Workshop in Diagnostic Immunohistochemistry
Oud St. Jan/ Old St. John – Brugge (Bruges), Belgium June 13th – 15th 2018

NordiQC External Quality Assurance in Immunohistochemistry

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark

NORTH DENMARK REGION

AALBORG (~ 200.000 inhabitants)

Aalborg University Hospital

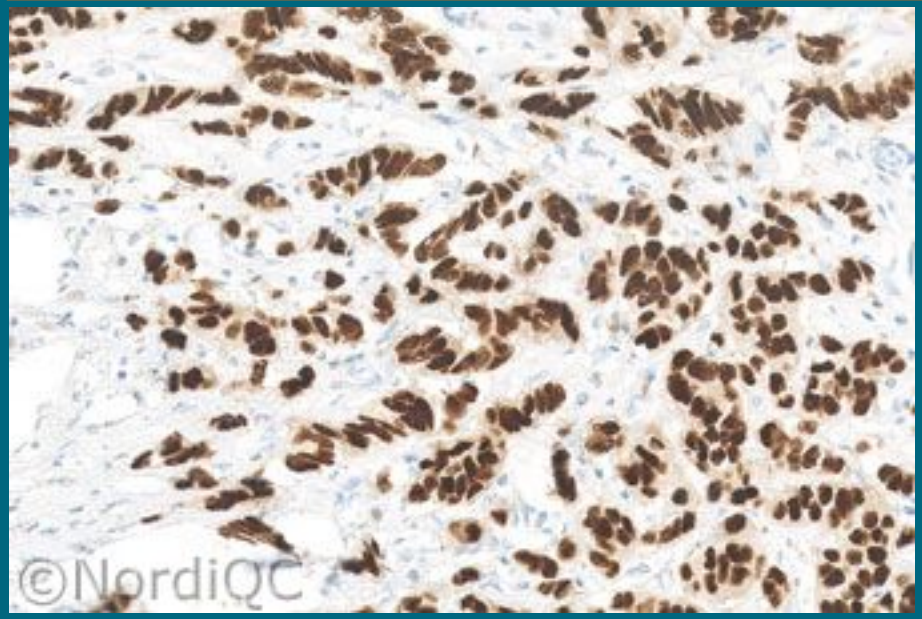
Institute of Pathology

Budolfi Church

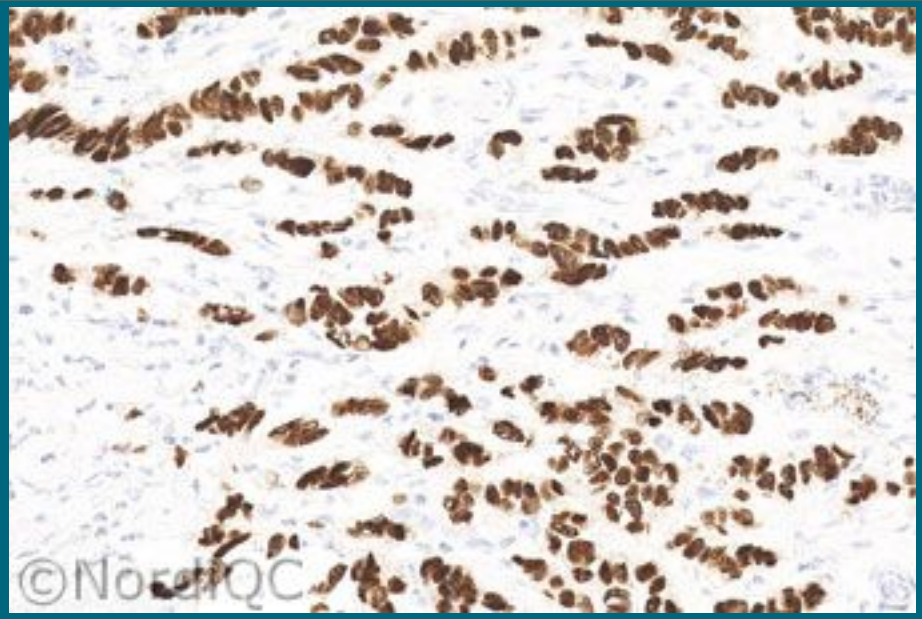
Aalborg Harbour Front

Aalborg House of Music

Serial sections stained for Estrogen receptor



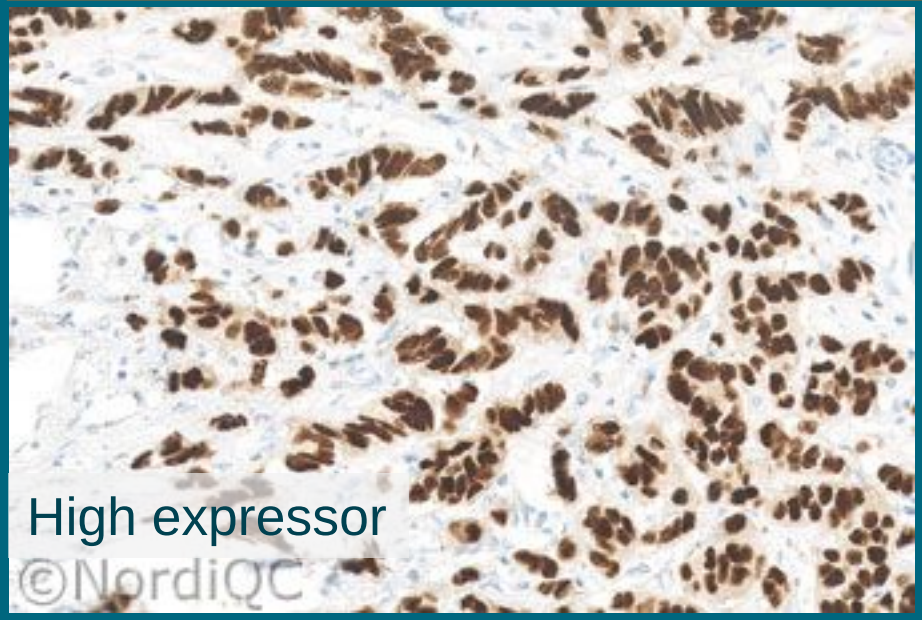
Lab. A

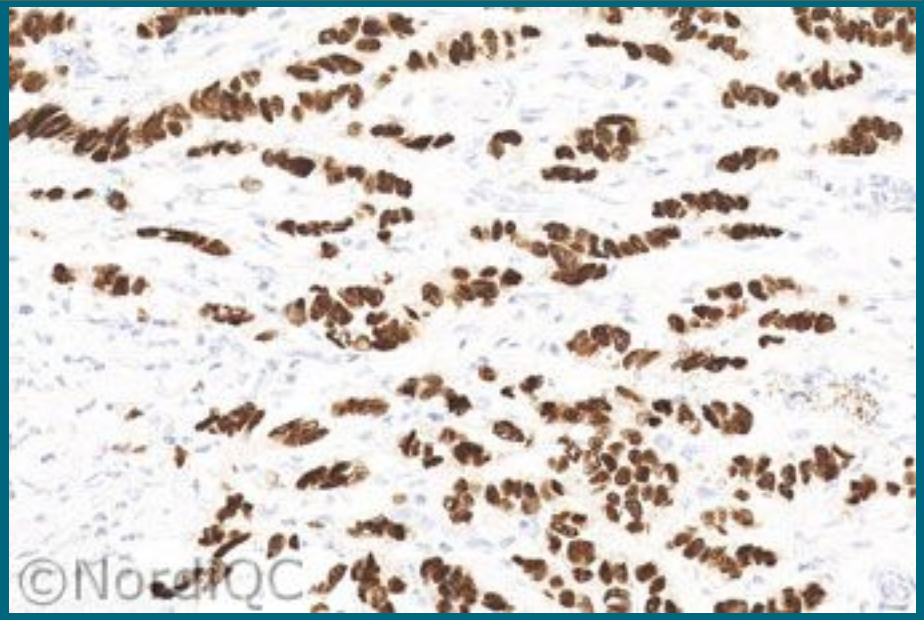


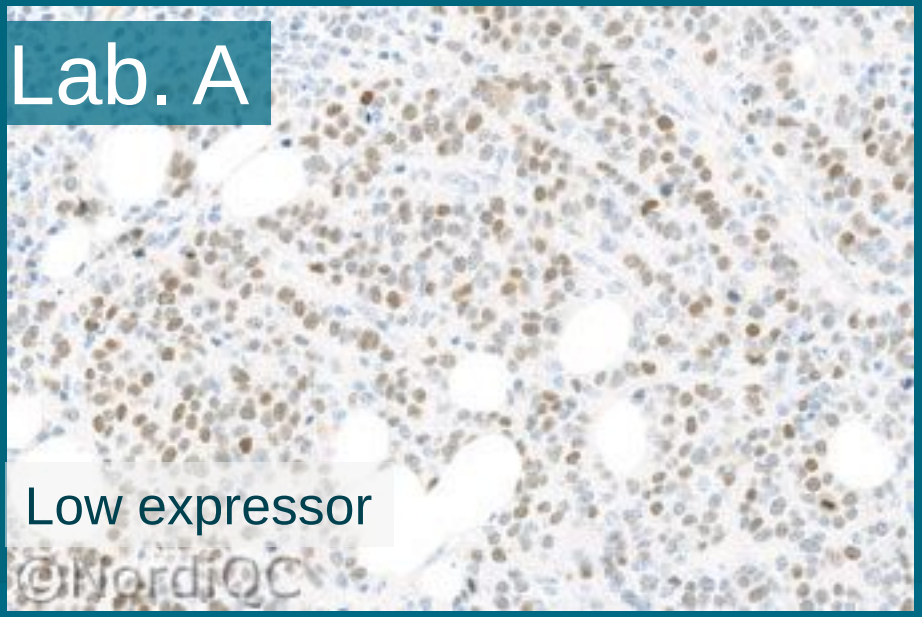
Lab. B

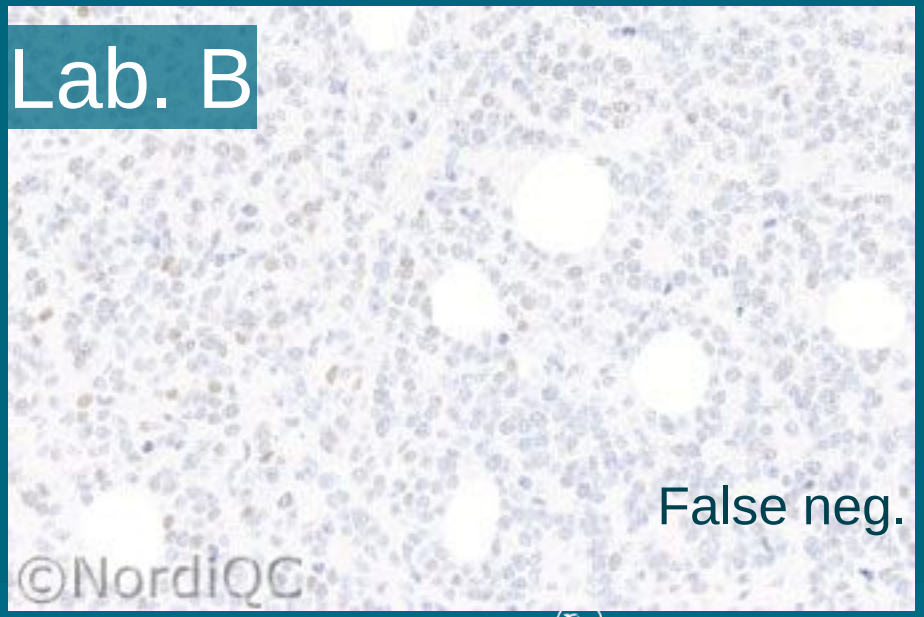
Optimally processed ductal breast carcinoma tissue

Serial sections stained for Estrogen receptor

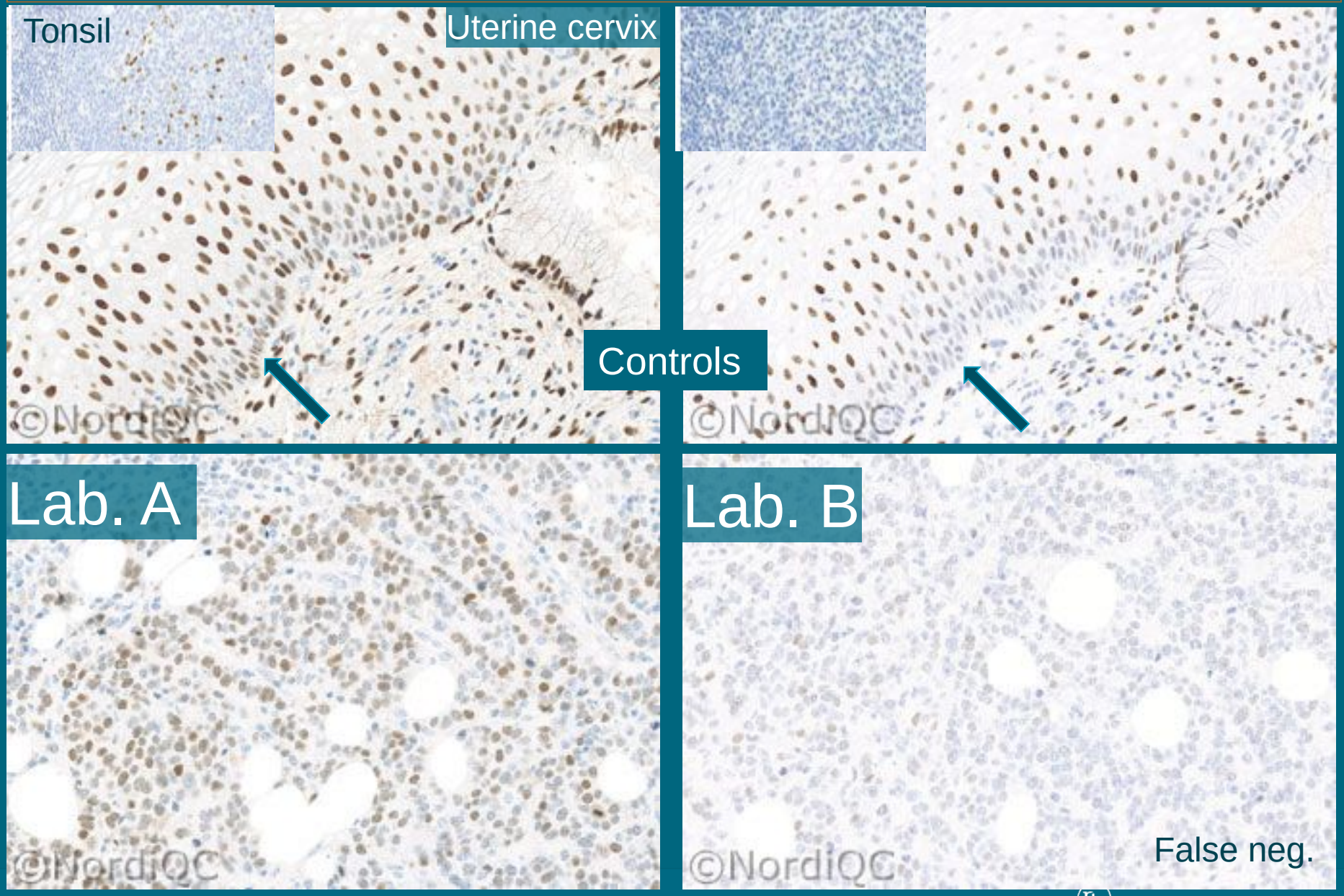




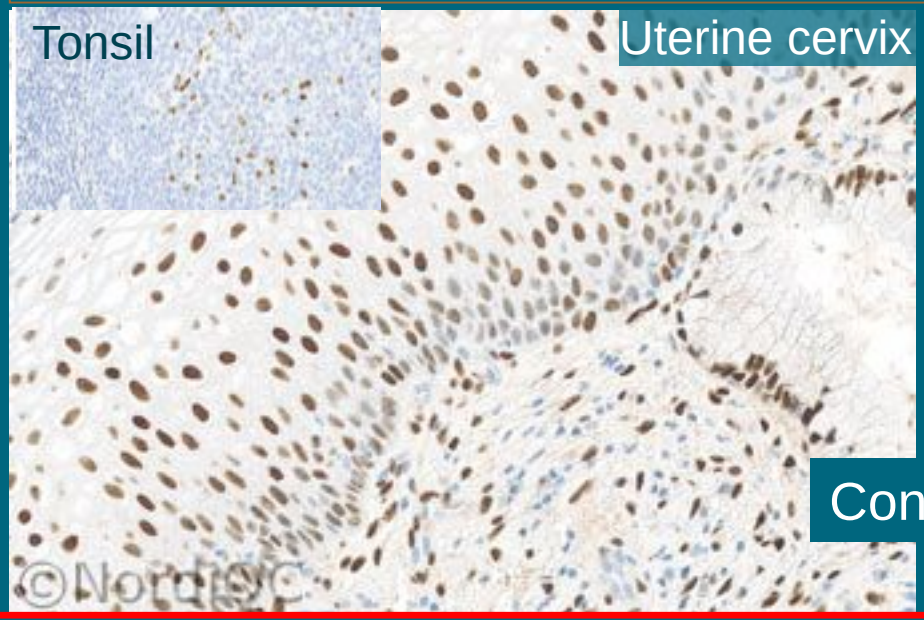


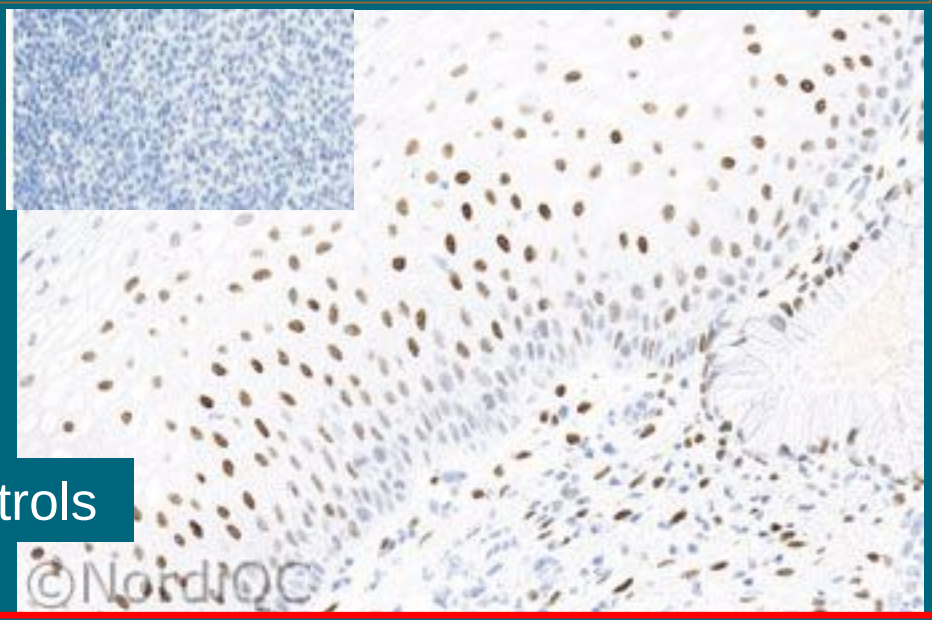


Serial sections stained for Estrogen receptor

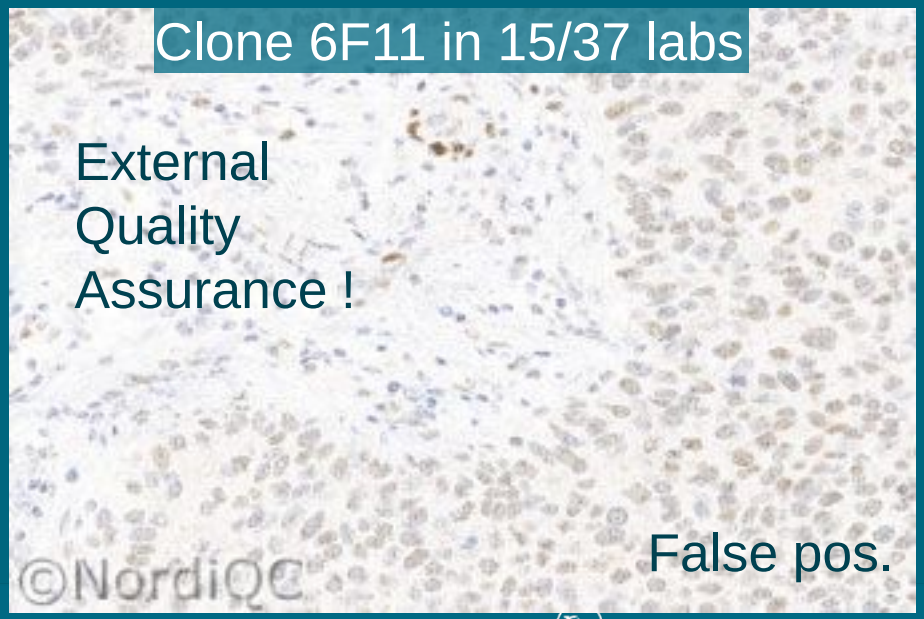


Serial sections stained for Estrogen receptor





Controls



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

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 / too dilute antibody clones/RTUs
  - Insufficient epitope retrieval
  - Insensitive visualization systems
  - Platform problems

Should be identified with proper controls

- Post-analytical issues
  - Interpretation criteria, interobserver variation …

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 ...

AALBORG UNIVERSITY HOSPITAL

www.nordiqc.org

Nordic immunohistochemical Quality Control

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

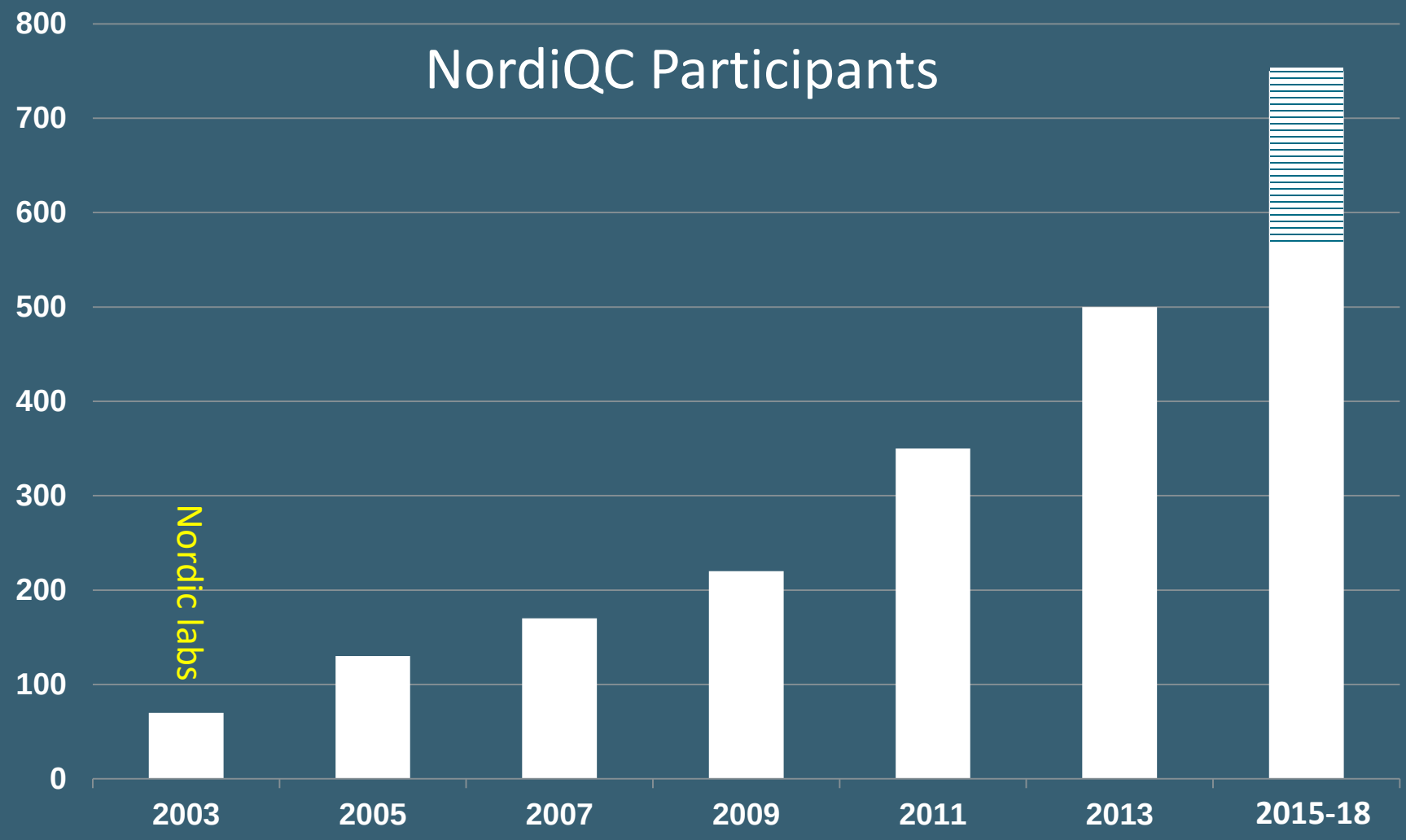
ANNUAL REVIEW ISSUE

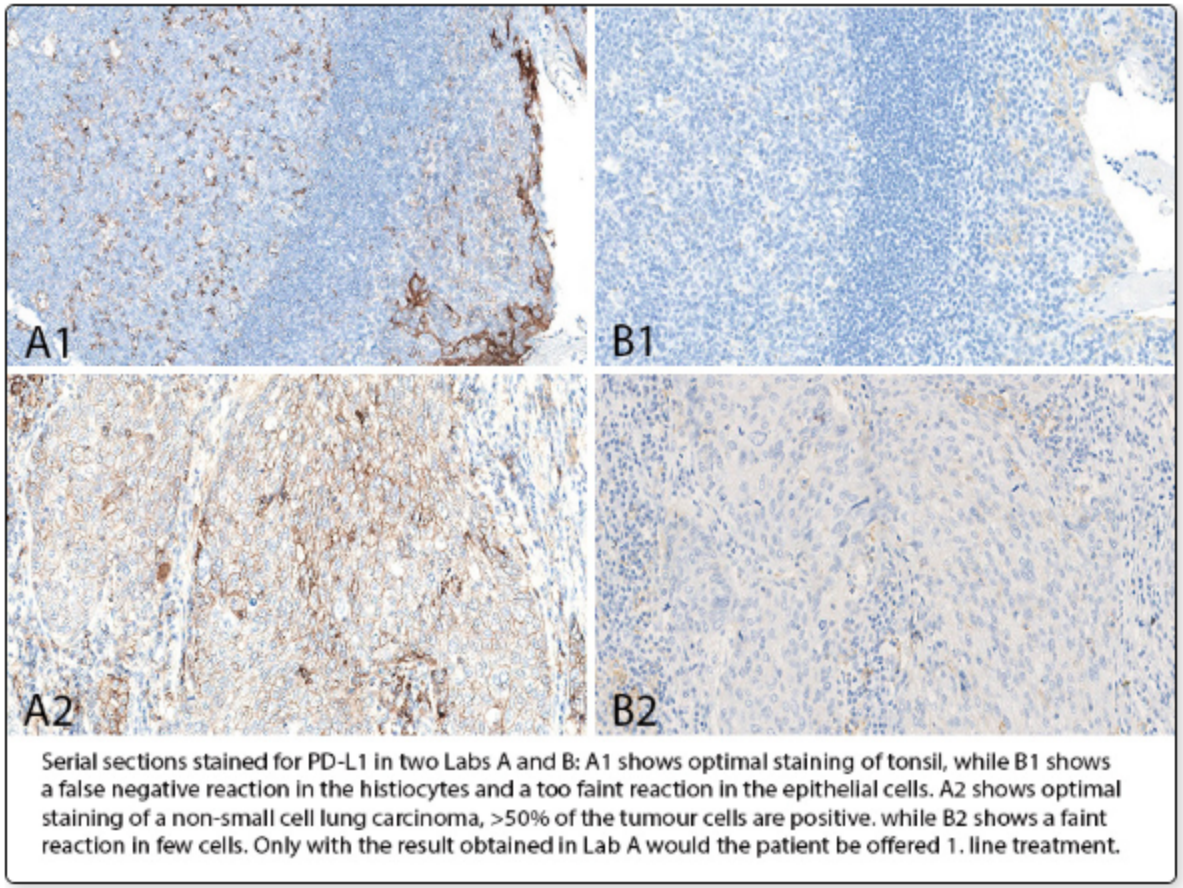
Free PMC Article

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

Mogens Vyberg [1,2] · Søren Nielsen [1]

Nordic immunohistochemical Quality Control





Serial sections stained for PD-L1 in two Labs A and B: A1 shows optimal staining of tonsil, while B1 shows a false negative reaction in the histiocytes and a too faint reaction in the epithelial cells. A2 shows optimal staining of a non-small cell lung carcinoma, >50% of the tumour cells are positive. while B2 shows a faint reaction in few cells. Only with the result obtained in Lab A would the patient be offered 1. line treatment.

Results - run 52, B25, H13 & C3

20-Apr-2018

The general results for the runs **52**, **B25**, **H13** & **C3** are available on the website. Individual results are available after logging in.

All news

Events

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

NordiQC Workshop in Diagnostic Immunohistochemistry
19–21 Sep 2018: Aalborg, DK

Academy of Immunohistochemistry
10–12 Oct 2018: Krakow, Poland

Important dates

Run 53
Publication of results
9 Jul 2018

Jobs

Scientist, IHC
Peter MacCullum Cancer Centre, Australia

Questions

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

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

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

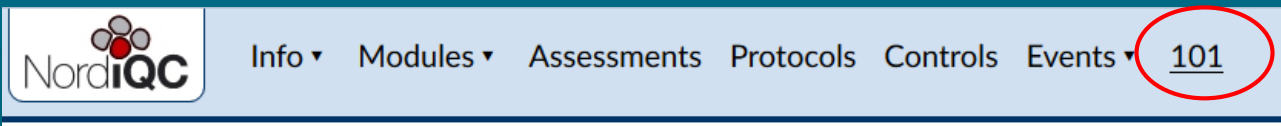
Epitope retrieval, HIER	(•) YES ( ) NO
Device	On Board / On Machine
HIER buffer	Ventana - Ultra CC1 (950-224)
Efficient Heating Time (minutes)	48
Max. heating temperature (Celcius)	99

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

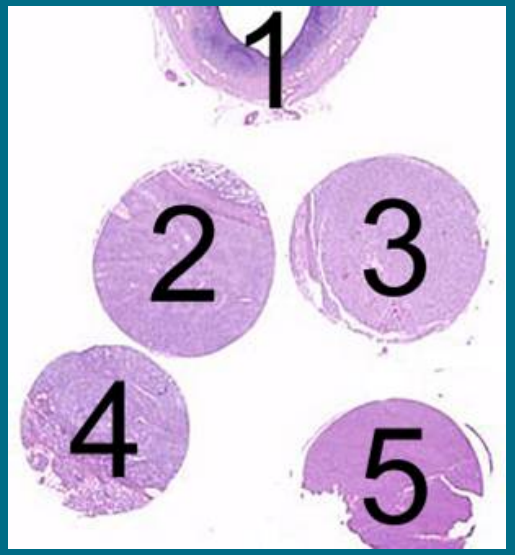
Test material

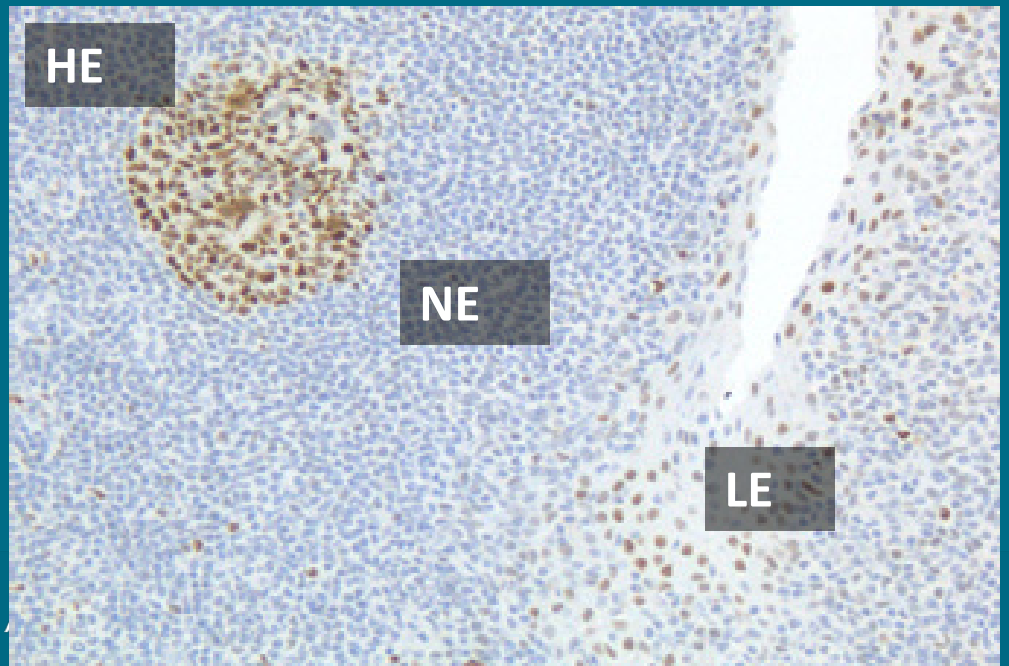
The slide to be stained for **Bcl-6** comprised:
1-2. Tonsils, 24 h/48 h

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.

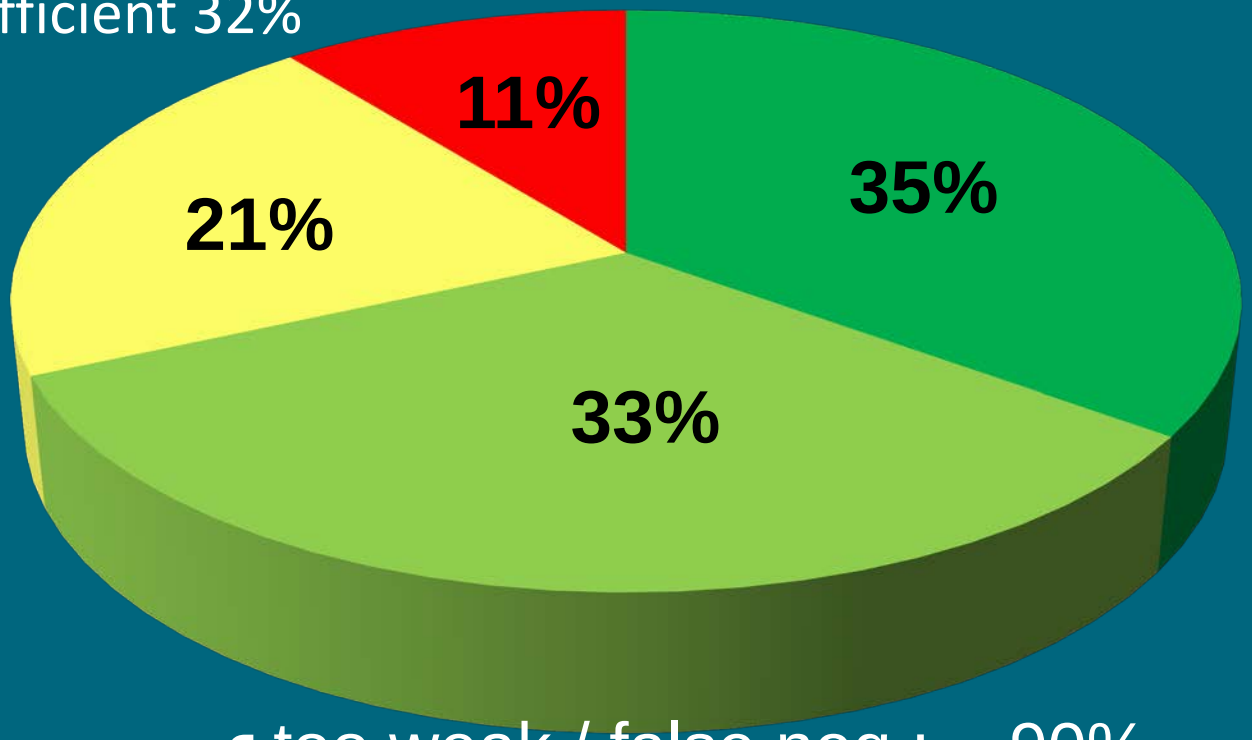
Marker	CD23	CR	CyD1	Ki67	Podop	TTF1
Assessment:	Poor	Optimal	Optimal	Good	Good	Borderline
Comments to the protocol:	False negative	-	-	Excessive counterstain	Weak	Weak*
Suggestions for improvement:	Consider change of primary Ab and recalibrate	-	-	-	-	Increase primary Ab conc. and/or prolong HIER

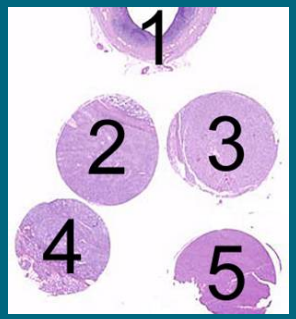
* Please read the epitope description and assessment summary carefully, as the choice of the Ab clone will influence the sensitivity and specificity.

NordiQC assessment results 2006 – 2015

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

Insufficient 32%



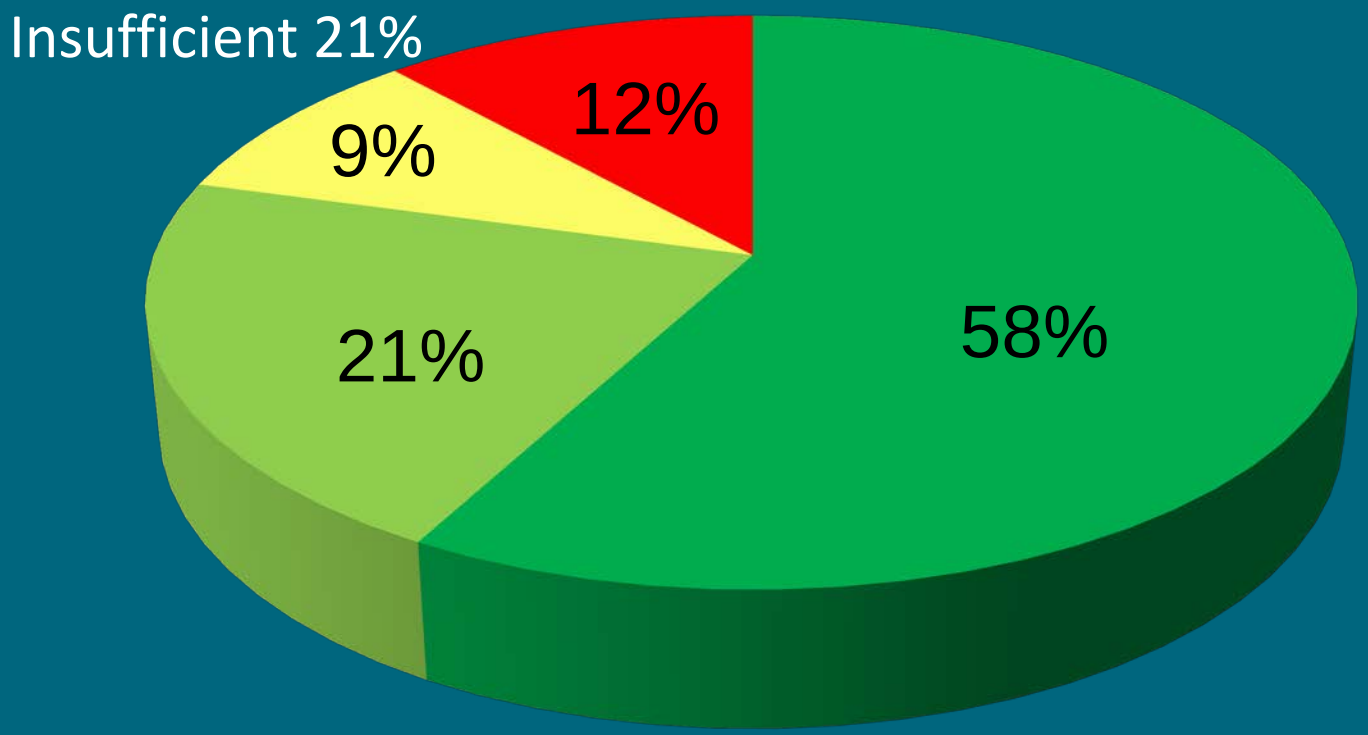


- Optimal
- Good
- Borderline
- Poor

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 Assay Performance Controls (CAPCs)

- essential to evaluate sensitivity
- essential to assure consistency

Normal tissues - easier to 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

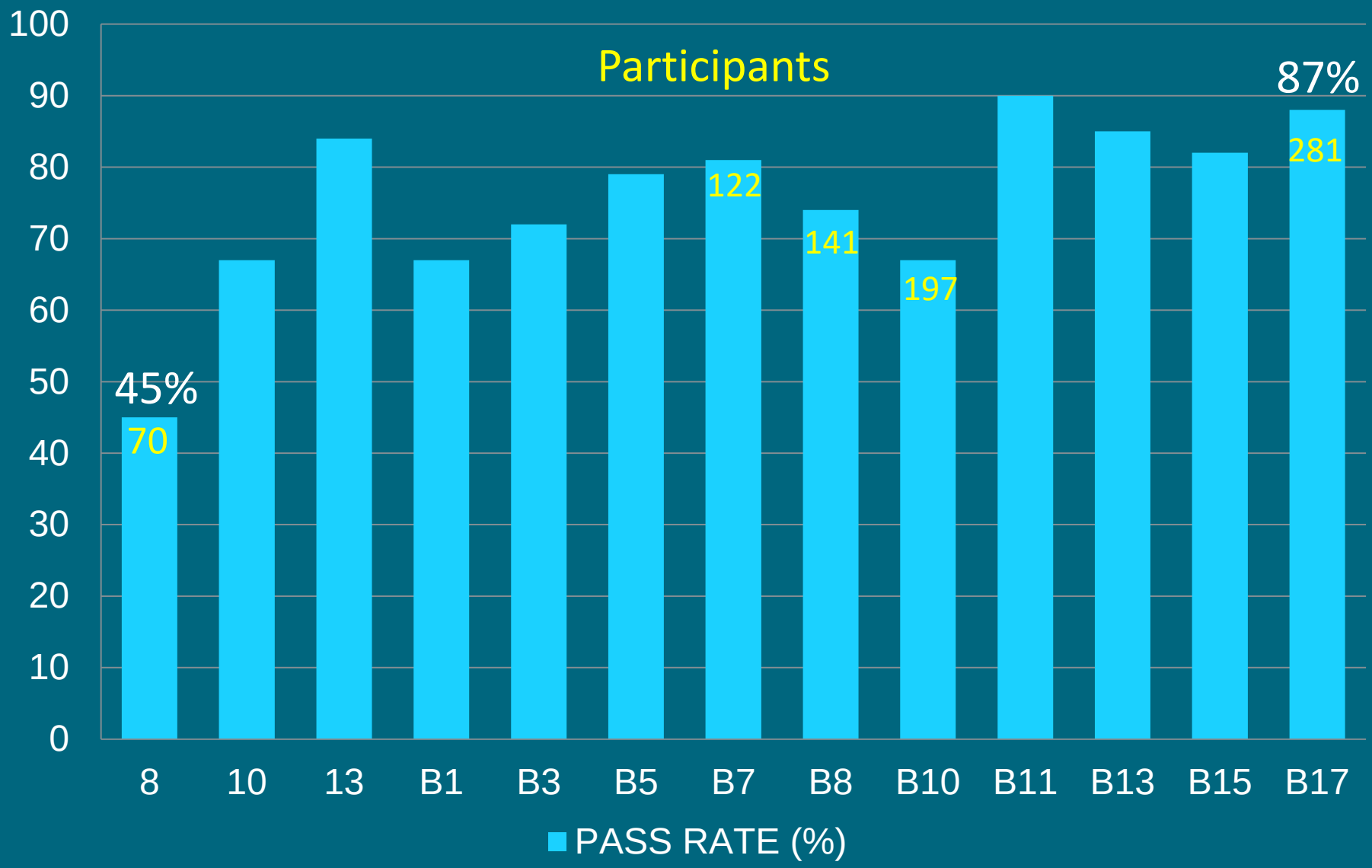
AIMM 2016-17

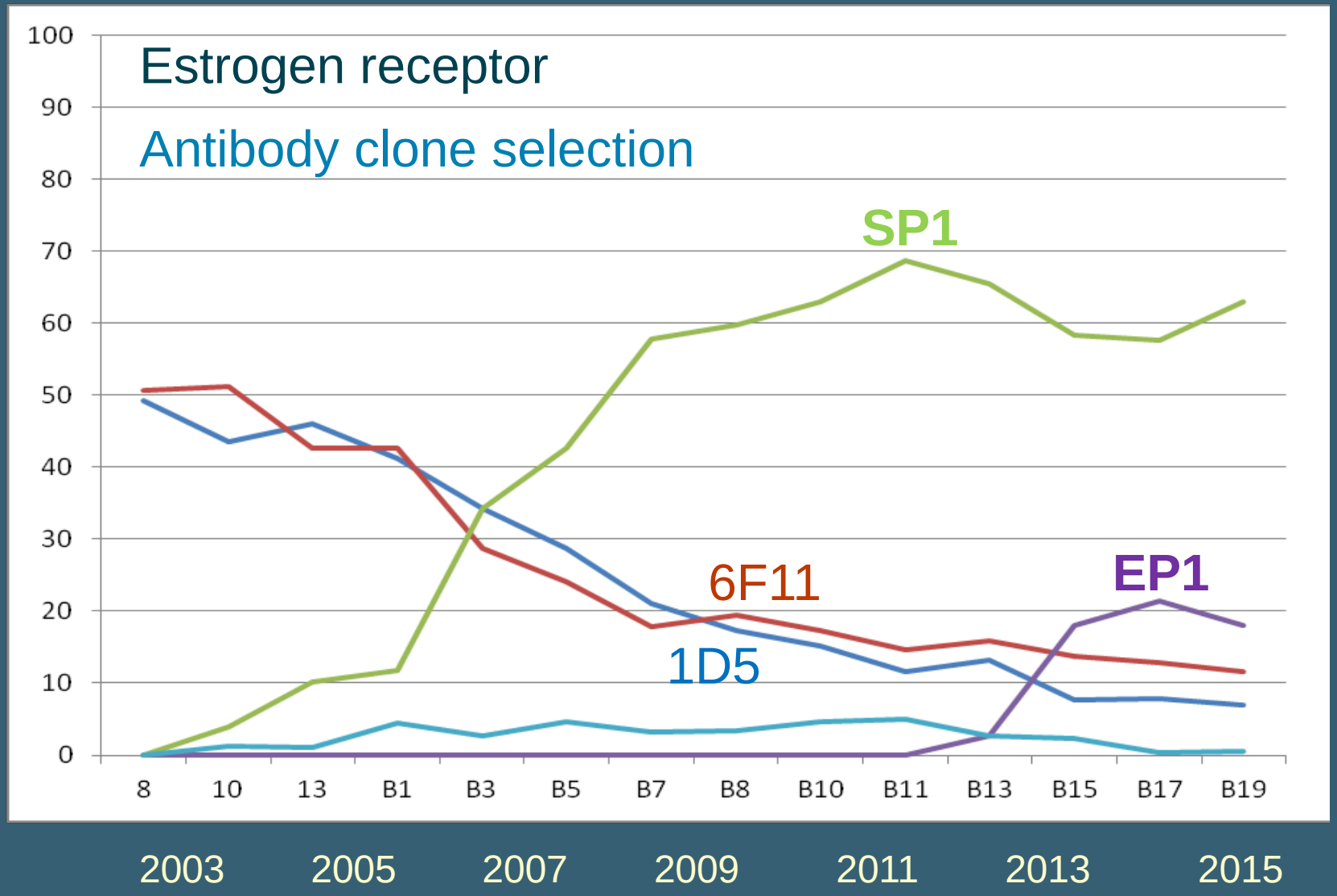
Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine – Part 2: Immunohistochemistry Test Performance Characteristics

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

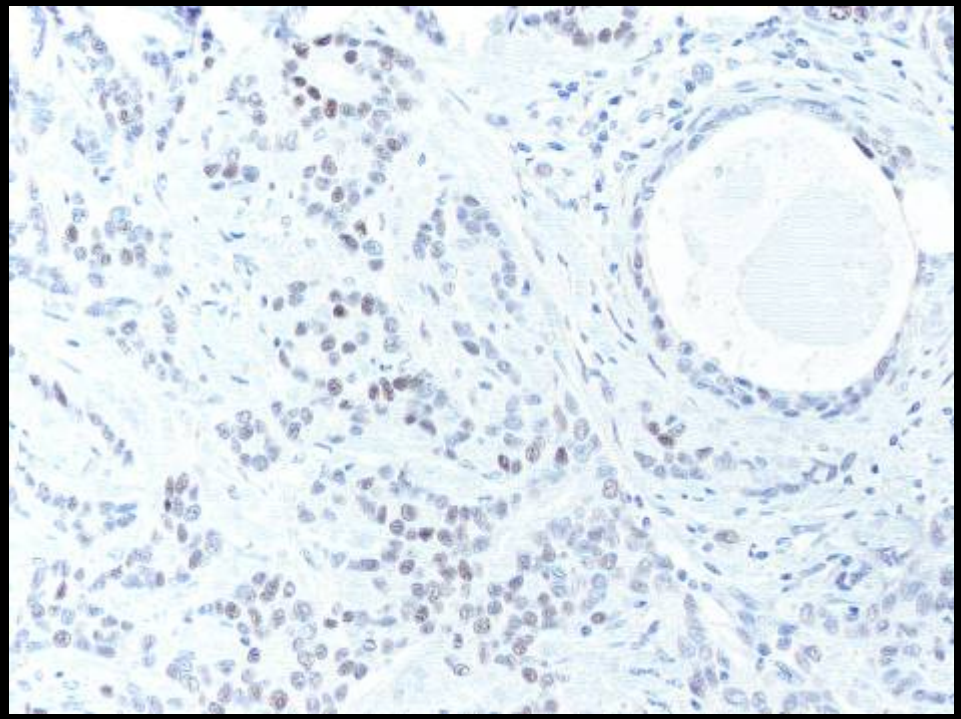
NordiQC EQA: Estrogen Receptor in 13 runs



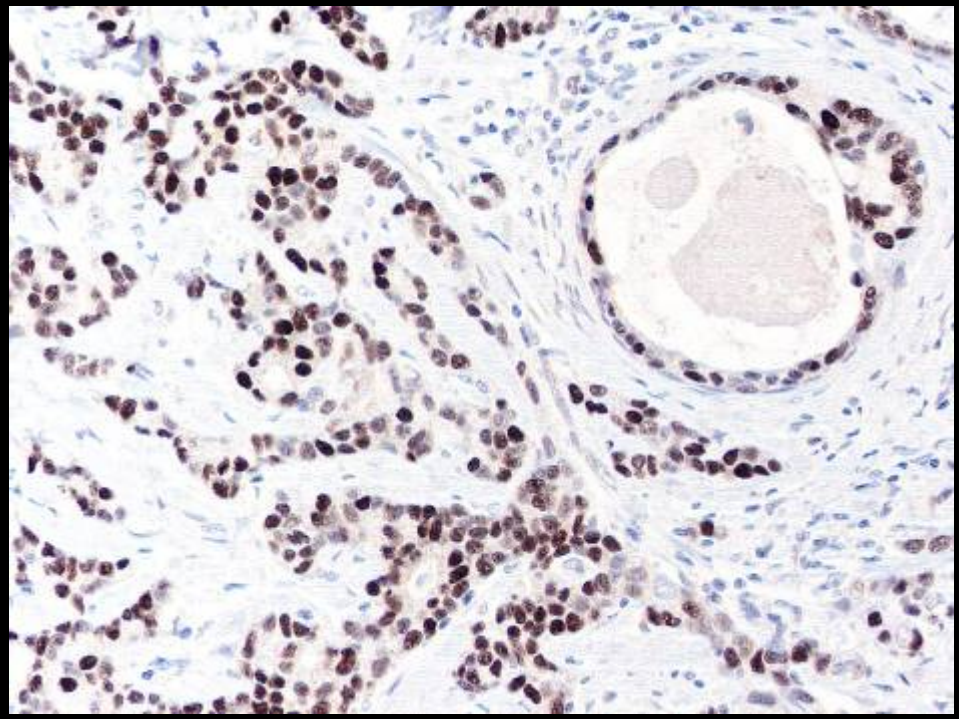

Estrogen receptor
Antibody clone selection
SP1
6F11
EP1
1D5
100
90
80
70
60
50
40
30
20
10
0
8
10
13
B1
B3
B5
B7
B8
B10
B11
B13
B15
B17
B19
2003
2005
2007
2009
2011
2013
2015

IHC – Optimal performance

ER 1D5 1:100
HIER Ci pH 6



2-step polymer



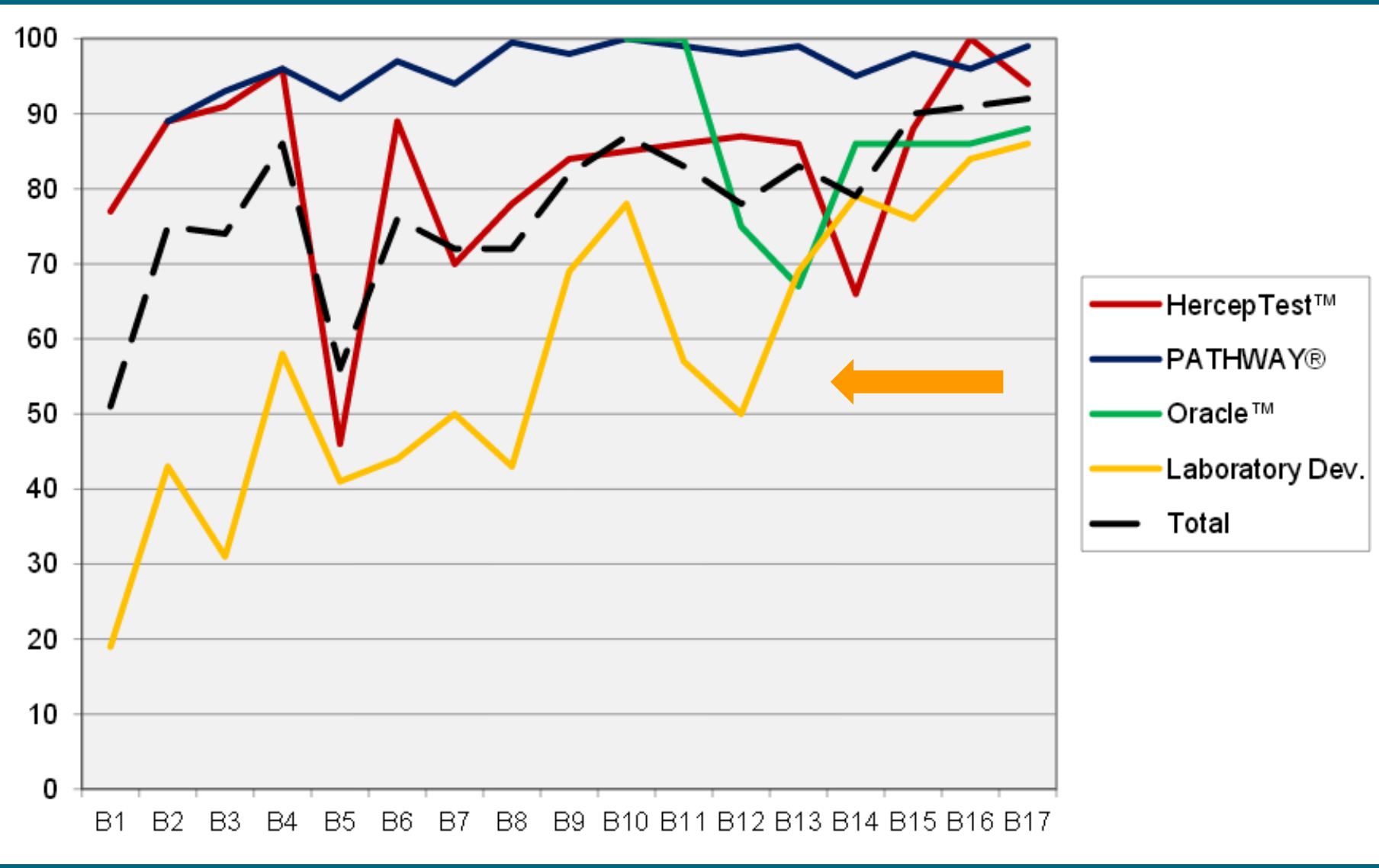
3-step polymer

Results of NordiQC recommendations

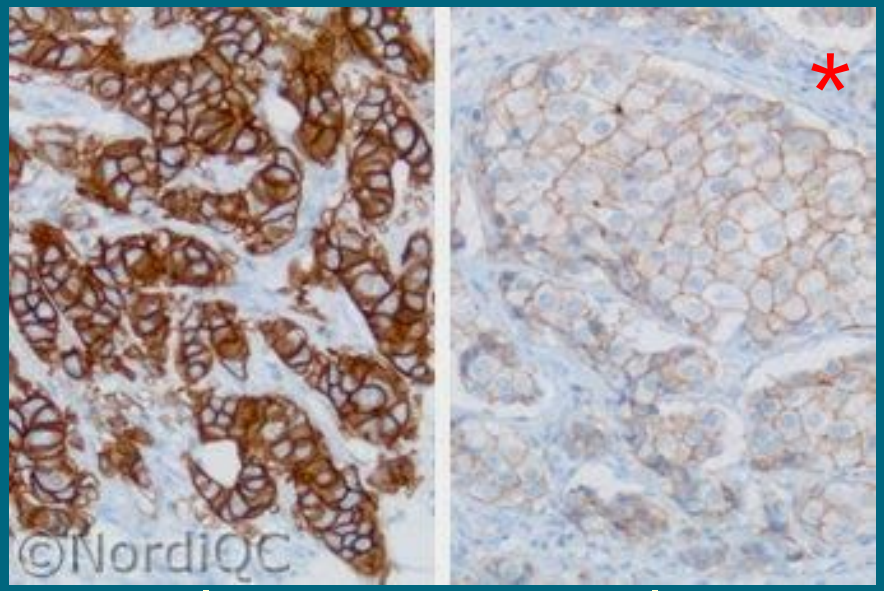
Pass rate (optimal + good) by participant status

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

HER-2 staining results in 17 runs

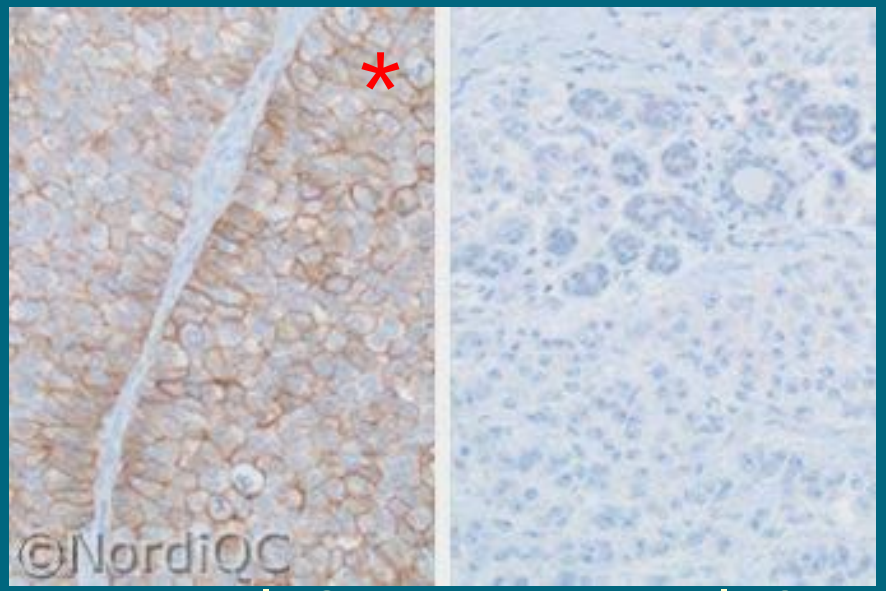


NordiQC runs for HER2 IHC

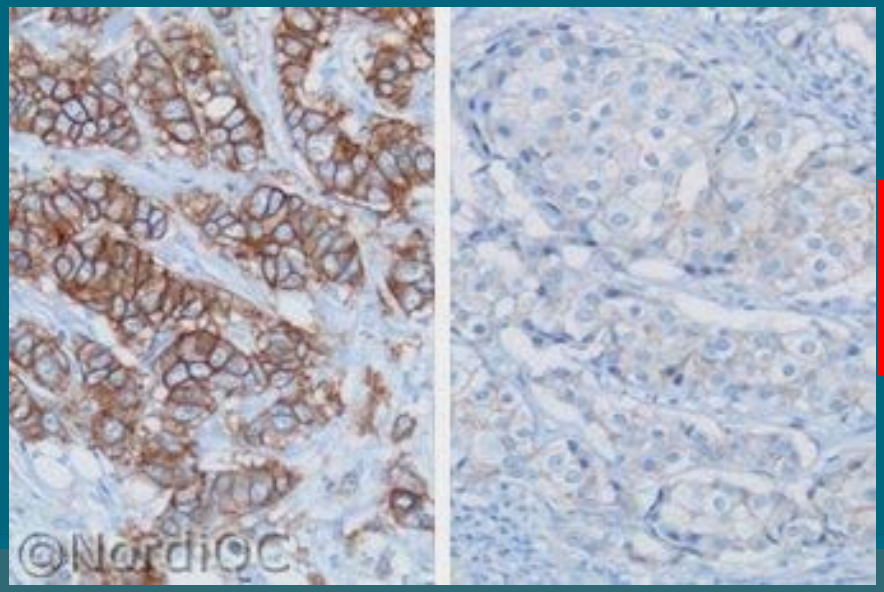


Ampl. 3+ Ampl. 2+

Optimal

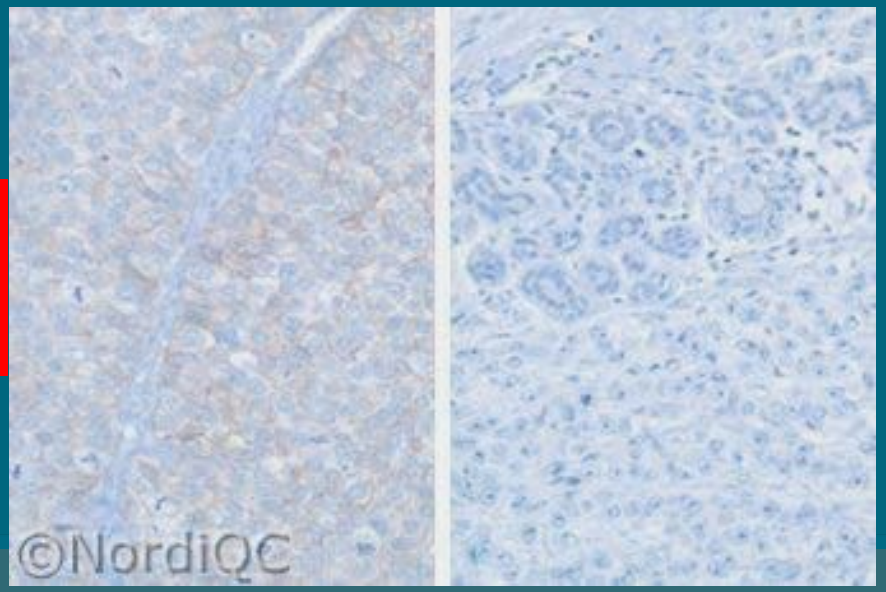


Unampl. 2+ Unampl. 0



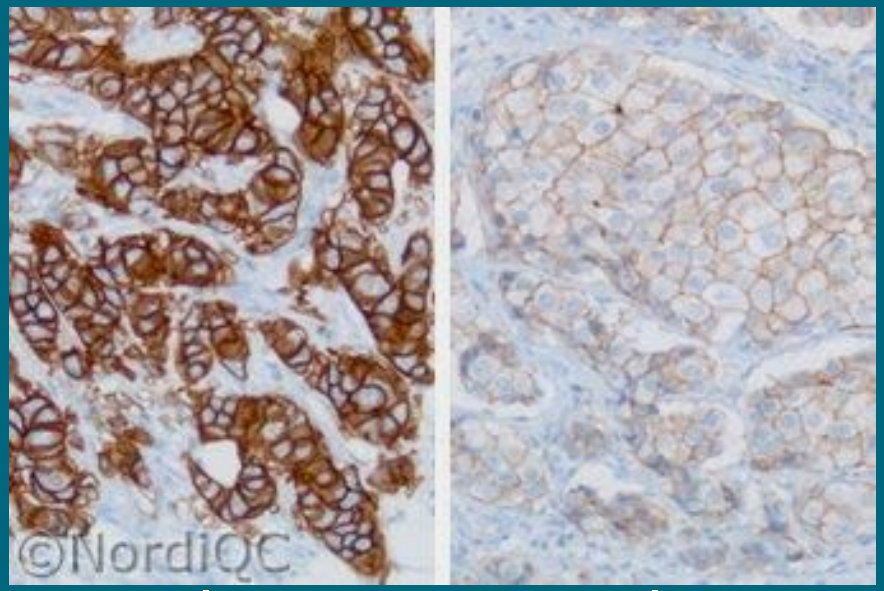
Ampl. 3+ Ampl. 1+

Poor



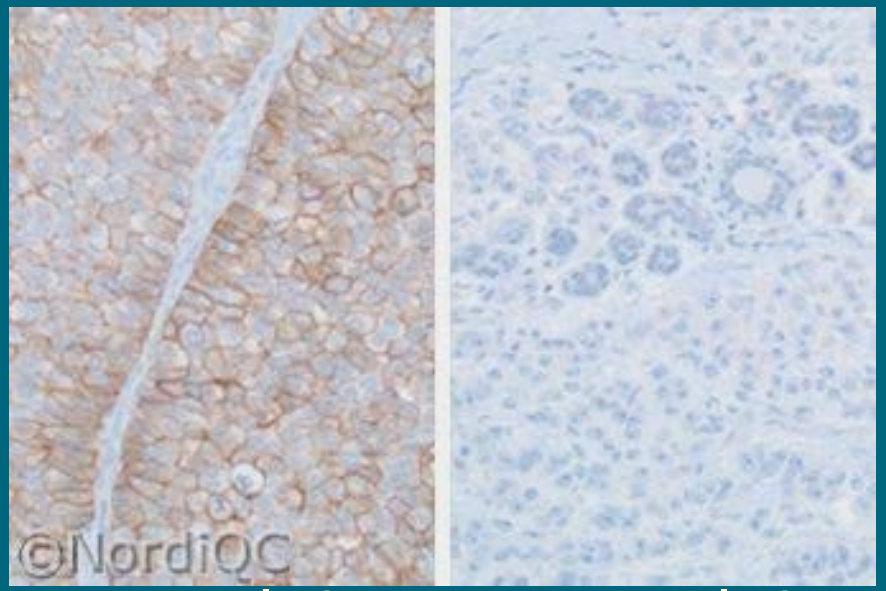
Unampl. 1+ Unampl. 0

NordiQC runs for HER2 IHC

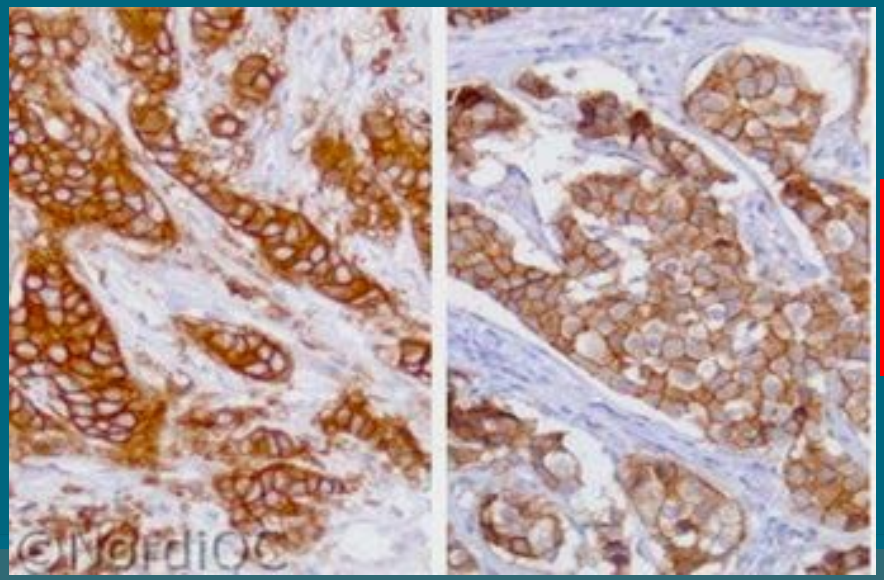


Ampl. 3+ Ampl. 2+

Optimal

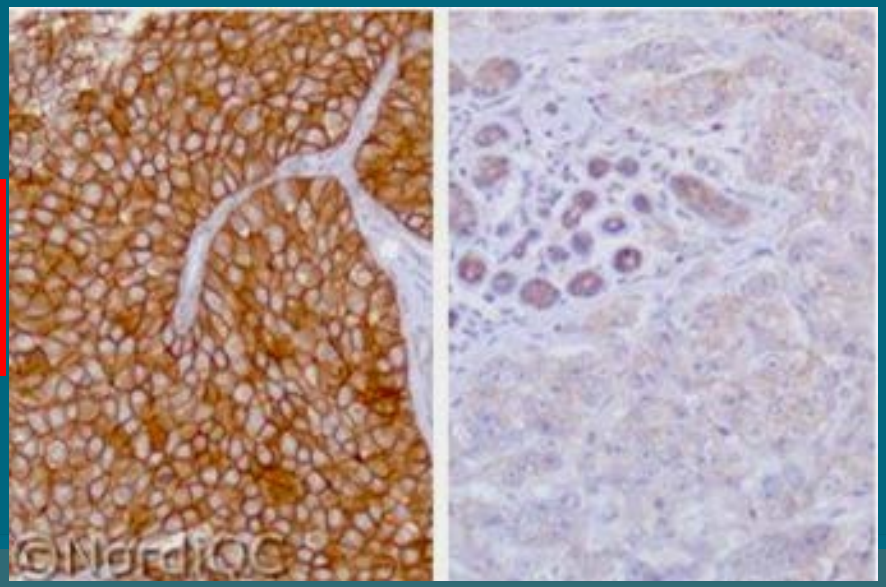


Unampl. 2+ Unampl. 0



Ampl. 3+ Ampl. 2+

Poor



Unampl. 3+ Unampl. 1

NordiQC runs for HER2 IHC

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

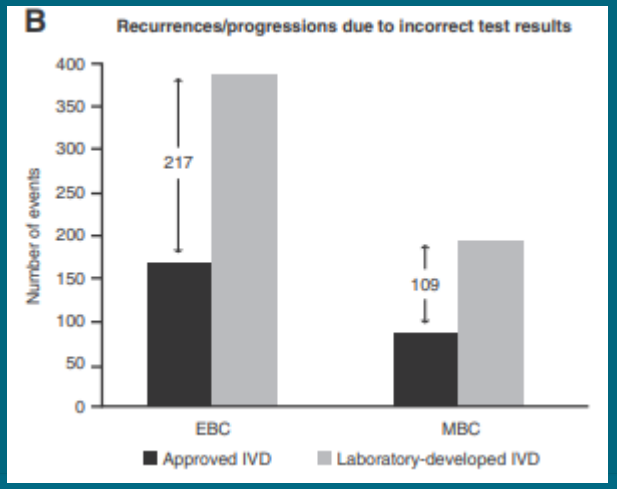
BMC Health Services Research

RESEARCH ARTICLE Open Access

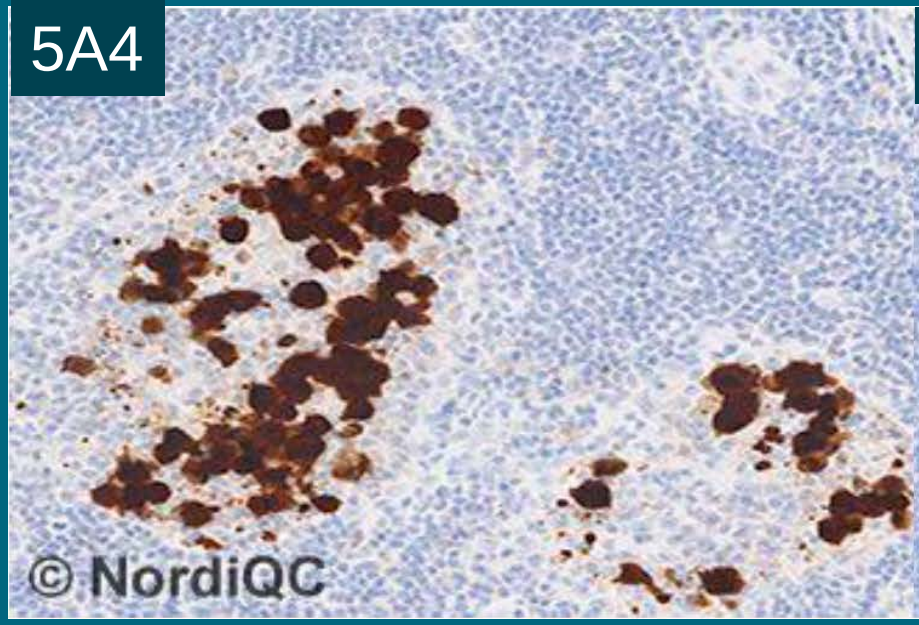
Immunohistochemical expression of HER2 in breast cancer: socioeconomic impact of inaccurate tests

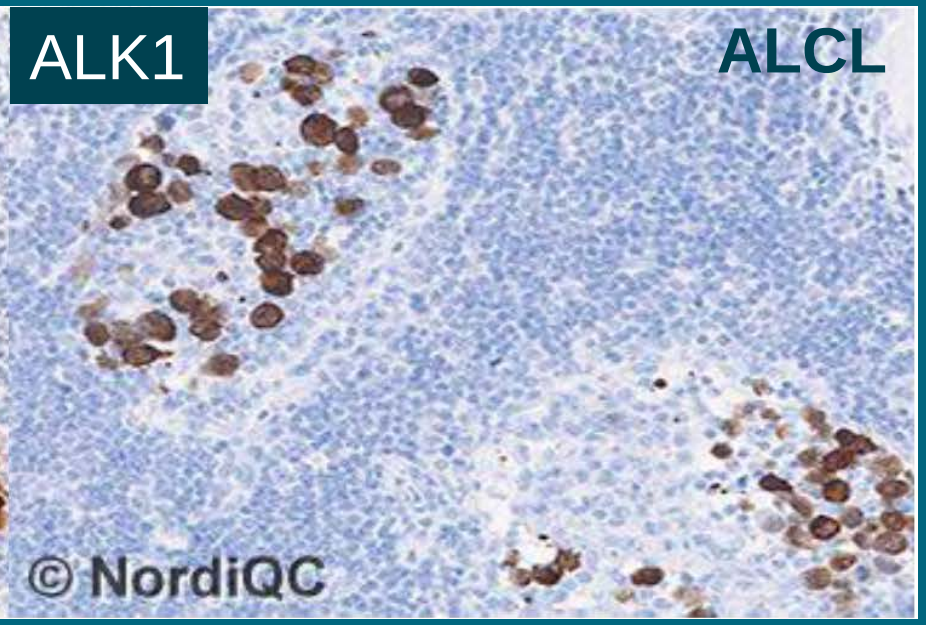
Mogens Vyberg[1*], Søren Nielsen[1], Rasmus Røge[1], Beth Sheppard[2], Jim Ranger-Moore[2], Eric Walk[2], Juliane Gartemann[3], Ulrich-Peter Rohr[3] and Volker Teichgräber[3]

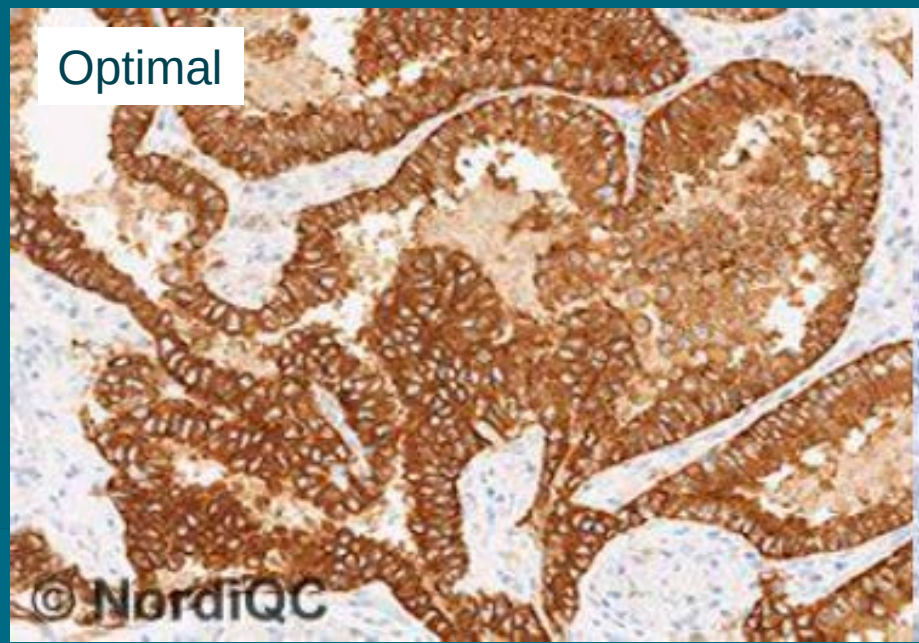
Every $1 saved by laboratories by using cheaper reagents could potentially result in approximately $6 additional costs to the healthcare system.

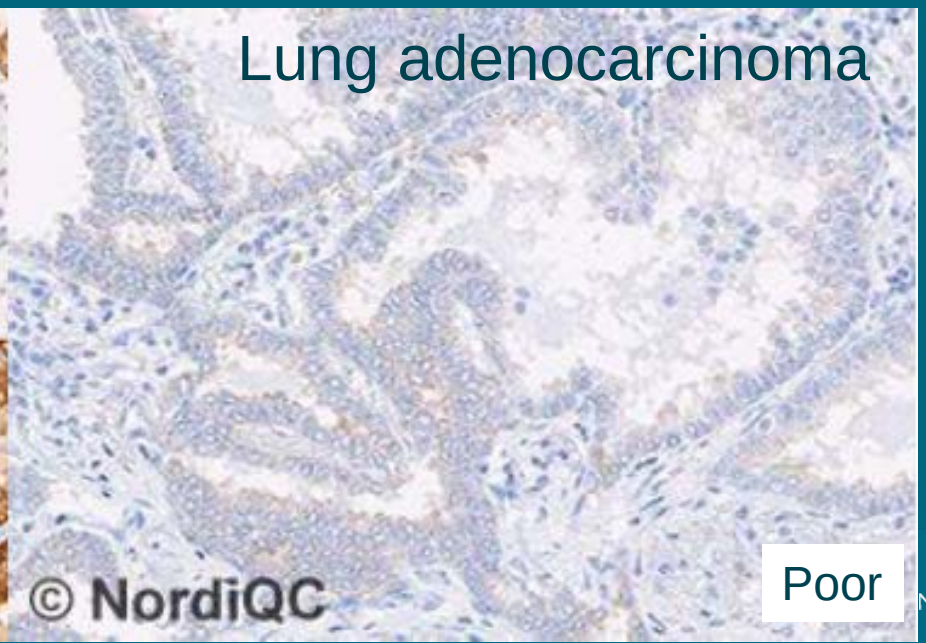


Lung ALK


5A4
© NordiQC


ALK1
ALCL
© NordiQC

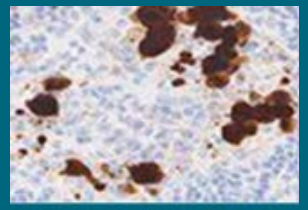

Optimal
© NordiQC

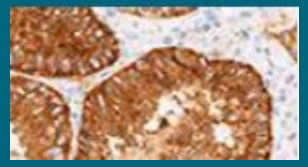

Lung adenocarcinoma
© NordiQC
Poor

Lung ALK

The immunoassay must fit for the purpose:

- Identify the antibody useful for the specific task



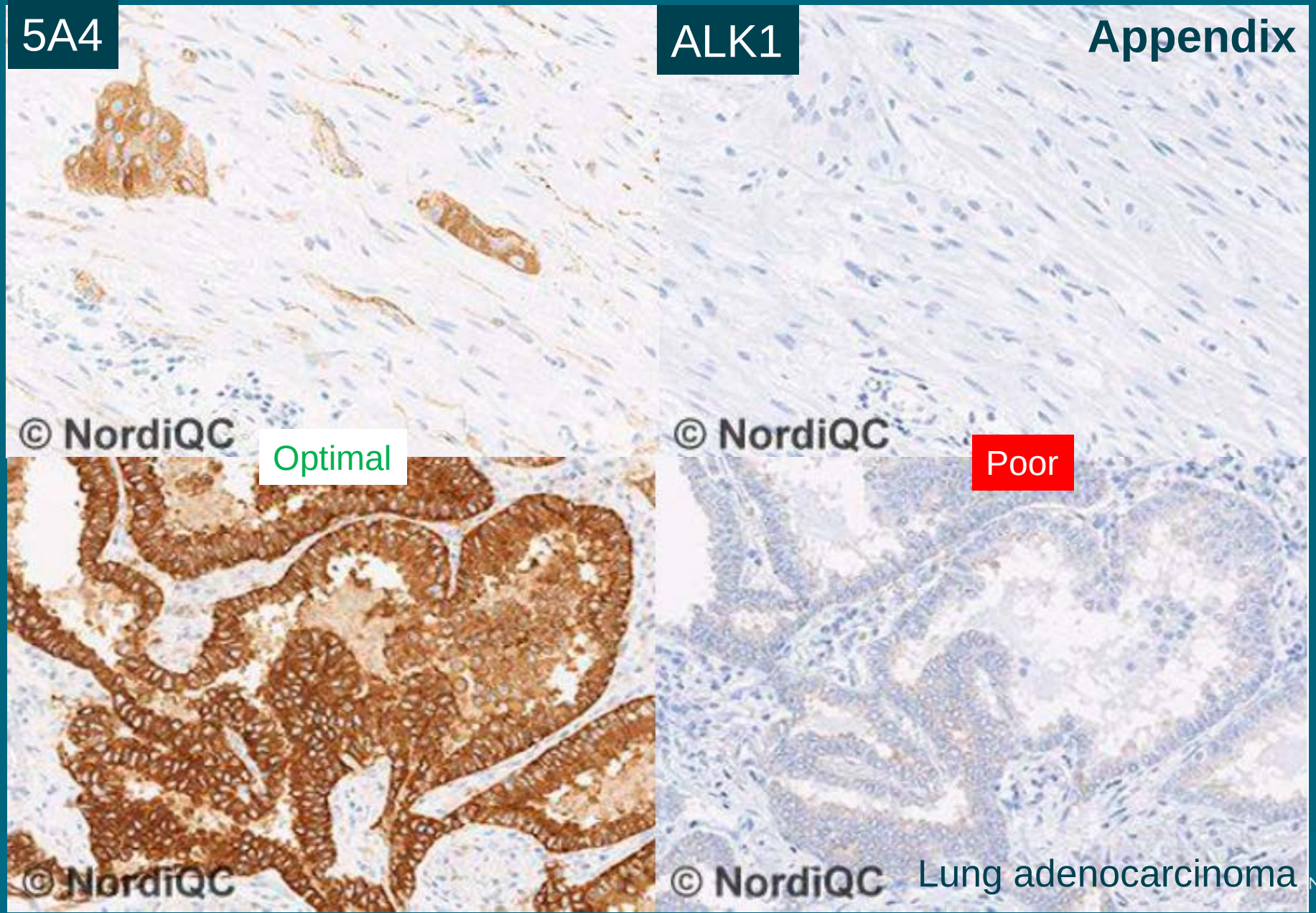


The right external controls must be used:

- 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



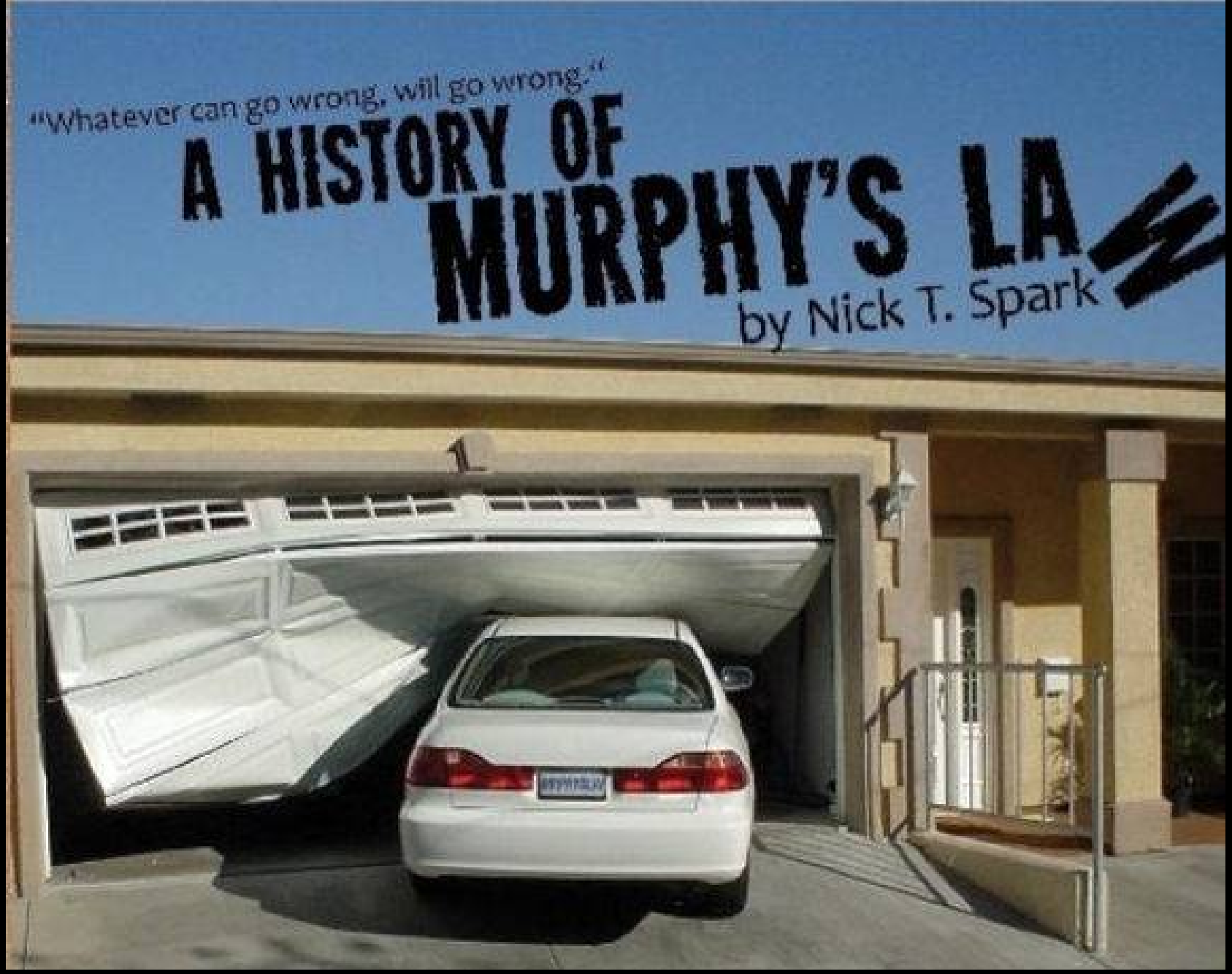
Results of NordiQC recommendations

419 advices for 11 markers

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

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 ?



When you believe in automation and stop thinking

Workshop in Diagnostic Immunohistochemistry
Oud St. Jan/ Old St. John – Brugge (Bruges), Belgium June 13th – 15th 2018

NordiQC External Quality Assurance in Immunohistochemistry

Mogens Vyberg
Professor of Clinical Pathology
Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark

NORTH DENMARK REGION