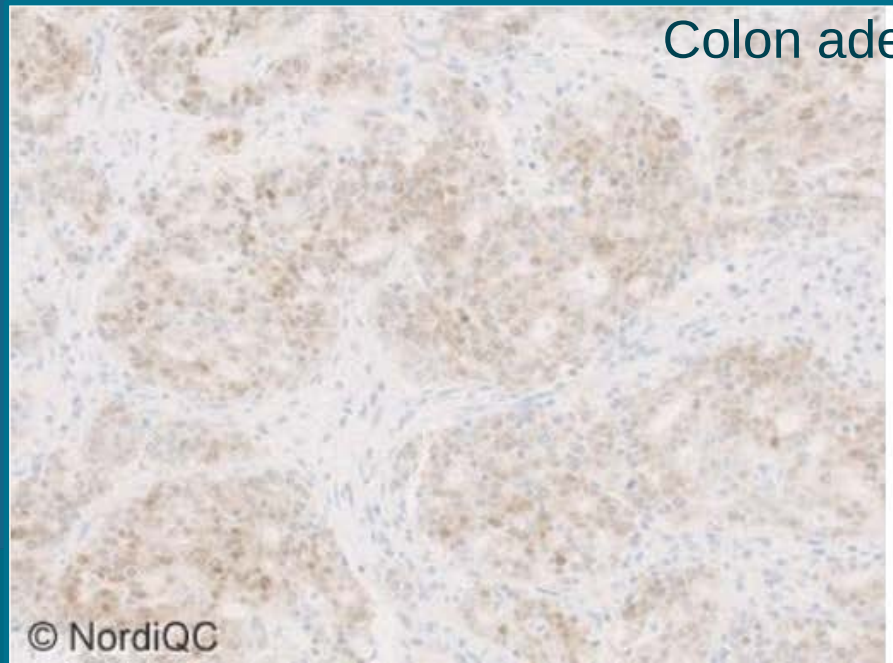
Pancreas

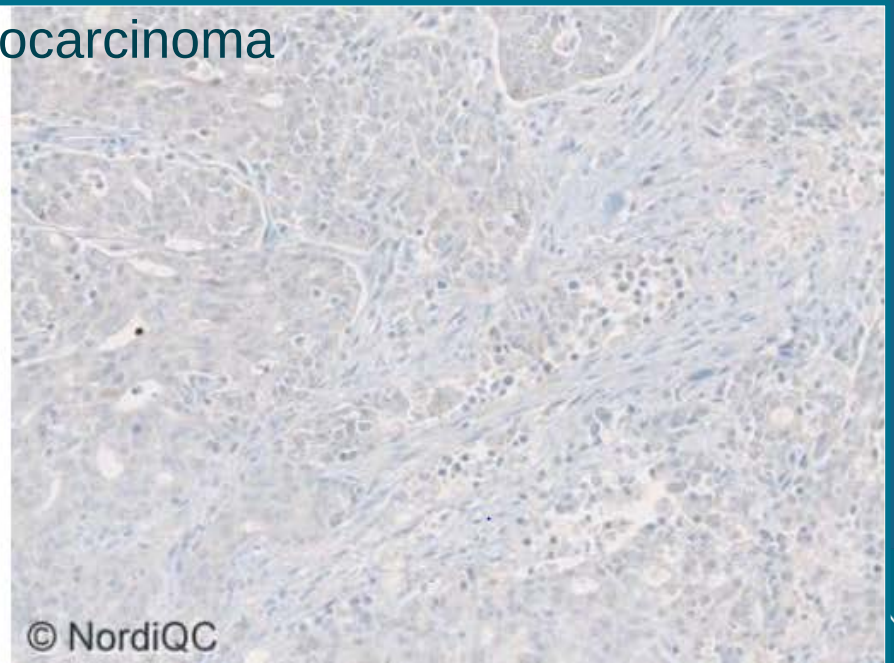
Poor:





Colon adenocarcinoma

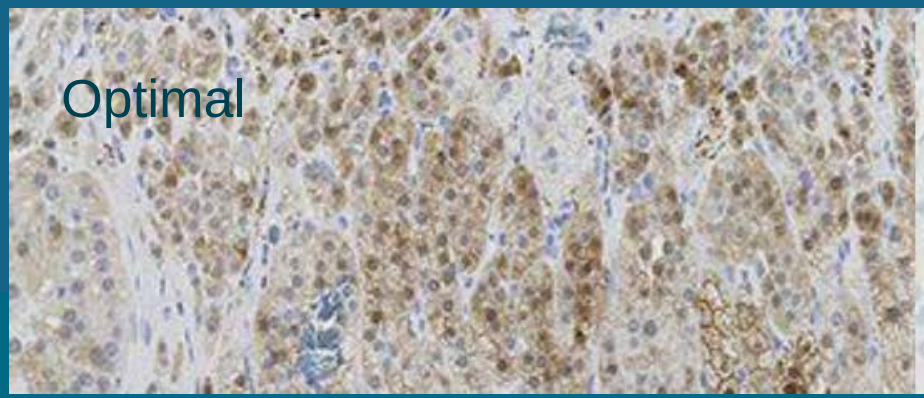


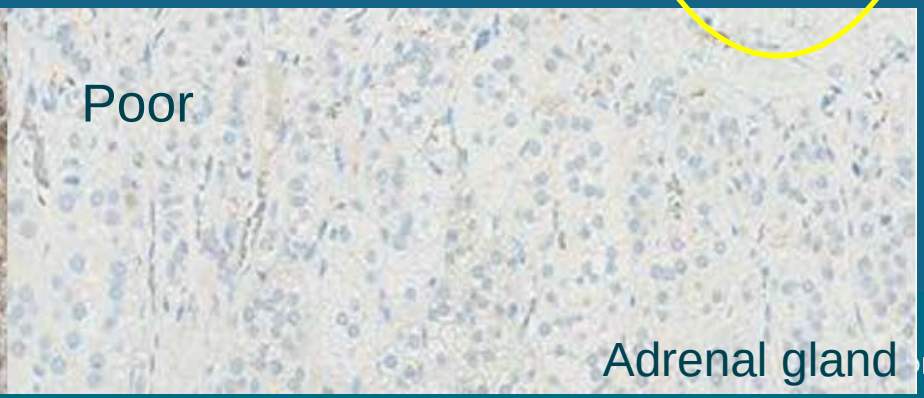


Calretinin - run 45

Table 1: **Antibodies and assessment marks for CR, run 45**

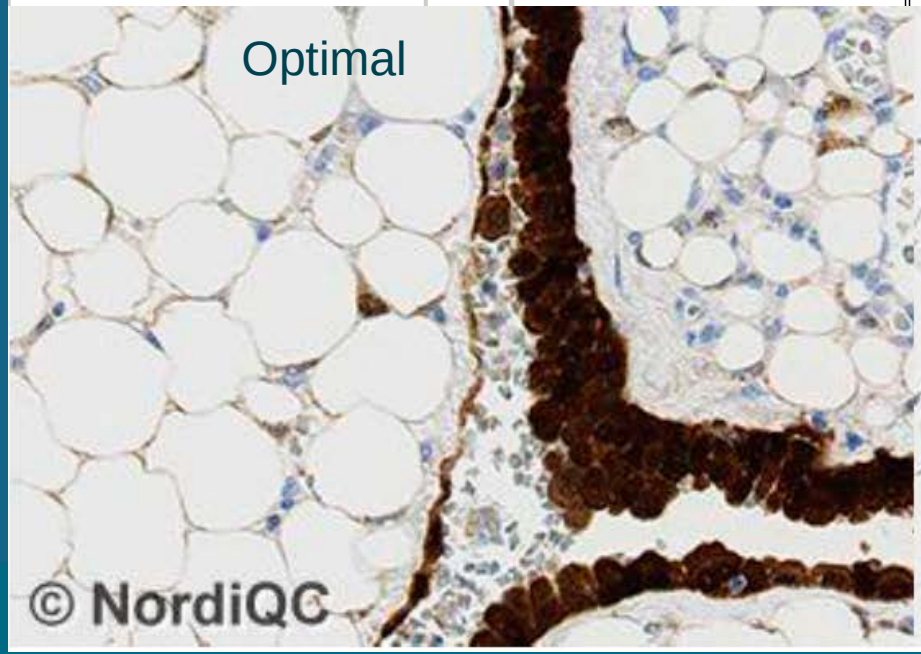
Concentrates	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mmAb clone **2E7**	1	Immunologic	1	0	0	0	-	-
mmAb clone **5A5**	21 1	Leica/Novocastra Immunologic	3	10	8	1	59%	56%
mmAb clone **CAL6**	6 1	Leica/Novocastra Monosan	4	1	0	2	71%	-
mmAb clone **DAK-Calret 1**	35	Dako	10	13	9	3	66%	87%
rmAb clone **SP13**	3 1 2	Thermo/Neomarkers Spring Bioscience Cell Marque	1	2	2	1	50%	-
pAb **18-0211**	16	Invitrogen/Zymed	2	8	6	0	63%	-
pAb **232A**	5	Cell Marque	0	1	2	2	20%	-
[illegible]	1	[illegible]	0	1	0	0		

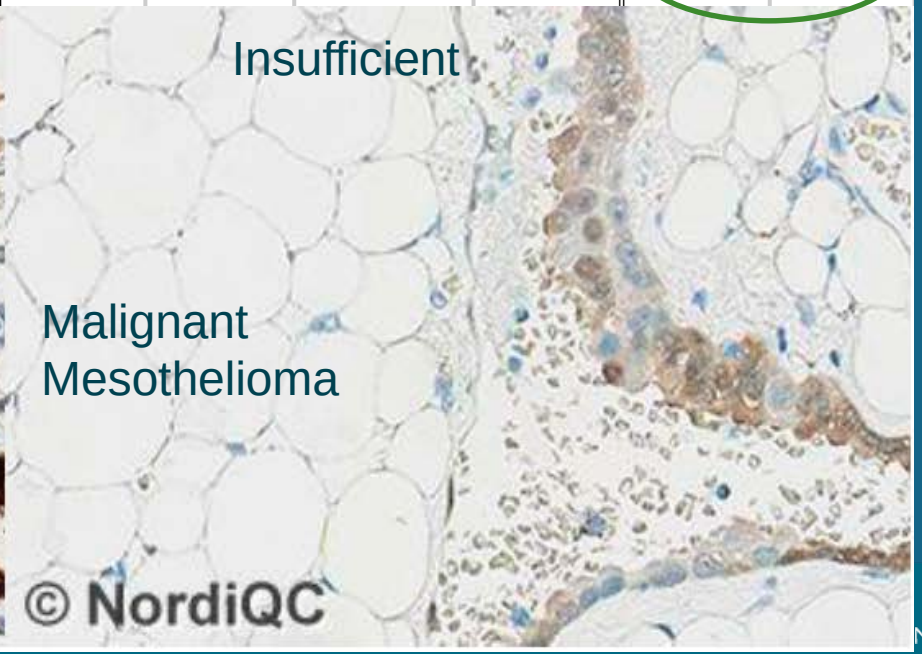




Calretinin – 113 labs using RTUs

Ready-To-Use antibodies								
mmAb clone **CAL6 PA0346**	8	Leica/Novocastra	2	3	2	1	63%	-
mmAb clone **DAK-Calret 1 IS/IR627**	38	Dako	9	17	10	2	68%	79%
rmAb **SP13 RMA-0524**	1	Maixin	1	0	0	0	-	-
rmAb **SP13 232R-18**	1	Cell Marque	0	1	0	0	-	-
rmAb **SP13 MAD-000315QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **SP65 790-4467**	64	Ventana	52	8	2	2	94%	94%

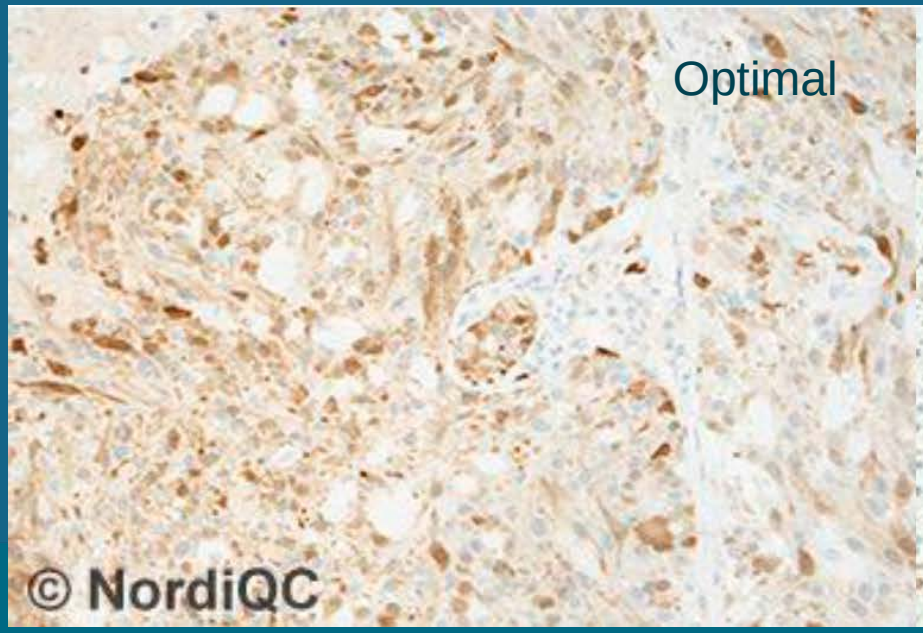


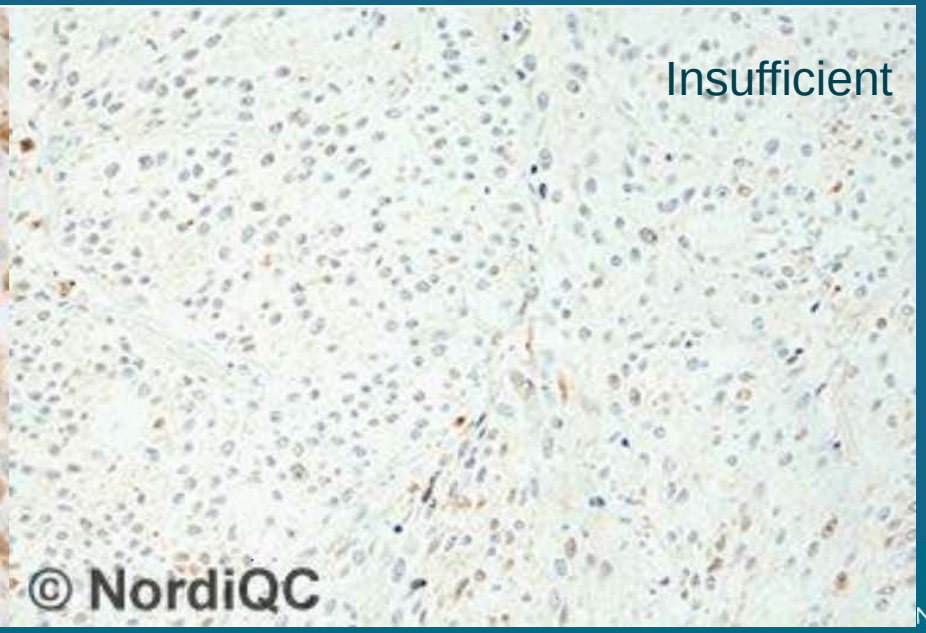


S-100

Ready-To-Use systems								
mAb clone **4C4.9 790-2914**	24	Ventana	2	8	9	5	42%	100%
pAb **IR504**	34	Dako	3	27	4	0	88%	95%
pAb **GA504**	13	Dako	5	6	2	0	85%	90%
pAb **760-2523**	26	Ventana	1	11	12	2	46%	100%

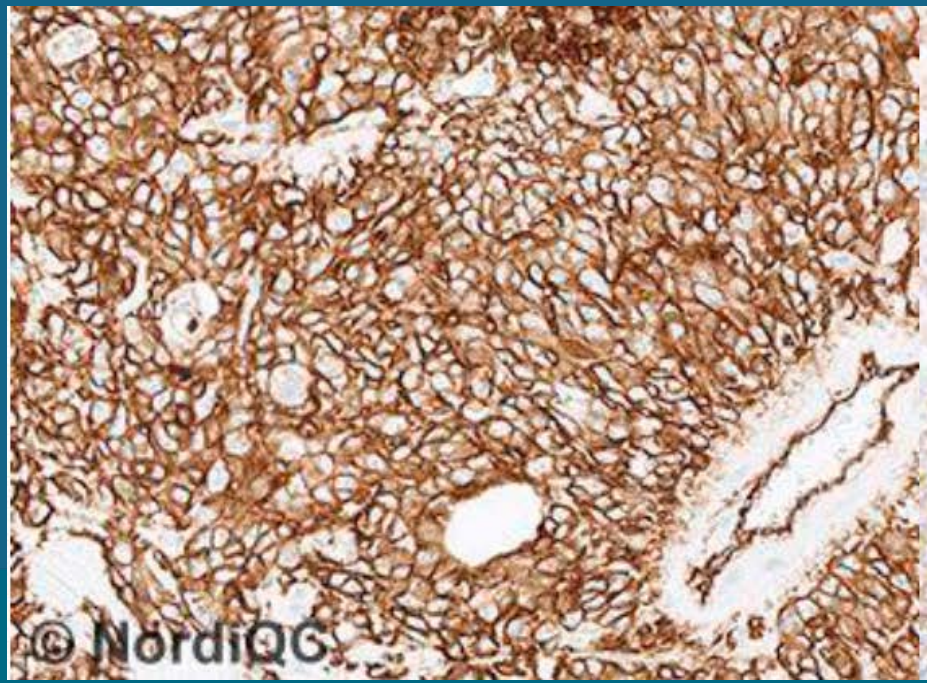
Mal. melanoma

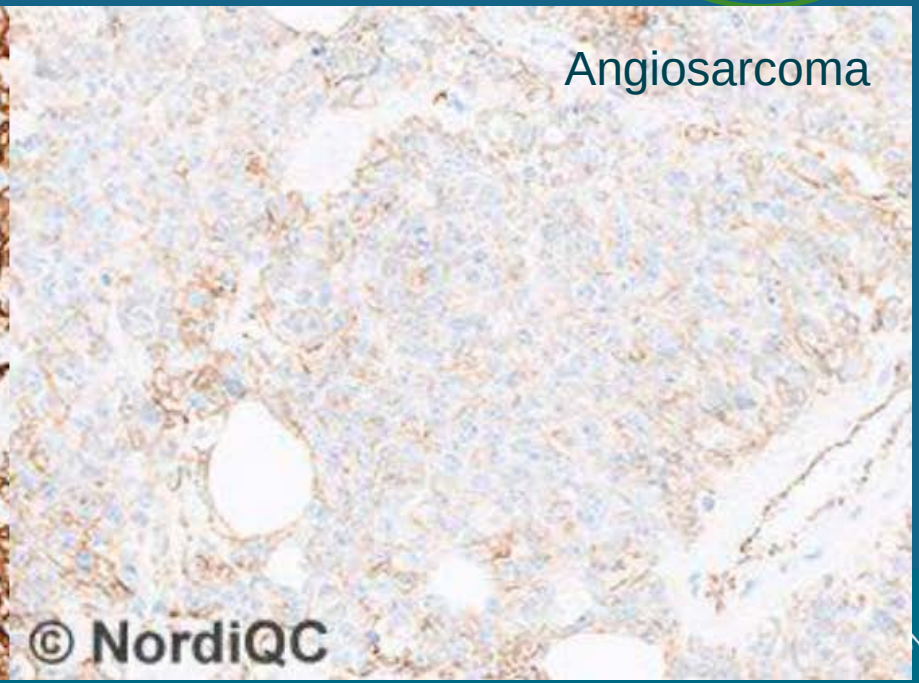




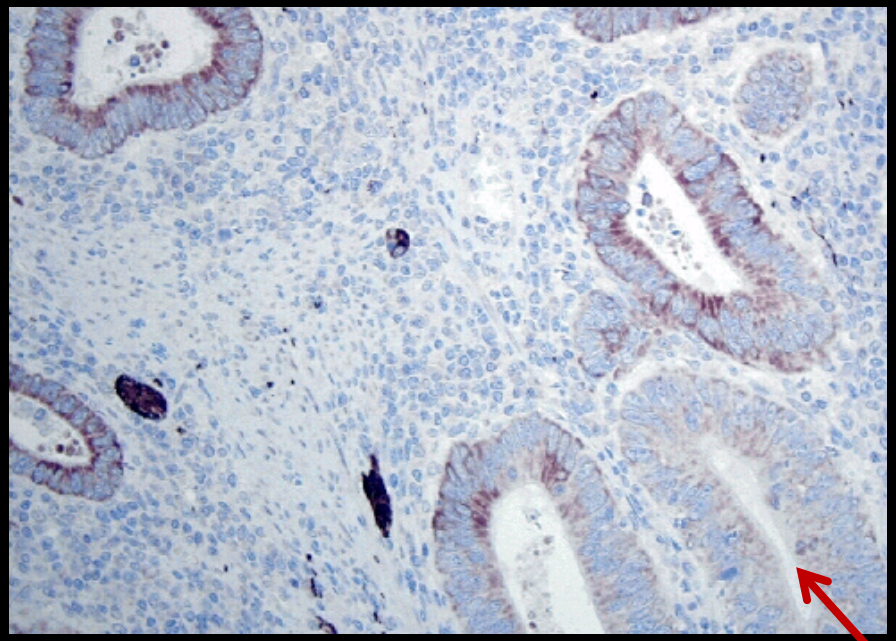
CD31

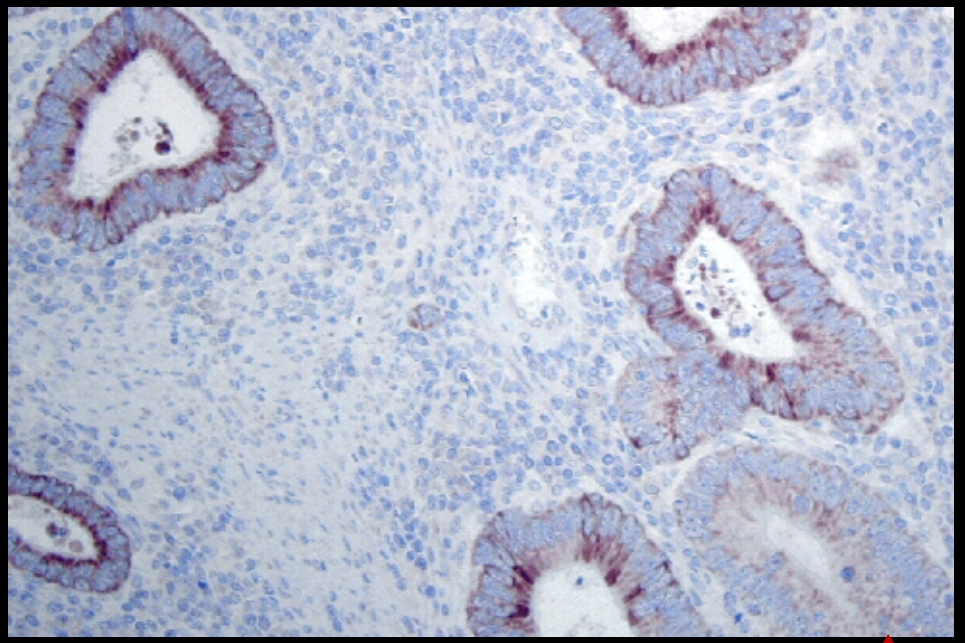
Ready-To-Use antibodies								
mAb clone **1A10** **PA0250**	5	Leica/Novocastra	0	0	0	5	0%	-
mAb clone **BC2** **PM347**	1	Biocare	0	1	0	0	-	-
mAb clone **JC70A** **760-4378**	54	Ventana/Roche	23	10	18	3	61%	67%
mAb **JC70A** **IR/IS610**	47	Dako/Agilent	27	15	3	2	89%	95%
mAb **JC70A** **GA610**	19	Dako/Agilent	15	4	0	0	100%	100%

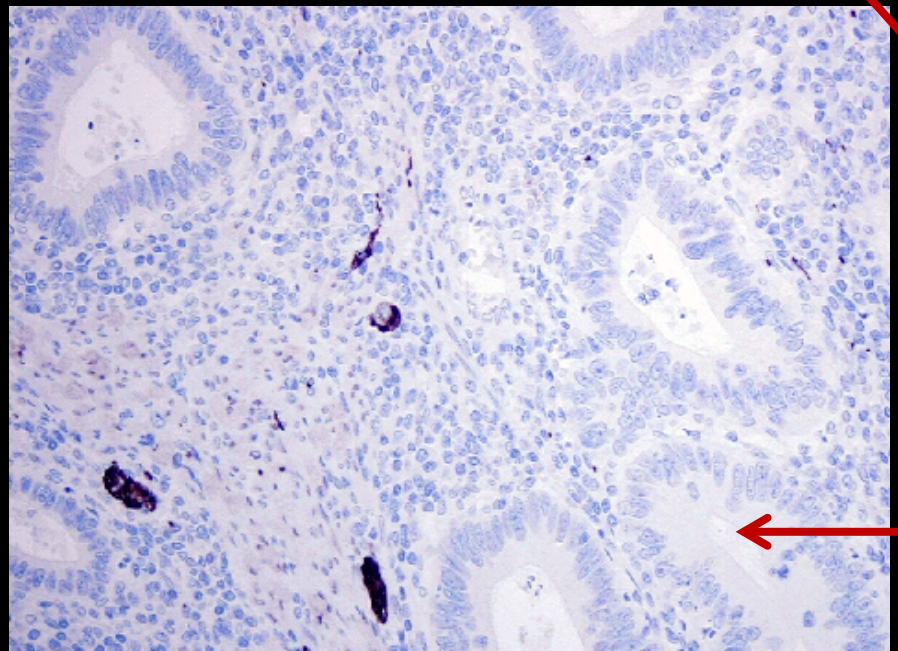




Biotin based system giving false postives







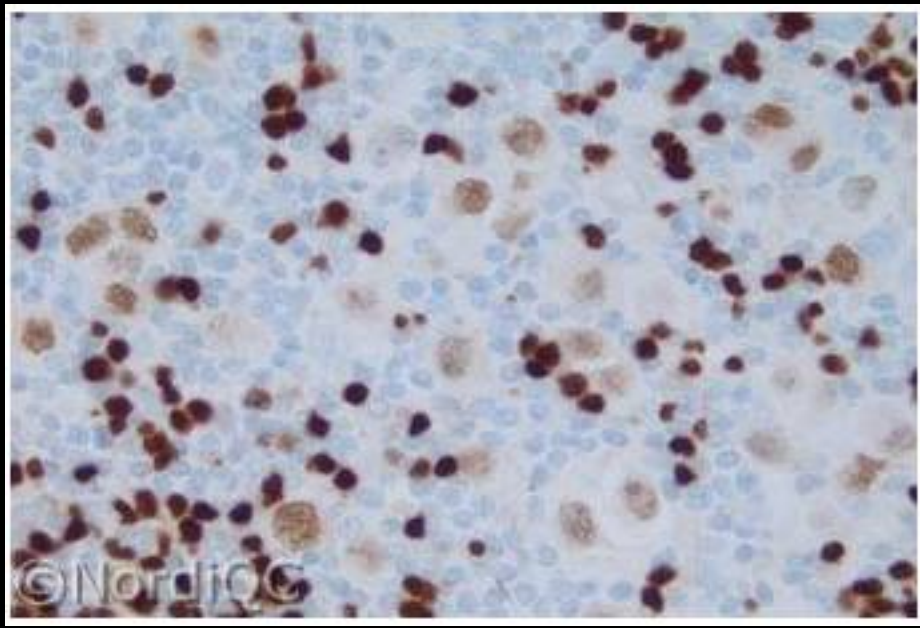
Synaptophysin
Labelled Steptavidin-Biotin system

No antibody
Labelled Streptavidin-Biotin system:
Neg. reagent control mandatory

Synaptophysin
Polymer based system

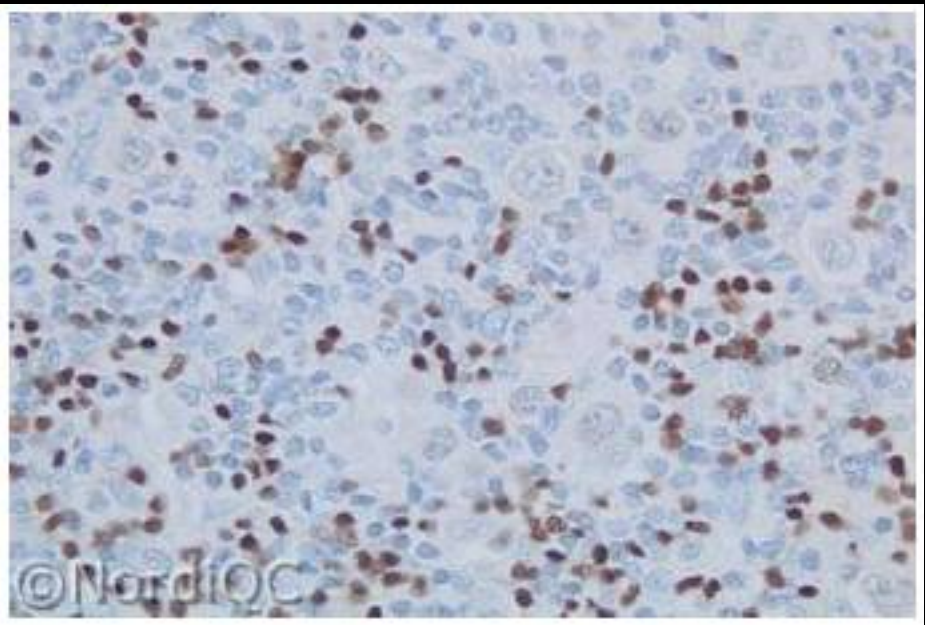
Platform dependent antibodies: PAX5

Hodgkin lymphoma NS



clone SP34
RTU VMS/CM

x200



clone 24
RTU VMS/CM

x200

IHC – Most common pitfalls

Original article

Call for a European programme in external quality assurance for bone marrow immunohistochemistry; report of a European Bone Marrow Working Group pilot study

J Clin Pathol 2009;**62**:547–551.

E E Torlakovic,[1] K Naresh,[2] M Kremer,[3] J van der Walt,[4] E Hyjek,[5] A Porwit[6]

Results: Eight different fixatives and nine different decalcification methods were used. While 93% of participants believed that they produced excellent results in BMTB IHC, only 4/19 (21%) laboratories did not have any poor results. CD117 and Ki-67, with 53% and 50% poor results, respectively, were the most problematic immunostains, while CD20 was the least problematic, with only 11% poor results.

Tailored recommendations

- Replace less successful antibodies (conc./RTU)
- Calibrate the antibody concentration
- Use HIER (instead of proteolysis or no retrieval)
- Increase HIER time / temperature / buffer pH
  - For 95% of epitopes pH 8-9 is preferable to pH 6
- Use a non-biotin based viz. system
- Use FDA approved kits instead of home-brews
- . . . . .
- Improve the internal QC: Identify the right controls – Select well defined normal low expressor cells/tissues

Results of NordiQC recommendations

419 advices for 11 markers

	No.	Improved	%
Positive	**268**	**195**	**73**
Negative	**151**	**21**	**14**

Conclusion

External Quality Assurance (EQA)

- Provides objective evidence of lab performance
- Identifies methodological errors
- Provides directions for improvements & controls

The results of the NordiQC work indicate that

- Improvement of IHC is strongly needed
- EQA schemes, industry and KOL must align - describing the requirements for optimal IHC performance.

Perspective

- Almost 1/3 of all IHC stains produced by NordiQC participants are still insufficient !
  - New labs
  - New antibodies, techniques, platforms
  - Increasing demands
- How many IHC stains produced by labs not participating in an EQA scheme are insufficient ?
- How many scientific publications are based on insufficient IHC stains ?
- What are the consequences for the patients ?

External Quality Assurance – ER

JNCI Journal of the National Cancer Institute Advance Access published June 10, 2008

NEWS

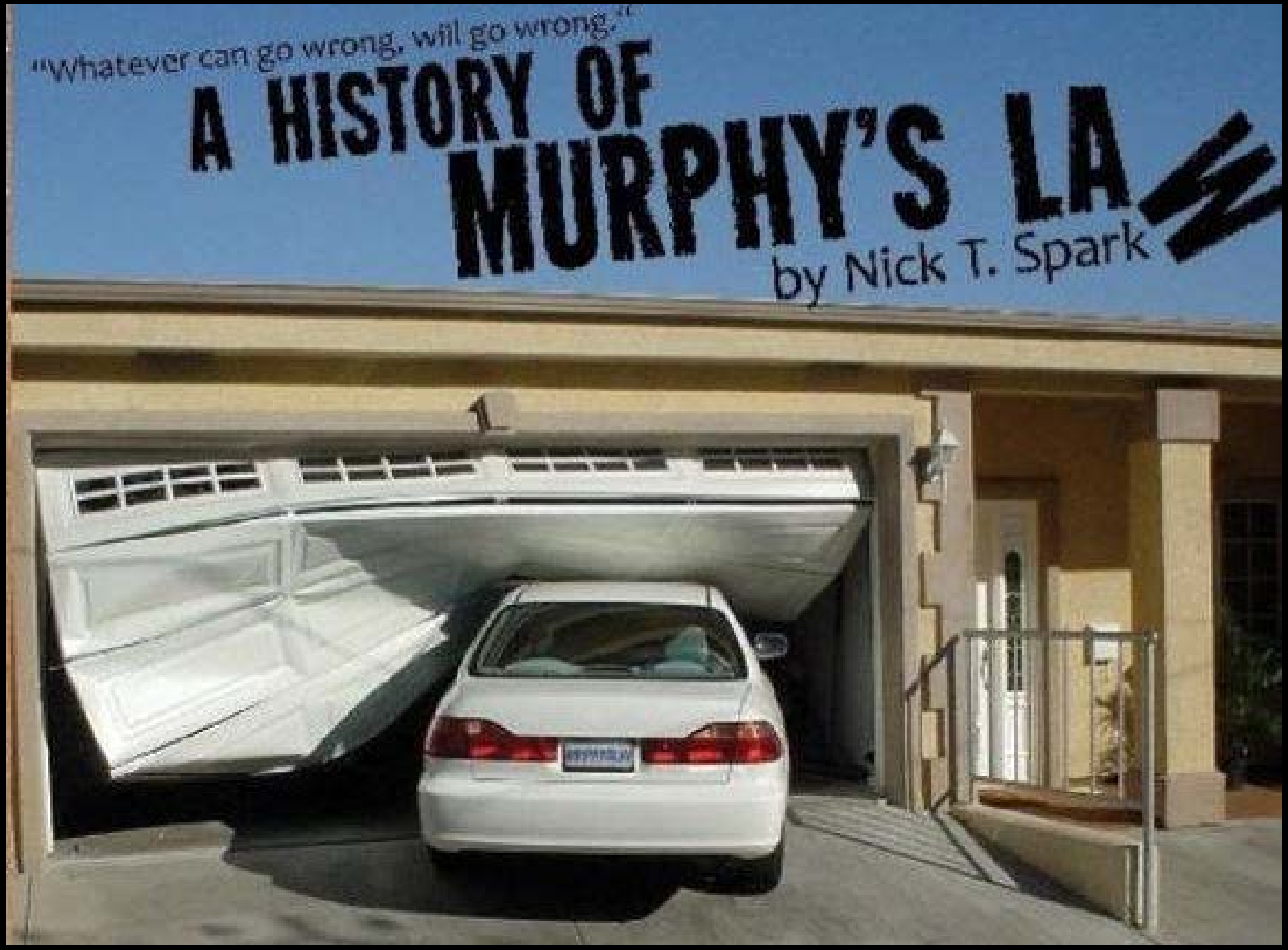
Breast Cancer Testing Scandal Shines Spotlight on Black Box of Clinical Laboratory Testing

By Karyn Hede

"Through the inquiry, the public learned that between 1997 and 2005 nearly 400 of about 1,000 breast cancer patients received incorrect test results of the ER status of their breast tumors."

"There are no good data on the quality of ER testing in the United States. The scary thing about the debacle in Canada is that we would never have known about this if results hadn't been checked in a central lab."

Craig Allred



When you believe in automation and stop thinking

International Symposium on Immunohistochemistry
January 4th - 7th, 2018
Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India
In collaboration with NordiQC, Alborg, Denmark and ISIMM, California, USA

The impact of proficiency testing on lab immunoassays

Thank you
for your attention

Mogens Vyberg
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Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark