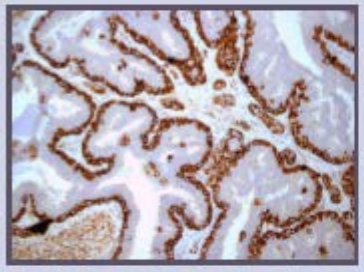
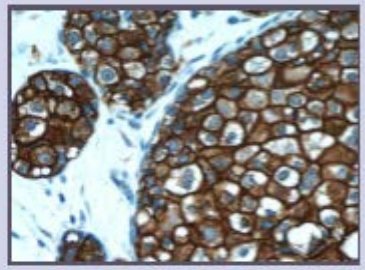




NordiQC

TATA MEDICAL CENTER

ISIMm
International Society for IHC and Molecular Morphology

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

QA of Prognostic / predictive markers in breast pathology

Søren Nielsen
Global Pathology Manager
Agilent Technologies

(Former Scheme Manager, NordiQC)

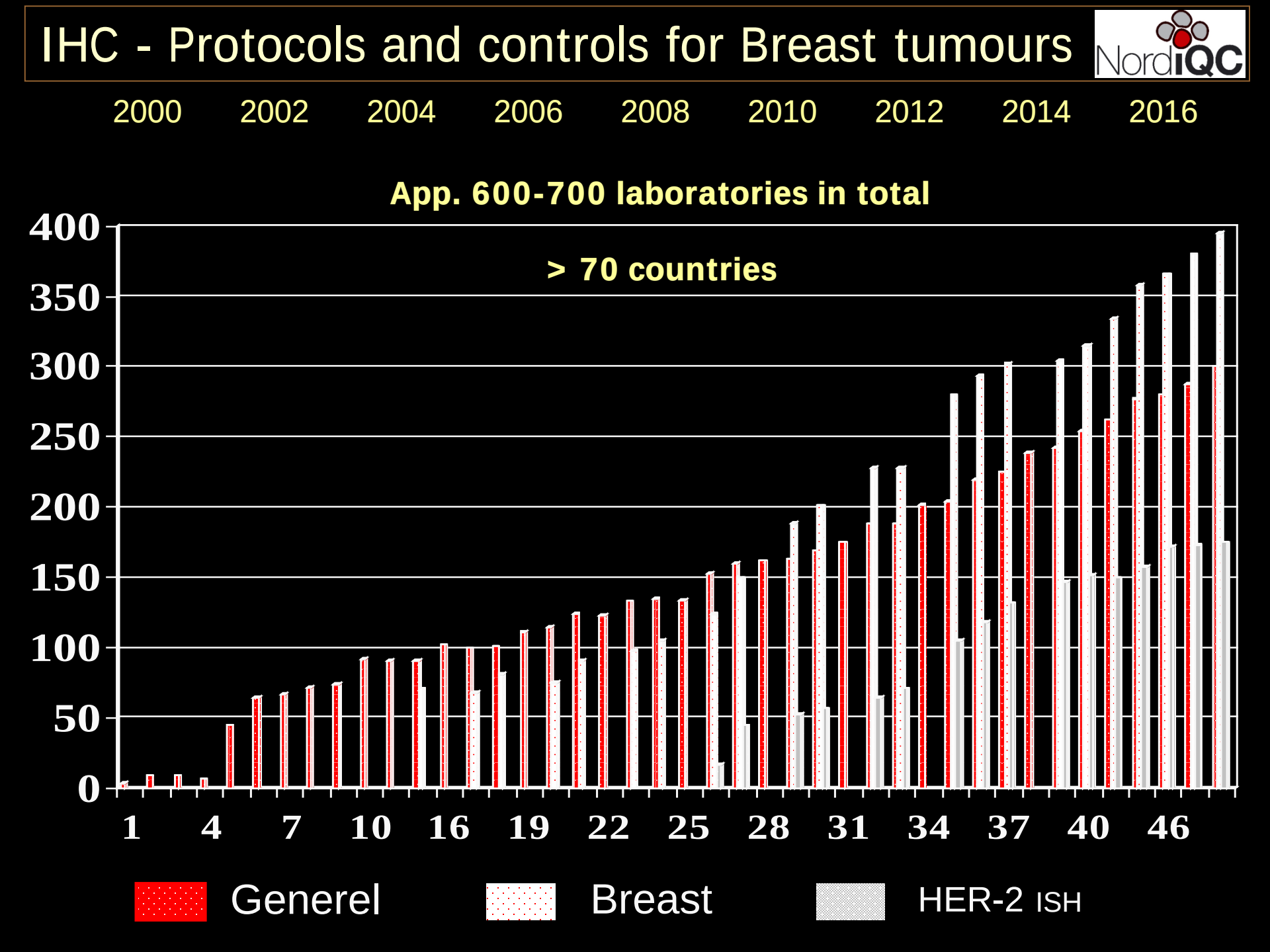
IHC - Protocols and controls for Breast tumours

Breast panel:

- GCDFP-15
- Mammaglobin
- Gata 3
- Smooth MHCM
- ASMA
- (p63)
- E-cadherin
- p120
- **ER**
- **PR**
- **HER-2**

- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

IHC - Protocols and controls for Breast tumours



IHC - Protocols and controls for Breast tumours

NordiQC

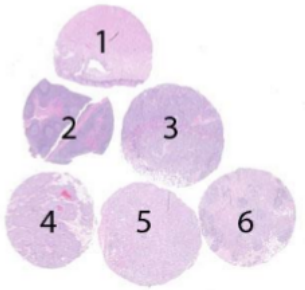
Assessment Run B23 2017

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	2 - 5%	Weak to strong
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	90 - 100%	Moderate to strong
5.	Breast carcinoma	50 - 70%	Weak to moderate
6.	Breast carcinoma	60 - 80%	Weak to moderate



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

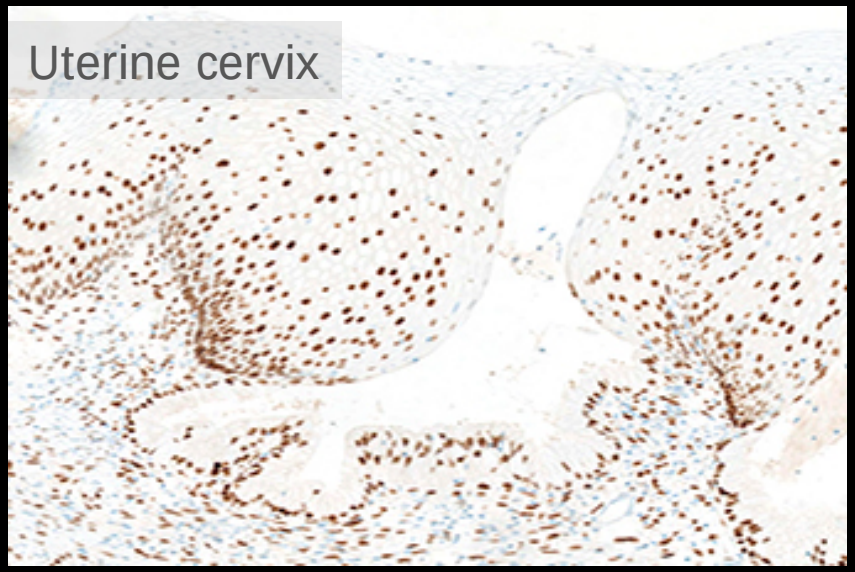
All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Yaziji et al. (1).

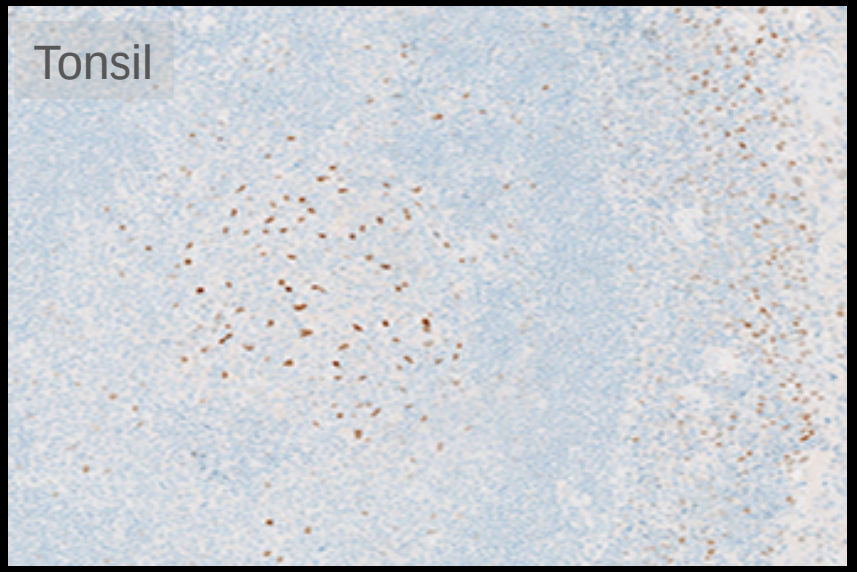
Focus:

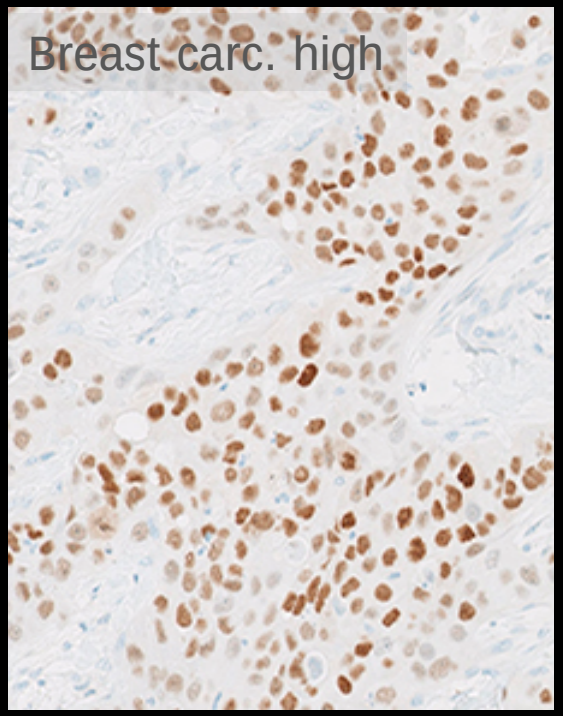
Appropriate technical quality; signal-to-noise, morphology etc

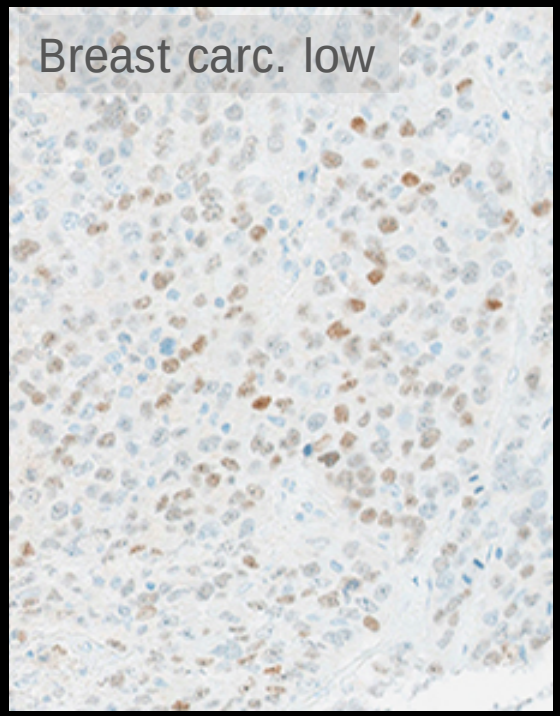
Appropriate analytical sensitivity and specificity – indicated by concordance of ER status in the included tumours to reference

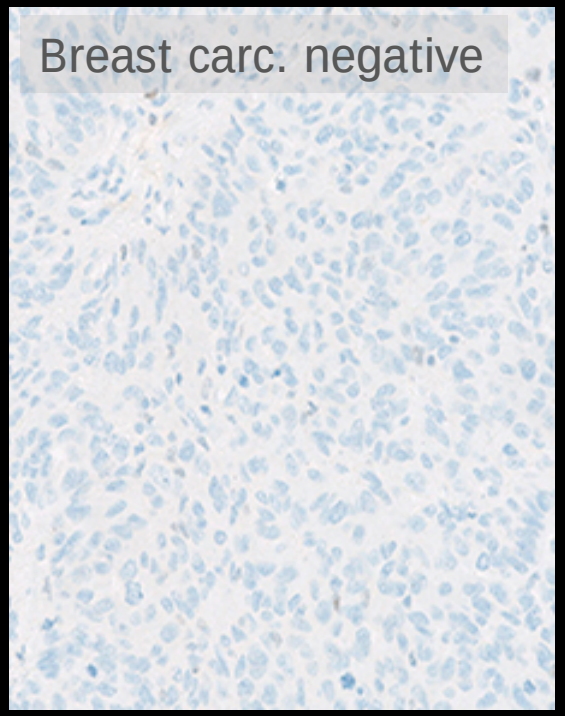
IHC - Protocols and controls for Breast tumours









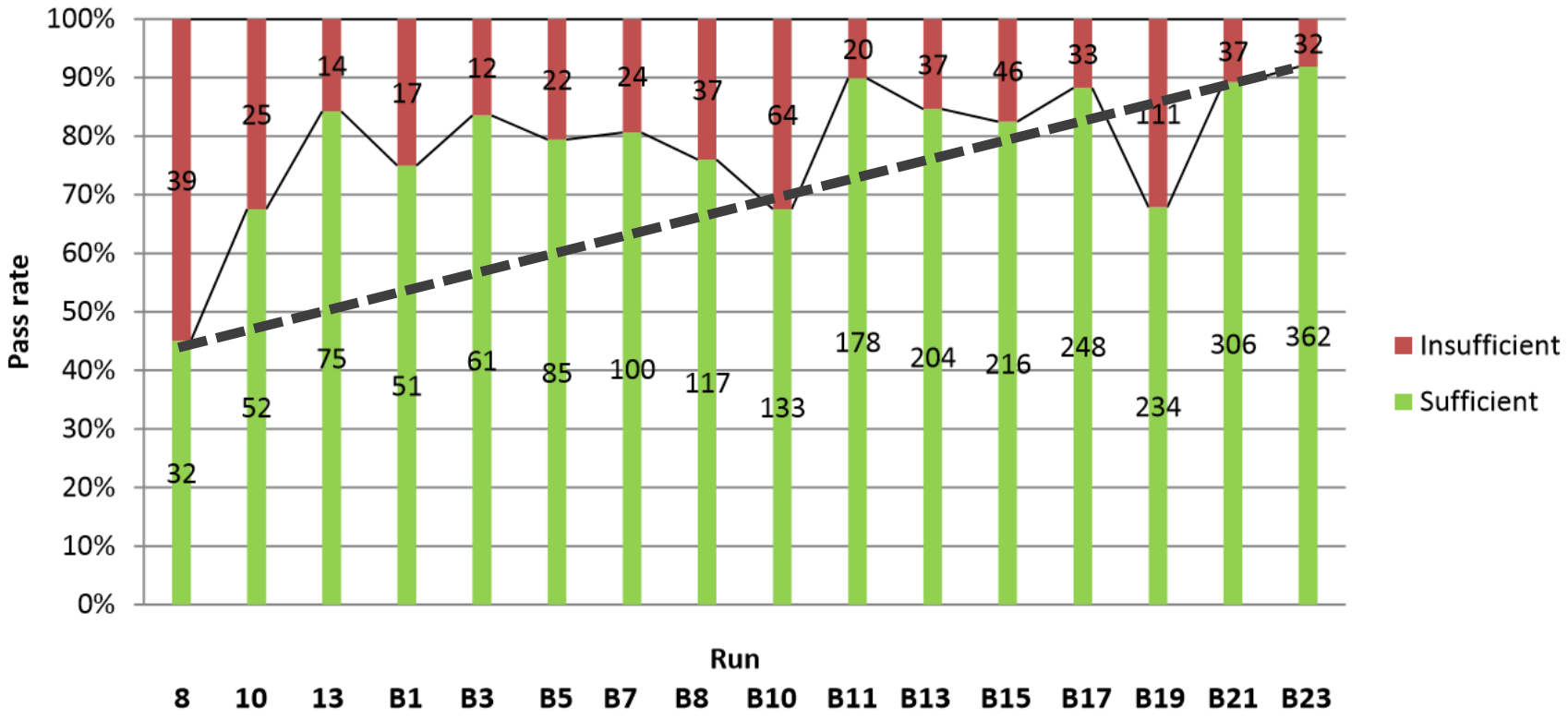


IHC - Protocols and controls for Breast tumours

Performance history

This was the sixteenth NordiQC assessment of ER. The proportion of sufficient results was similar compared to the latest run (see Figure 1).

Fig. 1. **Participant numbers and pass rates for ER during 16 runs**

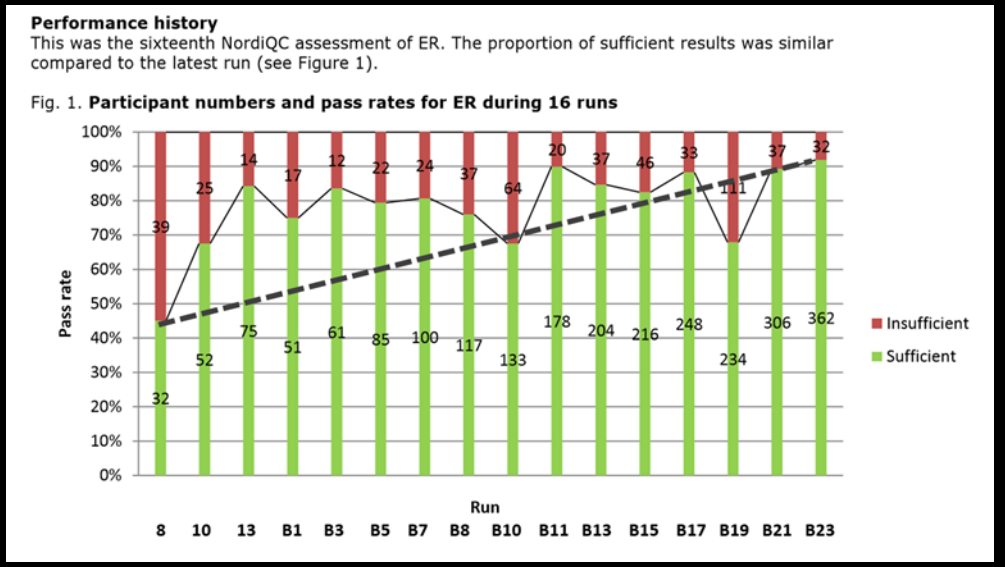


2003 2005 2007 2009 2011 2013 2015 2017

IHC - Protocols and controls for Breast tumours

Estrogen receptor;

Pass rate influenced by participation



	New participants	”Old” participants
Run B10, 2004	57% (n=61)	71% (n=134)
Run B15, 2010	70% (n=54)	86% (n=208)
Run B19, 2015	51% (n=86)	73% (n=259)

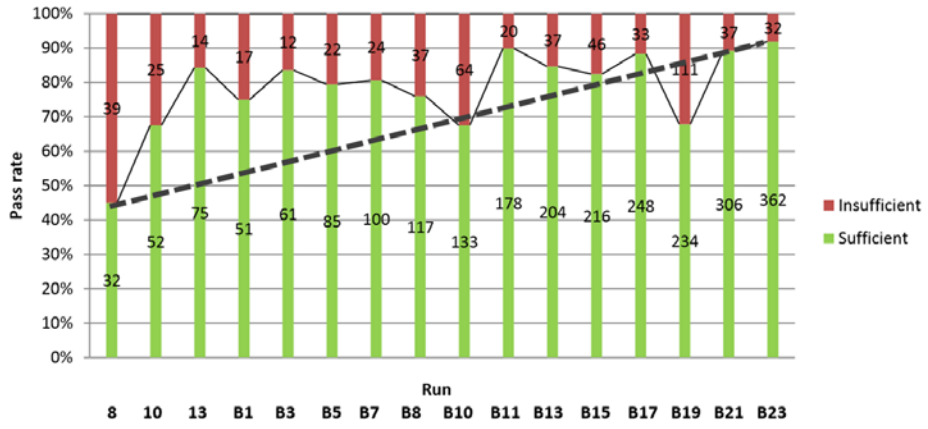
NordiQC

Estrogen receptor;

Pass rate influenced by protocol harmonization and availability of fully automated IHC systems

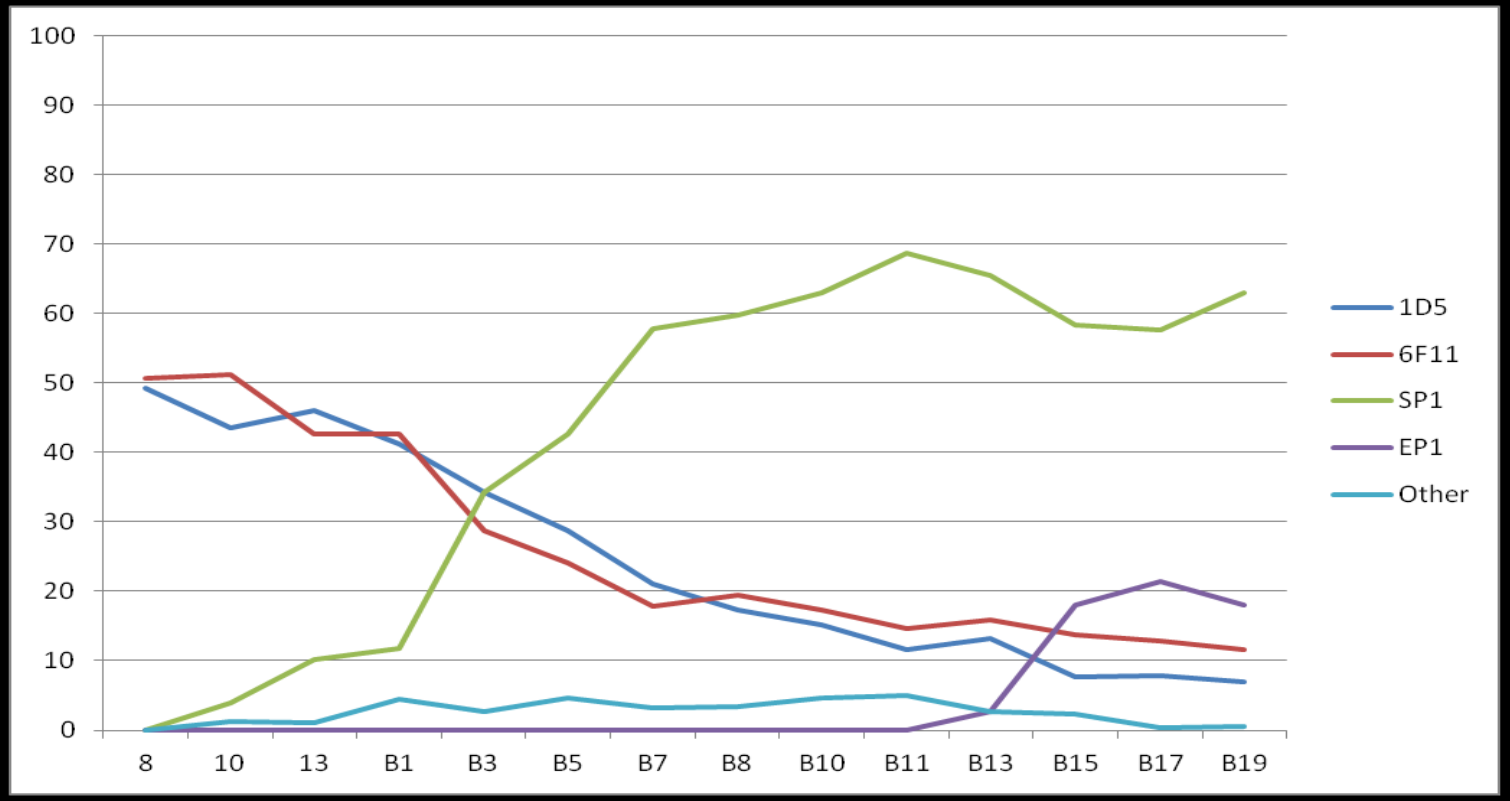
Performance history
This was the sixteenth NordiQC assessment of ER. The proportion of sufficient results was similar compared to the latest run (see Figure 1).

Fig. 1. **Participant numbers and pass rates for ER during 16 runs**



	2003 B8	2017 B23
Ready-To-Use format	21%	81%
HIER by in-house buffer	88%	5%
HIER by high pH	70%	94%
Polymer/multimer kit	56%	97%
Fully automated system	6%	78%

IHC - Protocols and controls for Breast tumours



VOLUME 24 · NUMBER 36 · DECEMBER 20 2006

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Immunohistochemical Detection Using the New Rabbit Monoclonal Antibody SP1 of Estrogen Receptor in Breast Cancer Is Superior to Mouse Monoclonal Antibody 1D5 in Predicting Survival

Maggie C.U. Cheang, Diana O. Treaba, Caroline H. Speers, Ivo A. Olivotto, Chris D. Bajdik, Stephen K. Chia, Lynn C. Goldstein, Karen A. Gelmon, David Huntsman, C. Blake Gilks, Torsten O. Nielsen, and Allen M. Gown

EP1: a novel rabbit monoclonal antibody for detection of oestrogen receptor α

Sunil Badve,[1] I Tudor Vladislav,[1] Betsy Spaulding,[2] Anna Strickland,[2] Sylvia Hernandez,[1] Lisa Bird-Turner,[1] Cecelia Dodson,[1] Bjorn Elleby,[2] Therese Phillips[2]

J Clin Pathol 2013;**66**:1051–1057.

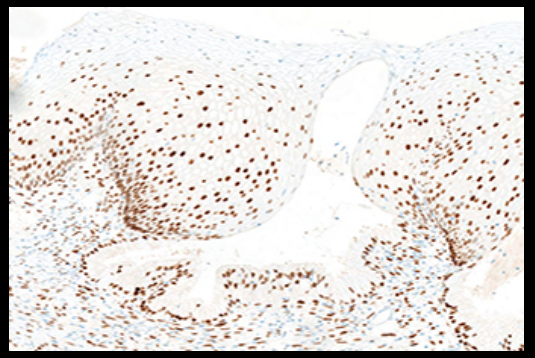
IHC - Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for ER, run B23**

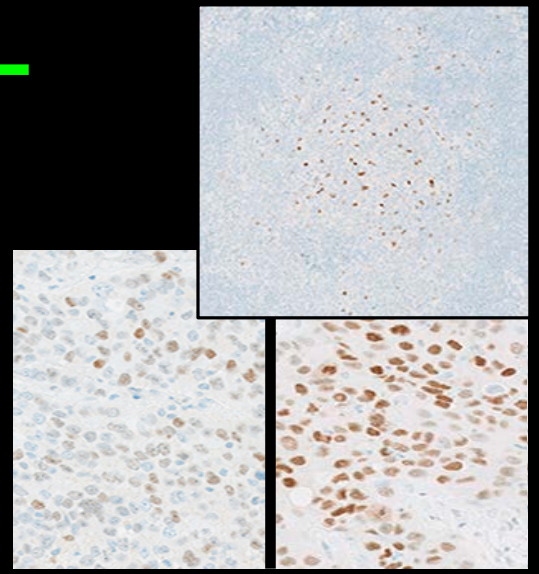
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1D5**	1	Dako/Agilent	0	0	0	1	-	-
mAb clone **6F11**	22 1	Leica/Novocastra Celnovte	10	11	1	1	91%	86%
rmAb clone **EP1**	15 1	Dako/Agilent Cell Marque	6	5	5	0	69%	89%
rmAb clone **SP1**	30 3 3 1	Thermo/Neomarkers Cell Marque Spring Bioscience Immunologic	27	7	2	1	92%	97%
Ready-To-Use antibodies								
mAb clone **1D5 IR/IS657**	4	Dako/Agilent	0	2	2	0	-	-
mAb clone **1D5 BMS008**	1	Zytomed	0	0	1	0	-	-
mAb clones **1D5 + ER-2-123 SK310**	2	Dako/Agilent	0	1	0	1	-	-
mAb clone **6F11 PA0009/PA0151**	14	Leica	7	6	1	0	93%	100%
rmAb **EP1 IR/IS084**	59	Dako/Agilent	34	21	3	1	93%	95%
rmAb **EP1 GA084**	16	Dako/Agilent	12	3	1	0	94%	92%
rmAb **EP1 AN710-5M**	1	Biogenex	0	1	0	0	-	-
rmAb **EP1 249R-2**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **SP1 790-4324/5**	209	Ventana/Roche	122	79	7	1	96%	95%
rmAb clone **SP1 249R-1**	3	Cell Marque	0	2	1	0	-	-
rmAb clone **SP1 KIT-0012**	2	Maixin	1	1	0	0	-	-
rmAb clone **SP1 RMPD001**	1	Diagnostic Biosystems	0	0	0	1	-	-
rmAb clone **SP1 ILM30142-R25**	1	Immunologic	1	0	0	0	-	-
rmAb clone **SP1 MAD-000306QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **SP1 M3011**	1	Spring Bioscience	1	0	0	0	-	-
rmAb clone **SP1 RM-9101-R7**	1	Thermo/Neomarkers	1	0	0	0	-	-
Total	394		222	140	25	7	-	
Proportion			56%	36%	6%	2%	92%	

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

HIER alk. pH
2- & 3-step kits
Carefully calib.



Uterine cervix;
all epithelial cells
Tonsil; scattered T-cells



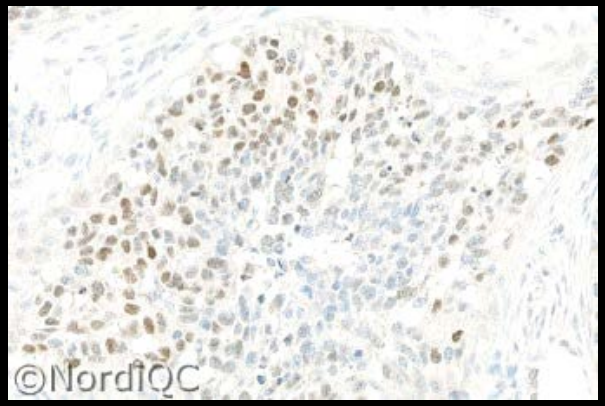
IHC - Protocols and controls for Breast tumours

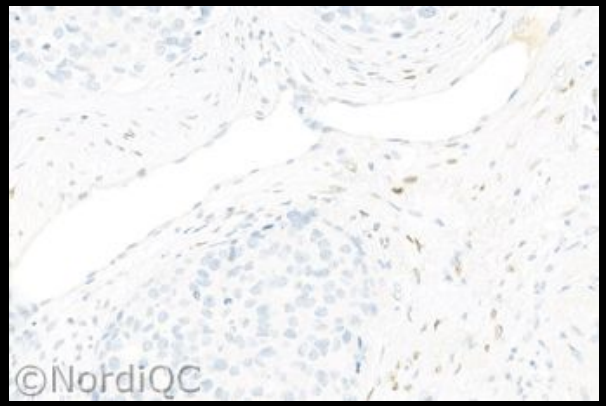
Estrogen receptor;

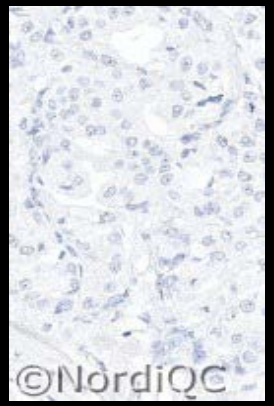
85% Weak / False negative

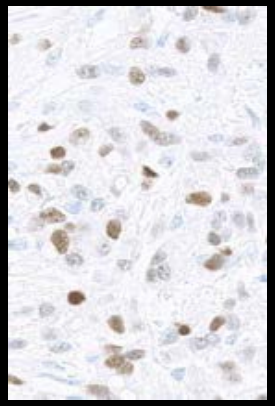
10% False positive

5% Impaired morphology, etc

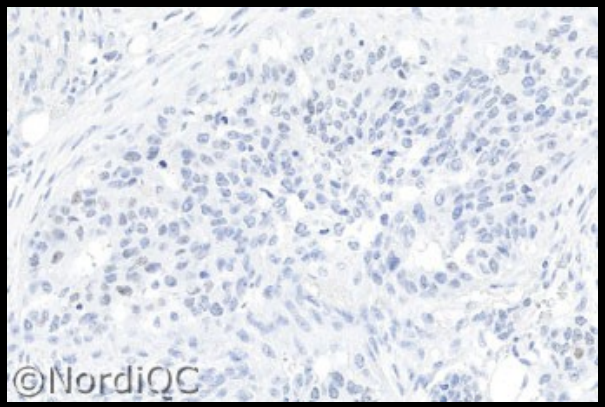


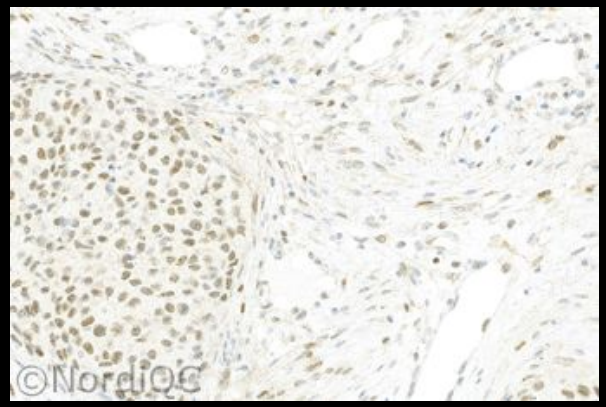


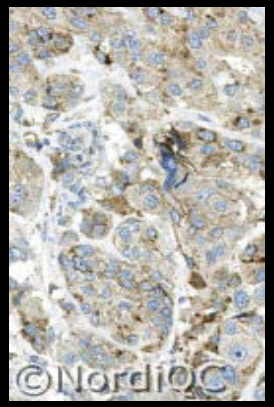


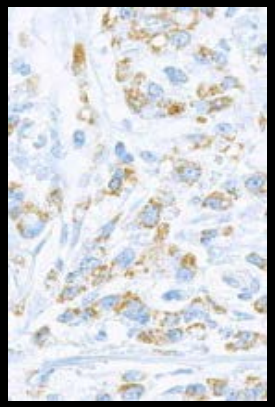


Suf.









Ins.

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

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

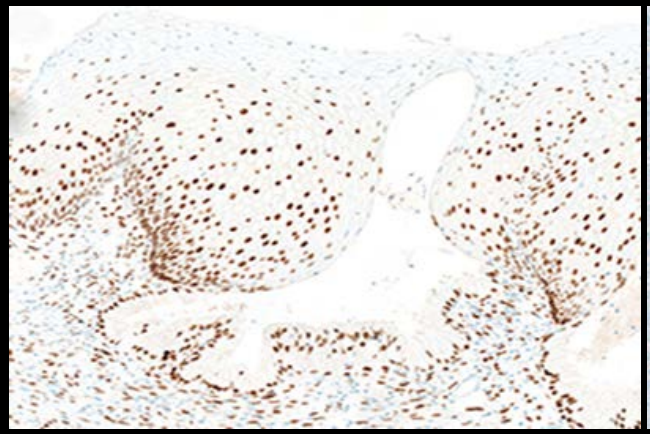
Clone 1D5 at high titre,
Biotin-based kits,
HIER in pressure cooker

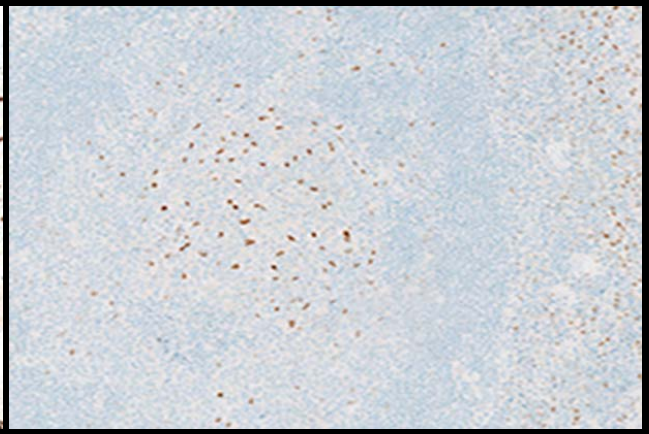
IHC - Protocols and controls for Breast tumours

Breast panel: Estrogen Receptor
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 6F11*	HIER Ci, High	1:50-200	2- & 3-step	Leica	3-step
rmAb EP1	HIER High	1:25–30	2- & 3-step	Dako	2- & 3-step
rmAb SP1	HIER High	1:30-100	2- & 3-step	Ventana	2- & 3-step

** Efficient HIER, high conc., 3-step pol. & low stringent washing can give aberrant nuclear staining Not seen on Ventana stainer, rarely on Autostainer and most commonly on Bond stainer.*





Use uterine cervix and tonsil to verify level of sensitivity and specificity;

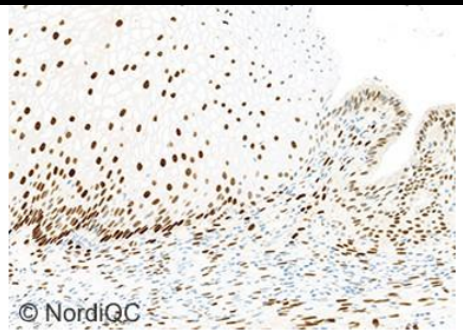


Fig. 1a
Optimal ER staining result of the uterine cervix using the rmAb clone SP1as Ready-To-Use format, Ventana 790-4324 with HIER in CC1 and UltraView as detection system. Virtually all the squamous and columnar epithelial cells show a moderate to strong, distinct nuclear staining reaction. The majority of the stromal cells are demonstrated and only endothelial and lymphoid cells are negative. Also compare with Figs. 2a – 4a, same protocol.

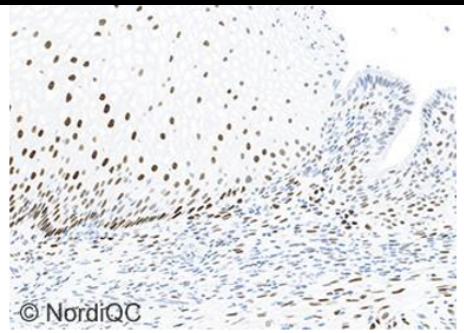


Fig. 1b
Insufficient ER staining result of the uterine cervix - same field as in Fig. 1a.
The proportion and intensity of the staining reaction in the squamous and especially in columnar epithelial cells is reduced. Also compare with Figs. 2b - 4b, same protocol.
The protocol was based on the rmAb clone EP1 applied with protocol settings giving a too low sensitivity – most likely due to a too dilute titre of the primary Ab and insufficient HIER.

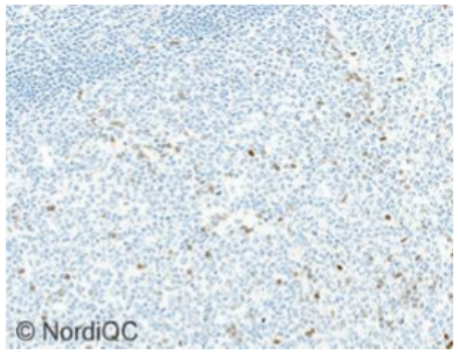


Fig. 2a
Optimal ER staining result of the tonsil using same protocol as in Fig. 1a
A weak to moderate nuclear staining reaction of dispersed germinal centre lymphocytes is seen. The nuclear staining reaction can be seen at low magnification, x100.
However note that the vast majority of lymphocytes are negative.

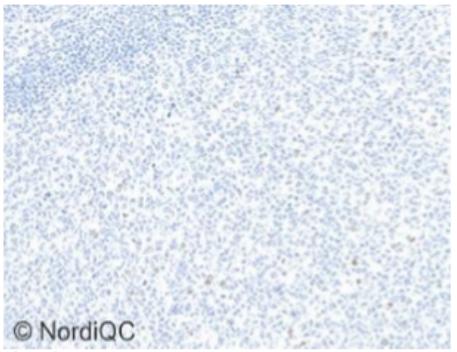


Fig. 2b
Insufficient ER staining result of the tonsil using same protocol as in Fig. 1b – same field as in Fig. 2a.
Compared to the result obtained in Fig. 2a, only a faint nuclear staining reaction in a significantly reduced proportion of germinal centre lymphocytes is seen.

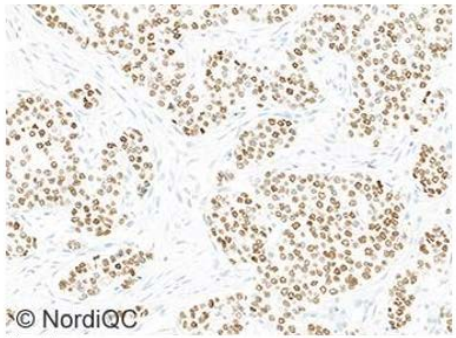


Fig. 3a
Optimal ER staining result of the breast ductal carcinoma no. 6 with 80 – 100% cells positive using same protocol as in Figs. 1a and 2a.
The vast majority of the neoplastic cells show a

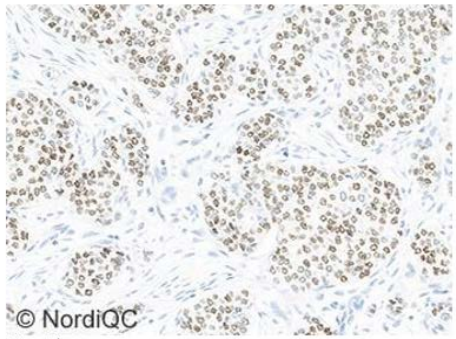


Fig. 3b
ER staining result of the breast ductal carcinoma no. 6 with 80 – 100% cells positive using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.

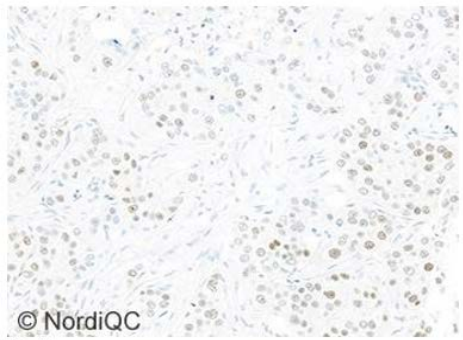


Fig 4a
Optimal ER staining result of the breast ductal carcinoma no. 4 with 40 – 60% cells positive using

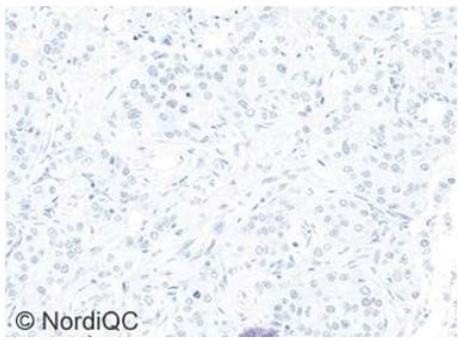


Fig 4b
Insufficient ER staining result of the breast ductal carcinoma no. 4 with 40 – 60% cells positive using

Use right performance controls

No tumour material

What is a high ER pos tumour..

IHC - Protocols and controls for Breast tumours

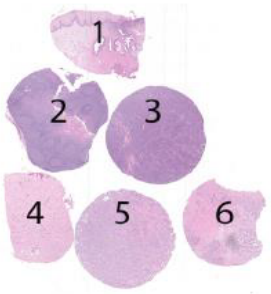
Assessment Run B20 2015
Progesterone Receptor (PR)

Recommended PR protocols Recommended PR control tissue

Material

The slide to be stained for PR comprised the following tissues:

No.	Tissue	PR-positivity*	PR-intensity*
1.	Uterine cervix	80 - 90 %	Moderate to strong
2.	Tonsil	Negative	Negative
3.	Breast carcinoma	Negative	Negative
4.	Breast carcinoma	40 - 60%	Weak to strong
5.	Breast carcinoma	60 - 80%	Weak to strong
6.	Breast carcinoma	80 - 100%	Moderate to strong



*PR-positivity and intensity as characterized by NordiQC reference laboratories using the mmAb clone 16 (Leica/Novocastra)

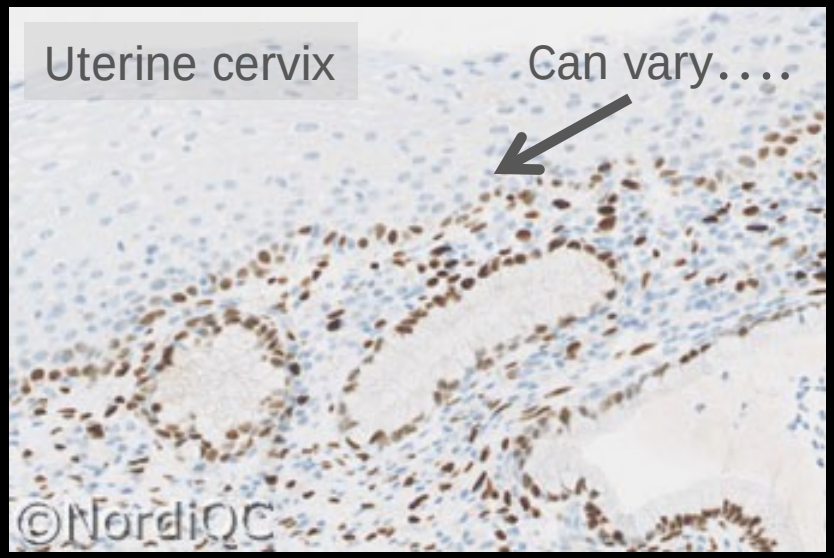
All tissues were fixed in 10% neutral buffered formalin for 24 – 48 hours and processed according to Yaziji et al. (1).

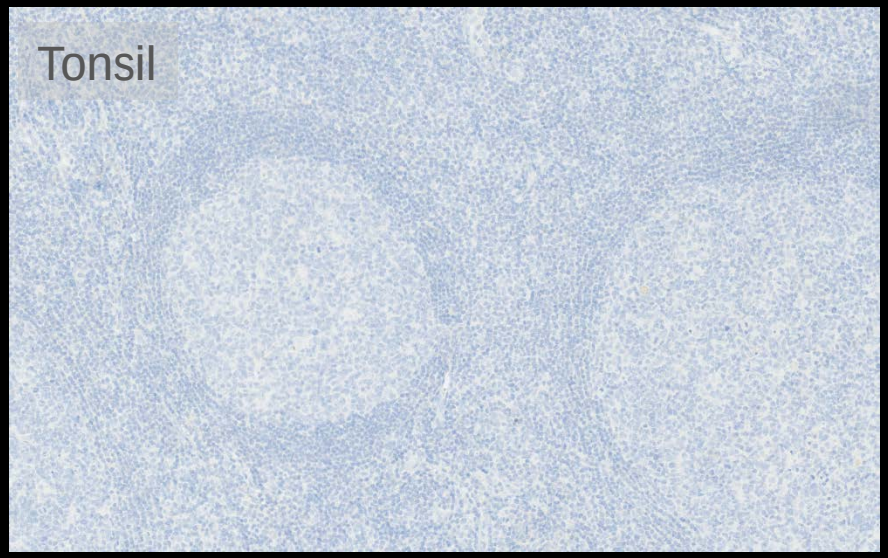
Focus:

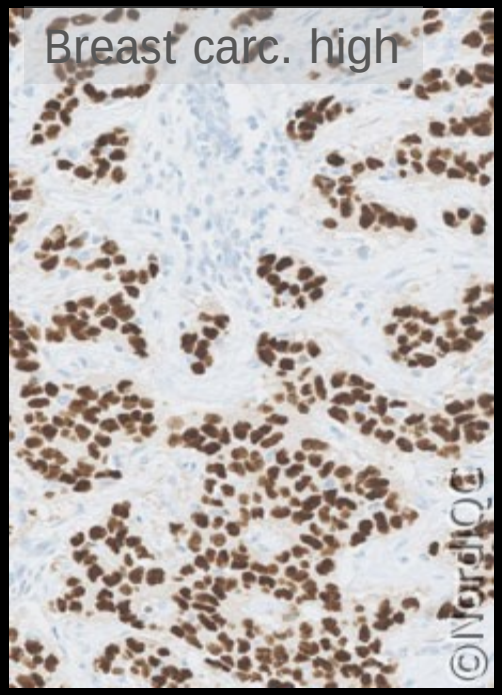
Appropriate technical quality; signal-to-noise, morphology etc

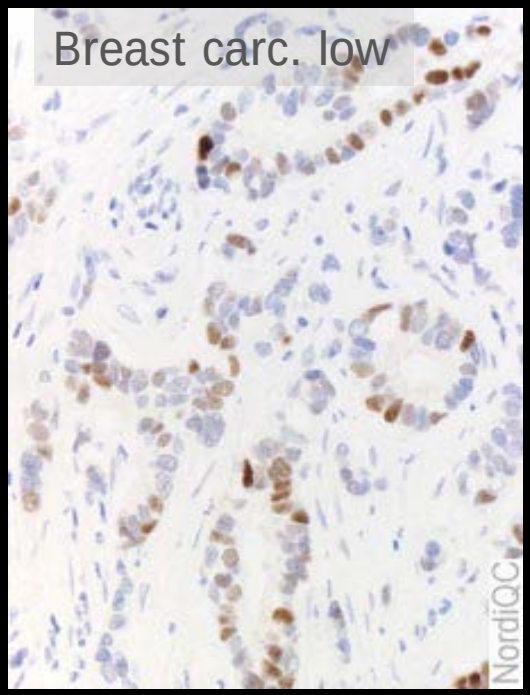
Appropriate analytical sensitivity and specificity – indicated by concordance of PR status in the included tumours to reference

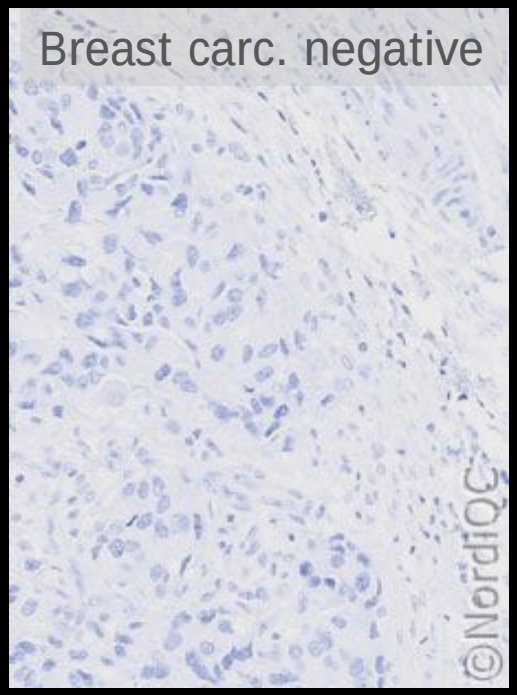
IHC - Protocols and controls for Breast tumours









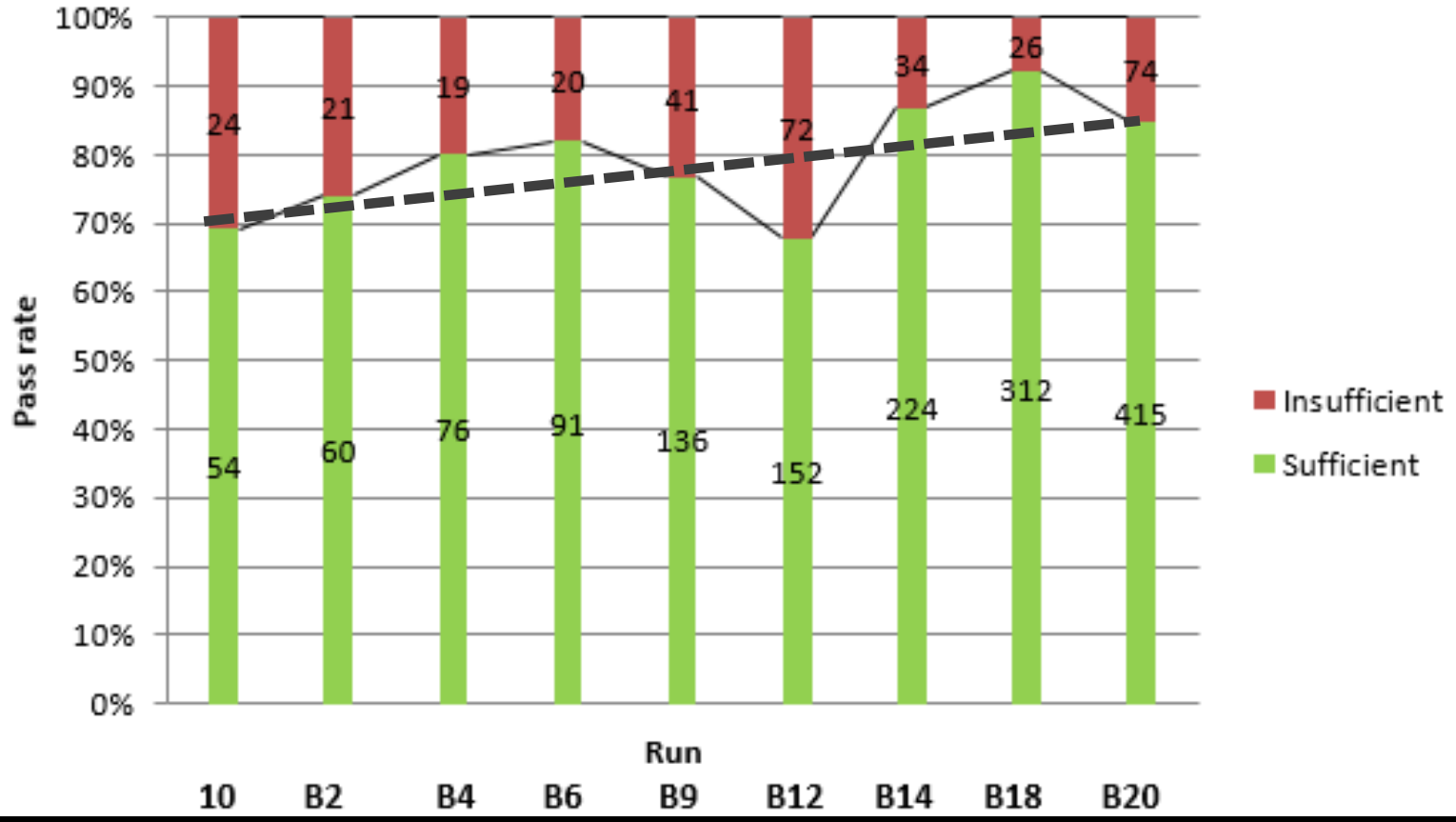


IHC - Protocols and controls for Breast tumours

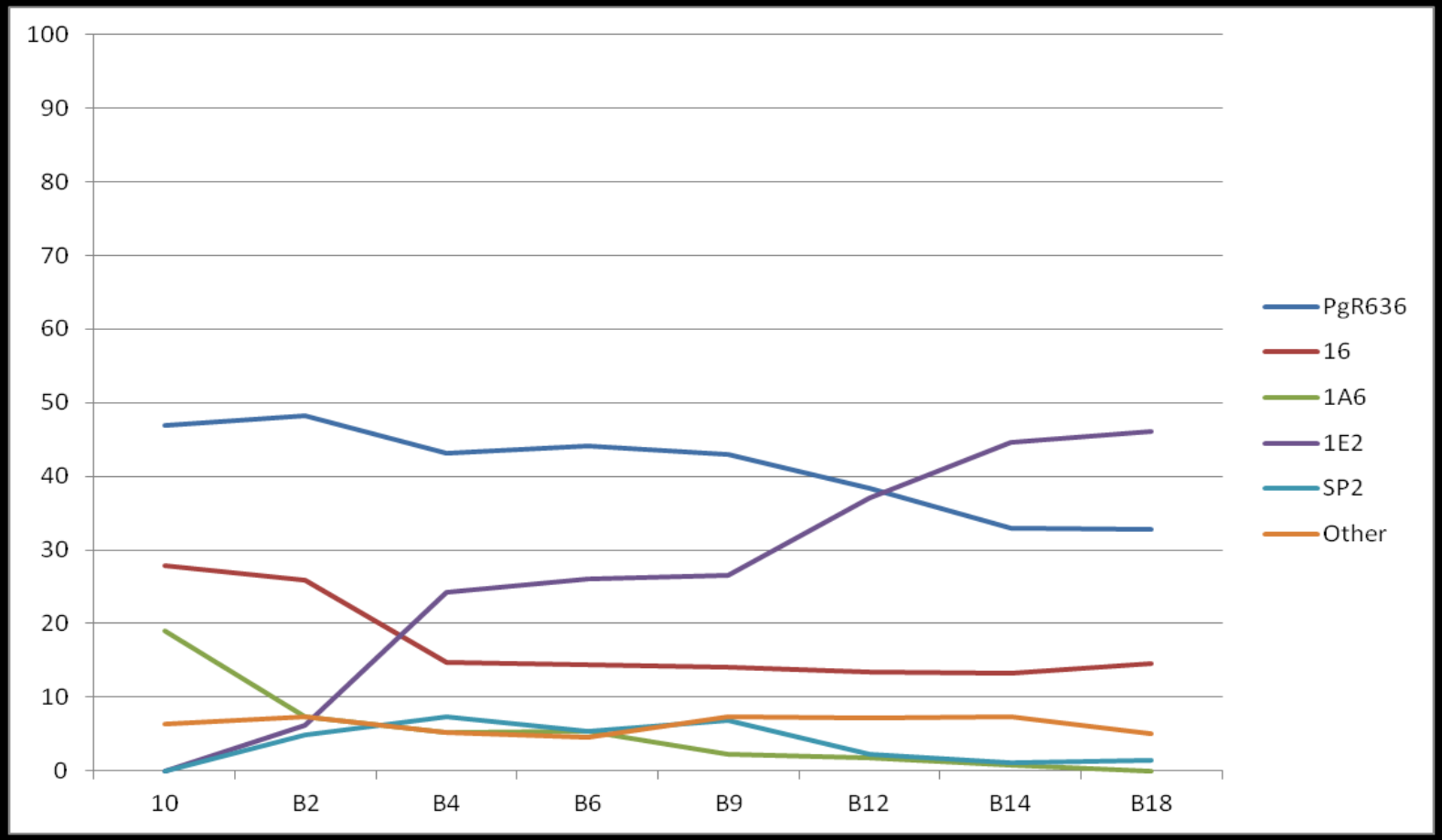
Performance history

This was the ninth NordiQC assessment of PR. A small decrease in the proportion of sufficient results was seen compared to the previous runs, as shown in figure 1:

Figure 1 – pass rate in the 9 NordiQC assessments for PR



IHC - Protocols and controls for Breast tumours



Comparison of different antibodies for detection of progesterone receptor in breast cancer

Steroids 67 (2002) 799–813

Michael Press[a,b], Betsy Spaulding[c], Susan Groshen[b], David Kaminsky[c], Margaret Hagerty[c], Lori Sherman[e], Kurt Christensen[e], Dean P. Edwards[e,*]

[a] Department of Pathology, University of Southern California School of Medicine, Los Angeles, CA 90033, USA
[b] Norris Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033, USA
[c] DAKO Corporation, Carpinteria, CA 93013, USA
[d] Department of Pathology, Eisenhower Medical Center, Palm Desert, CA, USA
[e] Department of Pathology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Campus Box B-216, Denver, CO 80262, USA

Potential for False-Positive Staining With a Rabbit Monoclonal Antibody to Progesterone Receptor (SP2)

Findings of the UK National External Quality Assessment Scheme for Immunocytochemistry and FISH Highlight the Need for Correct Validation of Antibodies on Introdu

Am J Clin Pathol 2008;129:398-409

Merdol Ibrahim, PhD,[1] Andrew Dodson, MSc,[2] Sarah Barnett, MSc,[1] David Fish, MSc,[2] Bharat Jasani, PhD,[1] and Keith Miller, MSc[1]

Key Words: Breast hormonal receptors; Progesterone receptor; Rabbit monoclonal antibody; SP2; Quality assurance; Antibody validation

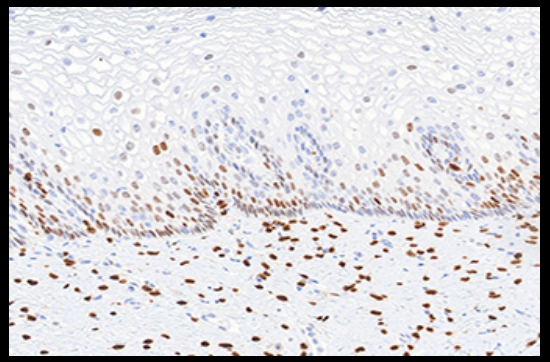
IHC - Protocols and controls for Breast tumours

Table 1: **Antibodies and assessment marks for PR, run B20**

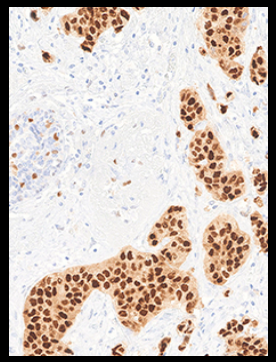
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	48 1 1	Leica/Novocastra Biocare Vector	39	7	3	1	92%	96%
mAb clone cocktail **16 + SAN27**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **1A6**	5	Leica/Novocastra	2	1	0	2	-	-
mAb clone **PgR 636**	68	Dako	48	15	4	1	93%	94%
mAb clone **PgR 1294**	17	Dako	13	4	0	0	100%	100%
rmAb clone **SP2**	5	Thermo/NeoMarkers	2	2	0	1	-	-
rmAb clone **SP42**	1	Zytomed	1	0	0	0	-	-
rmAb clone **Y85**	1 1	Cell Marque Thermo/NeoMarkers	1	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **16 PA0312**	13	Leica/Novocastra	11	2	0	0	100%	100%
mAb clone **16 MAD-000670QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb **PgR 636 IR/IS068**	78	Dako	60	15	1	2	96%	96%
mAb clone **PgR 1294 K4071/SK310**	4	Dako	1	3	0	0	-	-
mAb clone **PR88 AM328-5ME**	1	Biogenex	0	0	1	0	-	-
rmAb clone **1E2 790-2223/4296**	239	Ventana	85	98	53	3	77%	88%
rmAb clone **SP2 Kit-0013**	1	Maixin	1	0	0	0	-	-
rmAb clone **SP2 RM-9102**	1	Thermo/NeoMarkers	0	0	1	0	-	-
pAb E2071	1	Spring Bioscience	0	0	0	1	-	-
Total	489		266	149	63	11	-	
Proportion			54%	31%	13%	2%	85%	

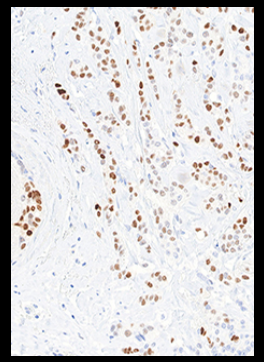
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
*discontinued product

HIER alk. pH
2- & 3-step kits
Carefully calib.



Uterine cervix –
all columnar epith. cells, and majority of basal squam. epith. cells (can be neg. in some pts).





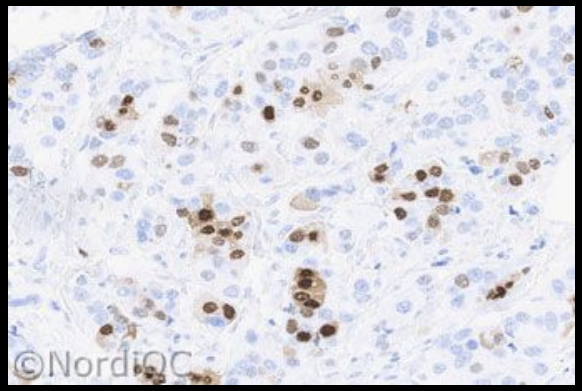
IHC - Protocols and controls for Breast tumours

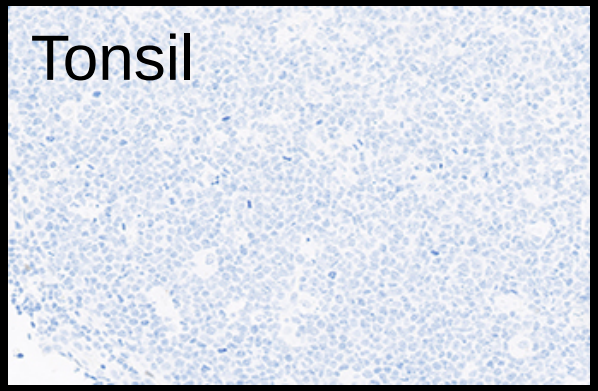
Progesterone receptor;

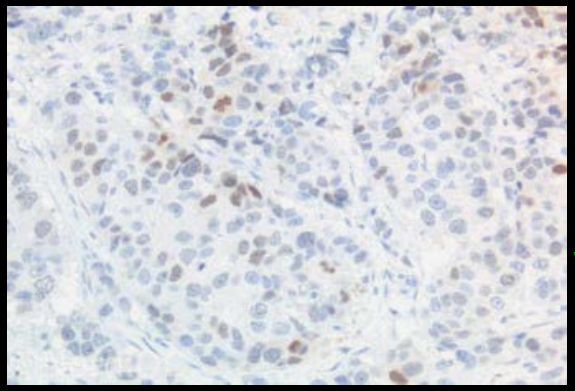
75% Weak / False negative

20% False positive

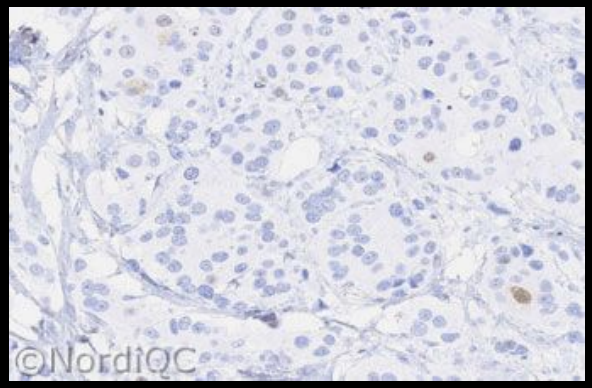
5% Impaired morphology, etc

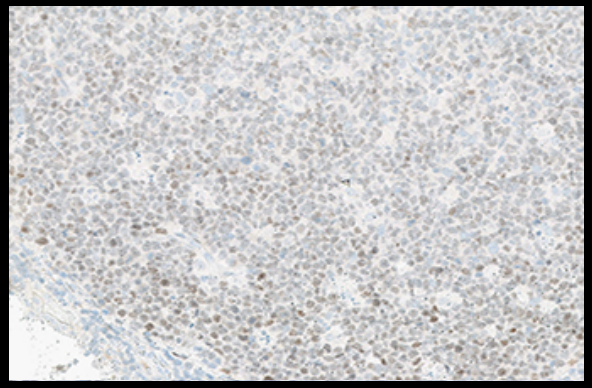


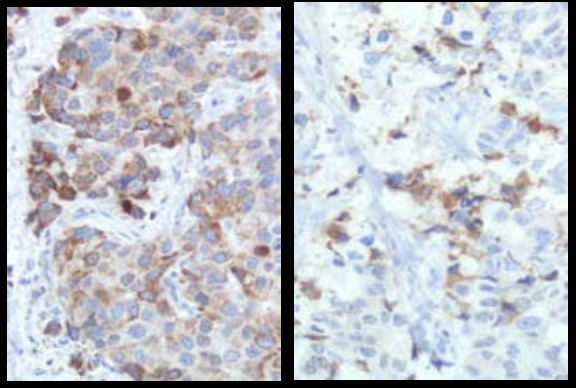




Suf.







Ins.

Too low titre (16, PgR636)
Insufficient HIER

Clone SP2 and 1E2.
1E2 mainly by off-label protocol (ext. sensitivity)

Clone 1A6,
Biotin-based kits,
HIER in pressure cooker

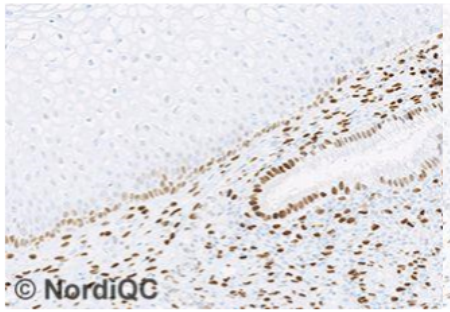


Fig. 1a
Optimal staining for PR of the uterine cervix using the mmAb clone 16 optimally calibrated at a titre of 1:50, efficient HIER for 48 min. in CC1 pH 8,5 using a 3-step multimer based detection system (OptiView, Ventana). The vast majority of basal squamous epithelial cells show a weak to moderate nuclear staining reaction, whereas the majority of columnar epithelial cells and stromal cells show a moderate to strong nuclear staining reaction.

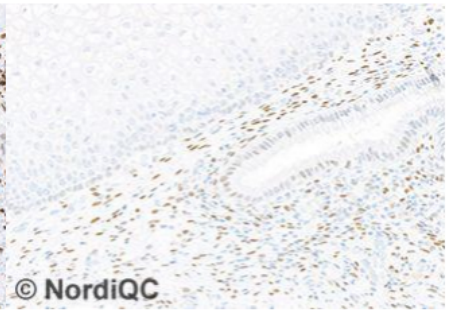


Fig. 1b
Insufficient staining for PR of the uterine cervix, using the mmAb clone 16 with protocol settings giving a too low sensitivity - same field as in Fig. 1a.
The stromal cells are demonstrated, but the squamous and columnar epithelial cells are virtually negative. Also compare with Figs. 2b - 3b – same protocol.

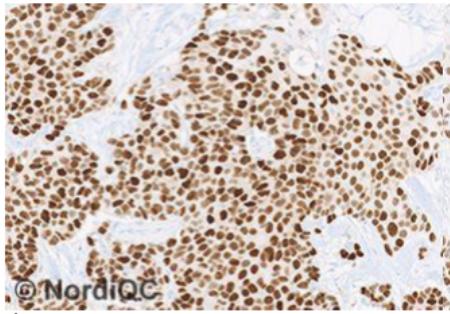


Fig. 2a
Optimal staining for PR of the breast carcinoma no. 6 with 80 - 100% cells positive using same protocol as in Fig. 1a.
A moderate to strong nuclear staining reaction is seen. A weak cytoplasmic staining reaction in the neoplastic cells is seen, but no background staining.

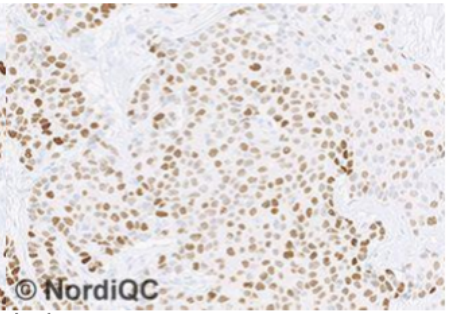


Fig. 2b
Staining for PR of the breast carcinoma no. 5 with 80 - 100% cells positive using same protocol as in Fig. 1b. - same field as in Fig. 2a.
A weak to strong distinct nuclear staining reaction in virtually all neoplastic cells is seen.
However also compare with Fig. 3b – same protocol.

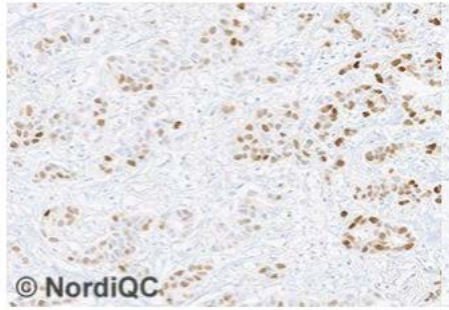


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 4 with 40 - 60% cells positive using same protocol as in Figs. 1a - 2a.
The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

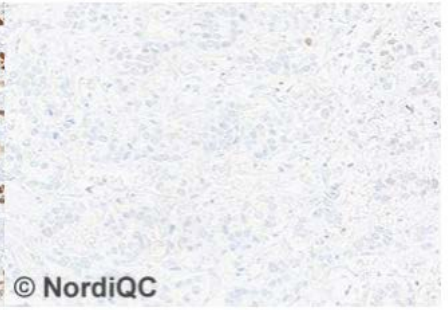


Fig. 3b
Insufficient staining for PR of the breast carcinoma no. 4 with 40 - 60% cells positive using same protocol as in Figs. 1b – 2b – same field as in Fig. 3a.
Only dispersed cells are demonstrated and a significant reduced proportion of cells are identified compared to the level expected.

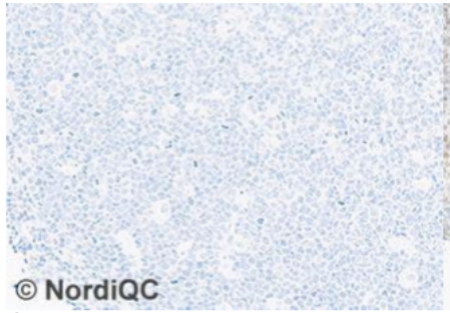


Fig. 4a
Optimal staining for PR of tonsil using same protocol as in Figs. 1a - 3a.
No nuclear staining reaction is seen.
This staining pattern was consistently seen for protocols based on mmAb clone 1A6, 16, PgR 636 and PgR 1294 irrespective of protocol settings applied.

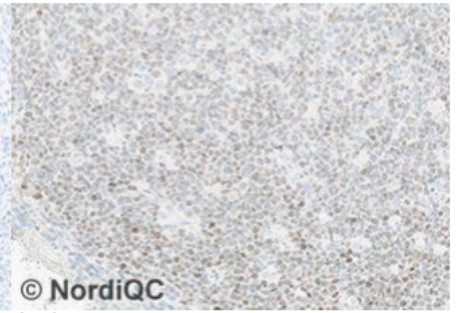


Fig. 4b
Insufficient staining for PR of tonsil – same field as in Fig. 4a.
The majority of germinal cells show a weak to moderate and aberrant false positive nuclear staining reaction.
This aberrant staining reaction was only seen for rmAb clones 1E2 (RTU, Ventana) and SP2.
For rmAb clone 1E2 prolonged antibody incubation time in combination with a reduced HIER time compared to the recommendations provided by Ventana seemed to enhance the aberrant staining pattern. In case of aberrant positive nuclear staining reaction in tonsil and otherwise an expected staining pattern in the other tissues was seen, the result was evaluated as borderline.
In case also an aberrant and false positive staining in the PR negative breast carcinoma was observed, the result was assessed as poor – see Fig. 5b.

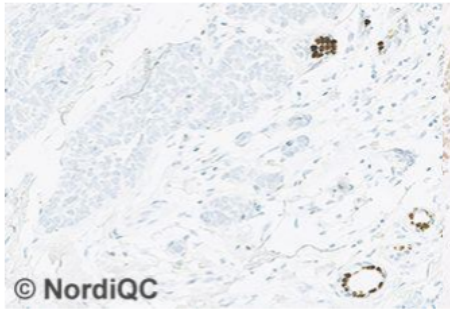


Fig. 5a
Optimal staining for PR of the breast carcinoma no. 3 expected to be negative using same protocol as in Figs. 1a - 4a.
No nuclear staining reaction in the neoplastic cells is seen. The PR status was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories. The tumour was ER negative. Normal glands serve as internal positive tissue control.

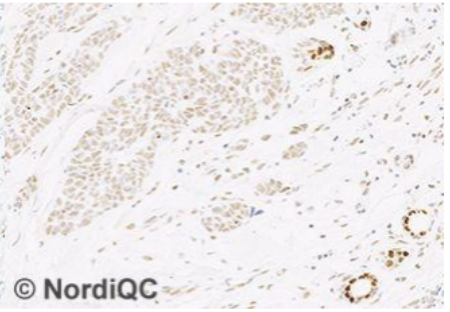


Fig. 5b
Insufficient staining for PR of the breast ductal carcinoma no. 3 expected to be negative – same field as in Fig. 5a.
Virtually all neoplastic cells show a weak to moderate and aberrant false positive nuclear staining reaction.
The protocol was based on the rmAb clone SP2, using HIER in an alkaline buffer and a 2-step polymer based detection system.

Use right performance controls

No tumour material

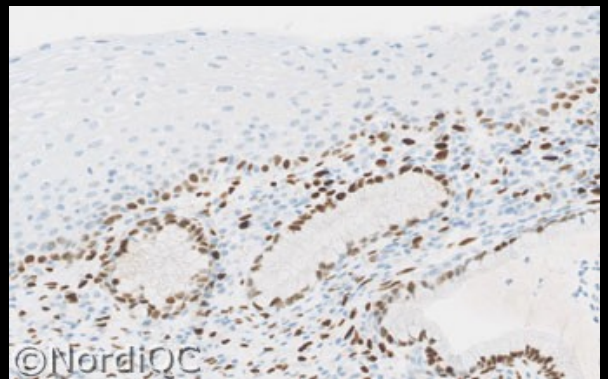
IHC – Protocols and controls for Breast tumours

Breast panel: Progesterone Receptor
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 16	HIER High	1:75-800	2- & 3-step	Leica	3-step
mAb PGR636*	HIER (High)	1:100-800	2- & 3-step	Dako	3-step
mAb PGR1294	HIER (High)	1:250–5.000	2- & 3-step	Dako	2-step
rmAb 1E2**	HIER High	-	-	Ventana	2-step

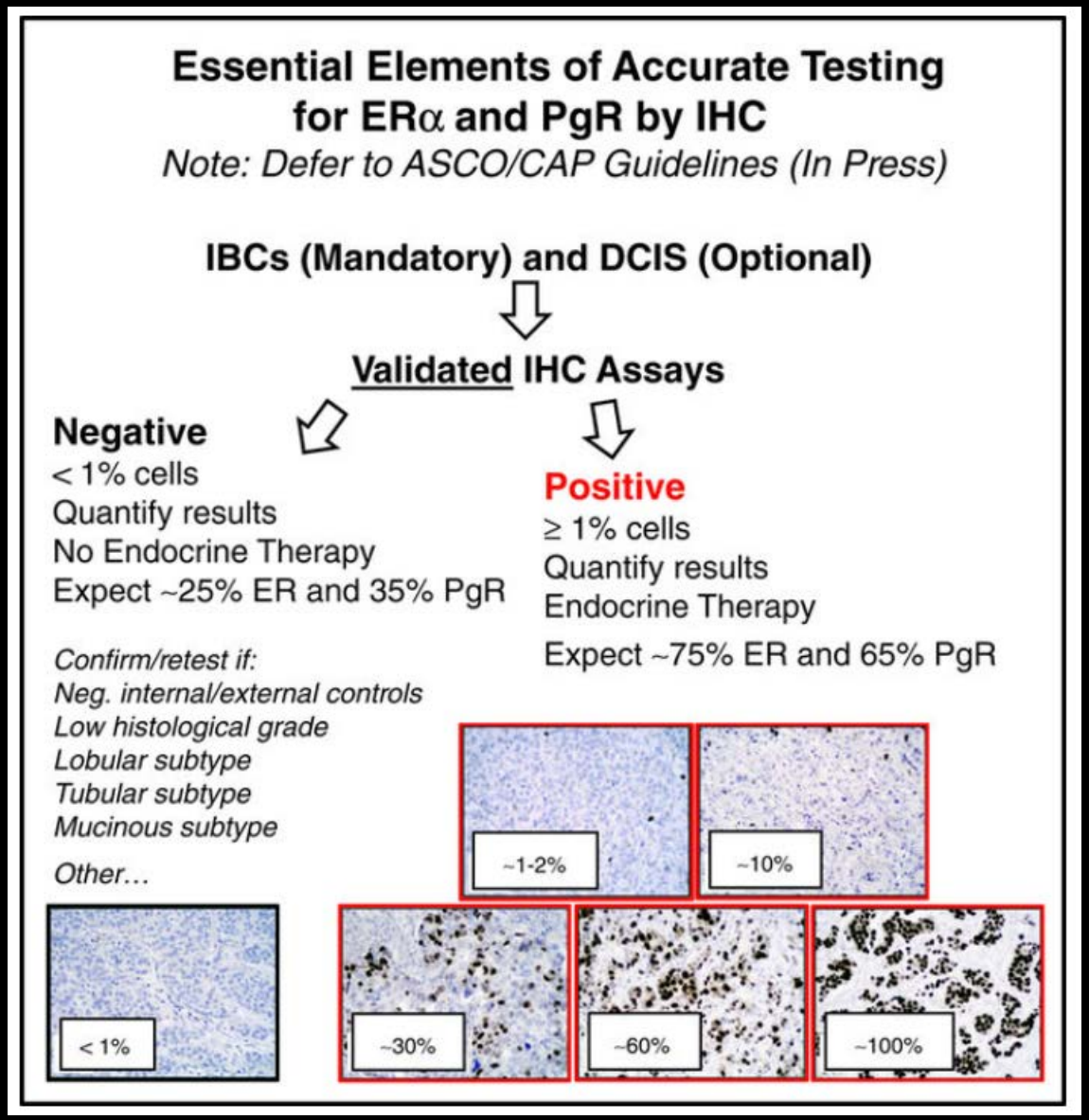
* *mAb clone PGR636 has shown to be less successful on Ventana BenchMark Ultra*

** *rmAb clone 1E2, RTU might provide aberrant false pos. result by 3-step protocols, reduced HIER and prolonged Ab incubation time compared to Ventana guidelines*



Use uterine cervix and tonsil to verify level of sensitivity and specificity;

IHC - Protocols and controls for Breast tumours



Use metrics

% of ER pos
% of PR pos

Some types must be pos
(Ductal I, Lobular etc)

IHC - Protocols and controls for Breast tumours

NordiQC

Assessment Run B23 2017

HER-2 IHC

Material

The slide to be stained for HER-2 comprised the following 9 materials:

	IHC: HER-2 Score* (0, 1+, 2+, 3+)	FISH: HER-2/chr17 ratio**
1. Cell line 1, Horizon Discovery***	3+	
2. Cell line 2, Horizon Discovery***	2+	
3. Cell line 3, Horizon Discovery***	1+	
4. Cell line 4, Horizon Discovery***	0	
5. Breast carcinoma, no. 1	3+	> 6.0 (clusters) (amplified)
6. Breast carcinoma, no. 2	2+	2.3 – 2.9 (amplified)
7. Breast carcinoma, no. 3	0-1+	1.1 – 1.4 (unamplified)
8. Breast carcinoma, no. 4	1-2+	1.3 – 1.7 (unamplified)
9. Breast carcinoma, no. 5	0-1+	1.2 – 1.4 (unamplified)

* HER-2 immunohistochemical score (see table below) as achieved by using the three FDA approved kits and antibodies, HercepTest™ (Dako), Oracle™ (Leica) and PATHWAY® (Ventana), in NordiQC reference laboratories.
** HER-2/chr17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)
*** The cell lines were not included in the assessment. Data will be analyzed subsequently by digital image analysis.

All carcinomas were fixed for 24 - 48 h in 10% neutral buffered formalin.

Focus:

Appropriate technical quality; signal-to-noise, morphology etc

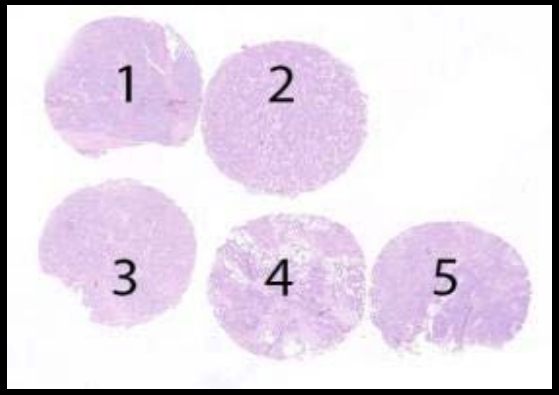
Appropriate analytical sensitivity and specificity – indicated by concordance to FISH status and IHC level established by reference data in all the included tumours.

IHC - Protocols and controls for Breast tumours

Table 1. **Assessment marks for IHC assays and antibodies run B23, HER-2 IHC**

FDA approved HER-2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	204	Ventana/Roche	186	13	2	3	98%	98%
CONFIRM™, rmAb clone **4B5, 790-4493**	15	Ventana/Roche	12	3	0	0	100%	100%
HercepTest™ **SK001**	33	Dako/Agilent	32	0	0	1	97%	97%
HercepTest™ **SK001**[4]	8	Dako/Agilent	8	0	0	0	100%	-
HercepTest™ **K5207**	2	Dako/Agilent	2	0	0	0	-	-
HercepTest™ **K5204**	2	Dako/Agilent	1	1	0	0	-	-
Oracle™ mAb clone **CB11, TA9145**	9	Leica	4	4	0	1	89%	88%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BS24**	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **CB11**	7 1	Leica/Novocastra Biogenex	4	4	0	0	100%	100%
rmAb clone **EP3**	1 1 1 1 1	Biocare Cell Marque Celnovte Thermo/NeoMarkers PathnSitu	3	2	0	0	100%	100%
rmAb clone **SP3**	17 3 1 1 1	Thermo/NeoMarkers Zytomed Cell Marque Spring Bioscience Thermo/Pierce	12	8	0	3	87%	100%
pAb clone **A0485**	50	Dako/Agilent	35	11	1	3	92%	93%
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11, NCL-L-CB11**	4	Leica/Novocastra	0	0	3	1	-	-
mAb clone **CB11, BMS014**	1	Zytomed	0	0	1	0	-	-
rmAb clone **EP3, AN726**	1	Biogenex	1	0	0	0	-	-
rmAb clone **EP3, RMPD049R**	1	Diagnostic Biosystems	1	0	0	0	-	-
rmAb clone **SP3, 237R**	2	Cell Marque	1	1	0	0	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostics	1	0	0	0	-	-
Ab clone **MXR001, RMA-0701**	2	Maixin	1	1	0	0	-	-
Total	372		304	49	7	12	-	-
Proportion			82%	13%	2%	3%	95%	-

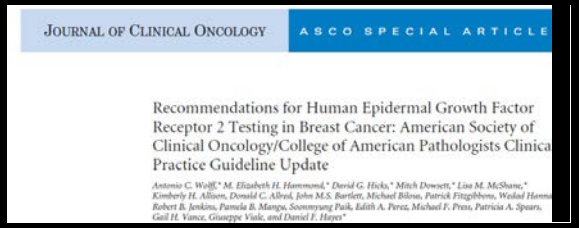
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.
4) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Leica Bond and Dako Omnis)



	IHC: HER-2 Score* (0, 1+, 2+, 3+)
1.Breast carcinoma	2-3+
2.Breast carcinoma	0-1+
3.Breast carcinoma	1-2+
4.Breast carcinoma	3+
5.Breast carcinoma	0-1+***

	FISH: HER-2 gene/chr 17 ratio**
1.Breast carcinoma	2.3 – 2.8 (a)
2.Breast carcinoma	0.9 – 1.3 (u)
3.Breast carcinoma	1.2 – 1.5 (u)
4.Breast carcinoma	> 6.0 (clusters) (a)
5.Breast carcinoma	1.2 – 1.5 (u)

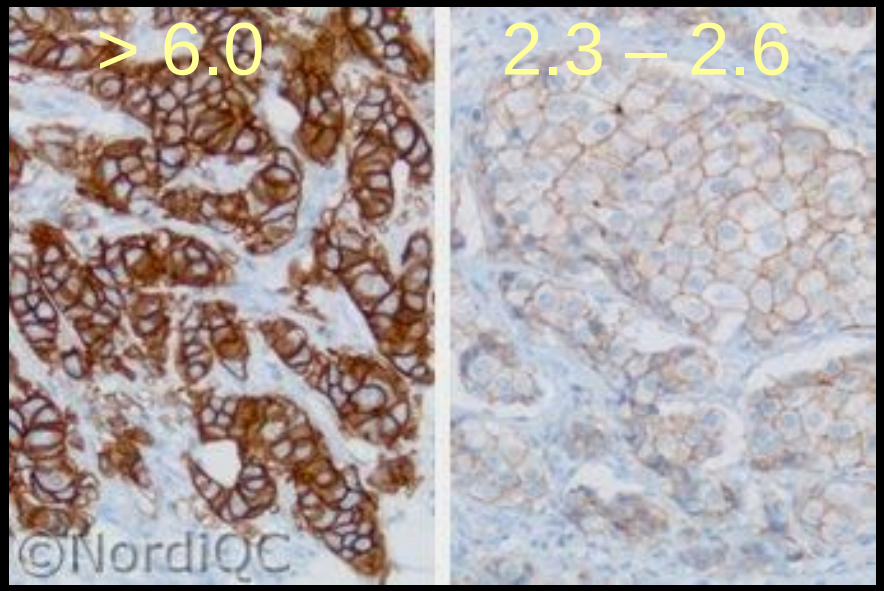
Material processed according to ASCO/CAP


JOURNAL OF CLINICAL ONCOLOGY ASCO SPECIAL ARTICLE

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinica Practice Guideline Update

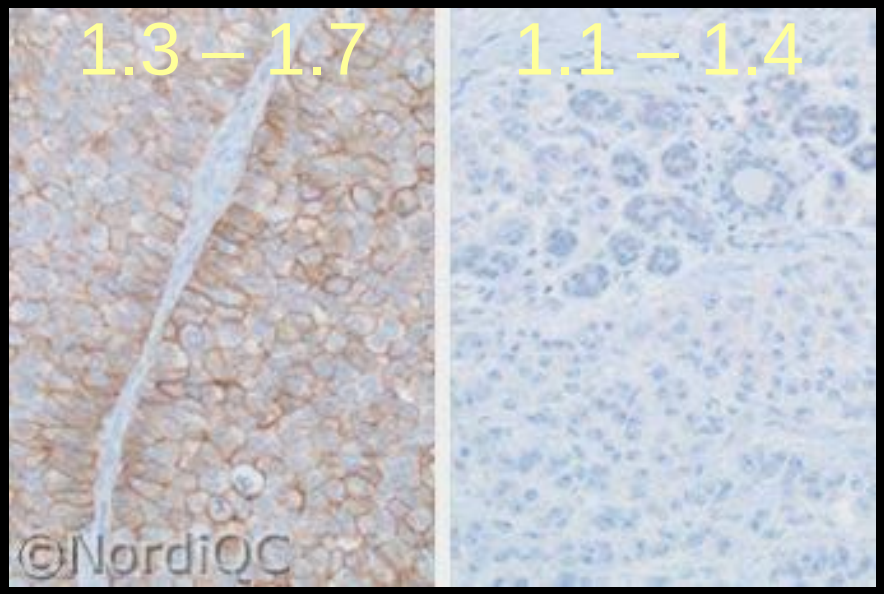
Antonio C. Wolff, M. Elizabeth H. Hammond, David G. Hicks, Mitch Dowsett, Lisa M. McShane, Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes

IHC - Protocols and controls for Breast tumours

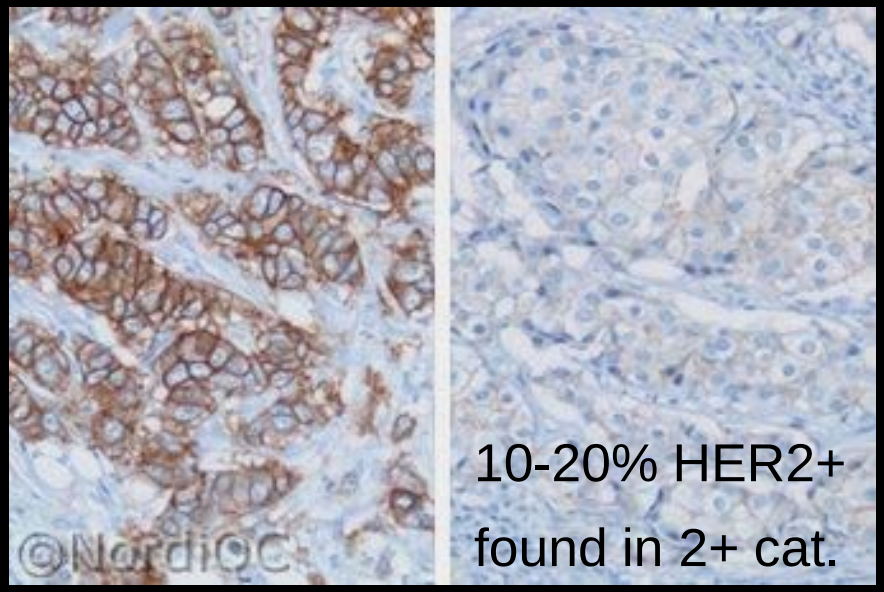


Ampl. 3+ Ampl. 2+

Optimal

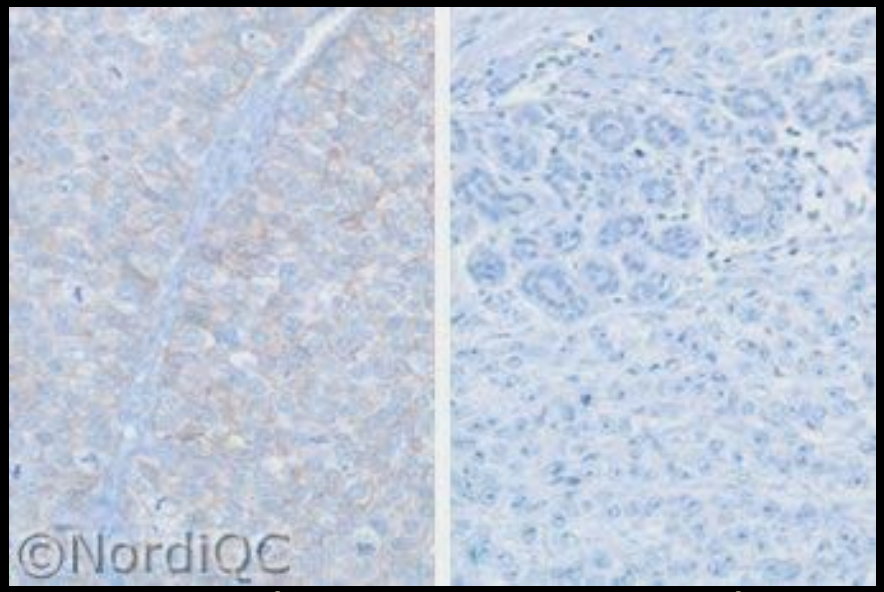


Unampl. 2+ Unampl. 0



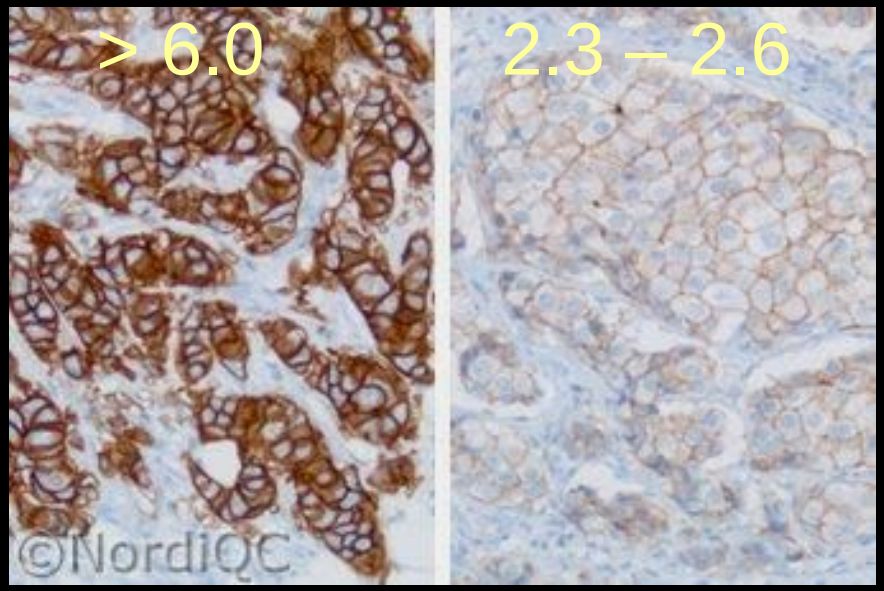
Ampl. 3+ Ampl. 1+

Poor



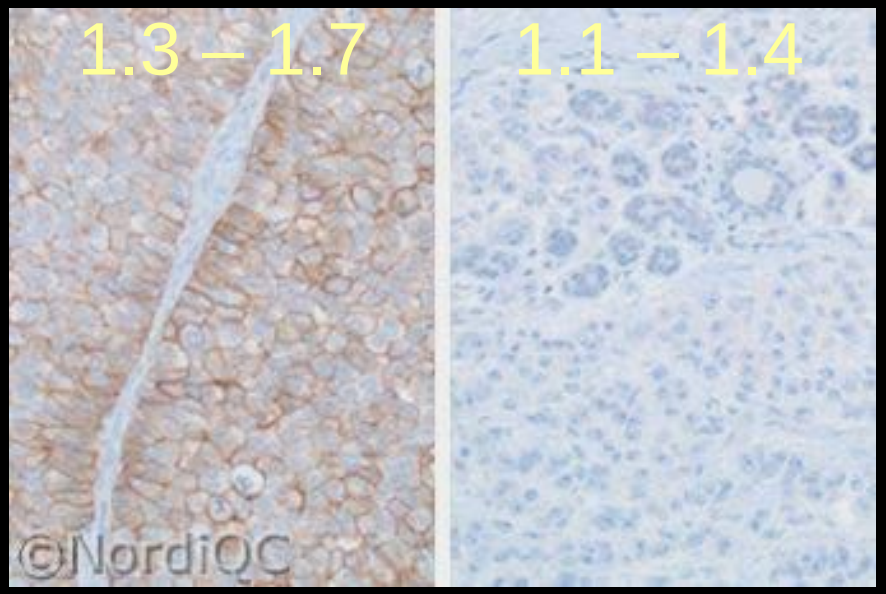
Unampl. 1+ Unampl. 0

IHC - Protocols and controls for Breast tumours

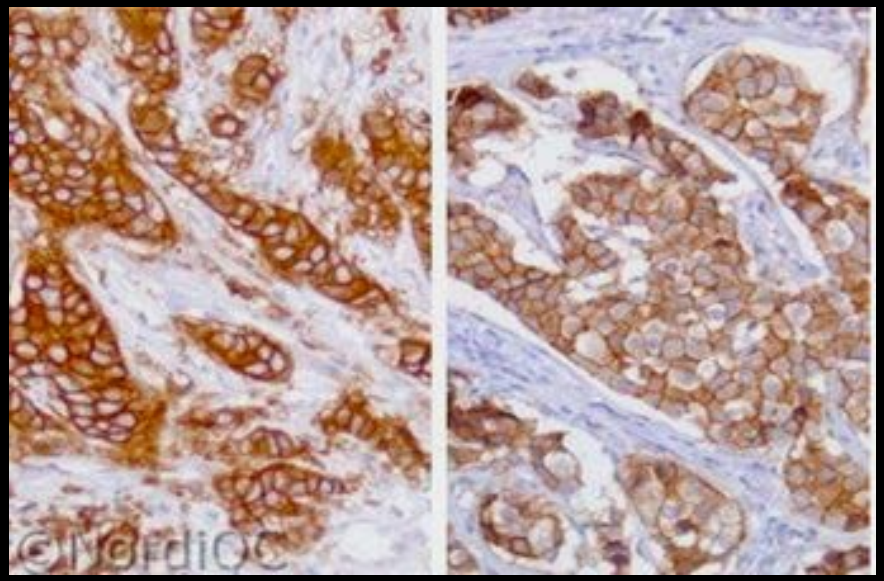


Ampl. 3+ Ampl. 2+

Optimal

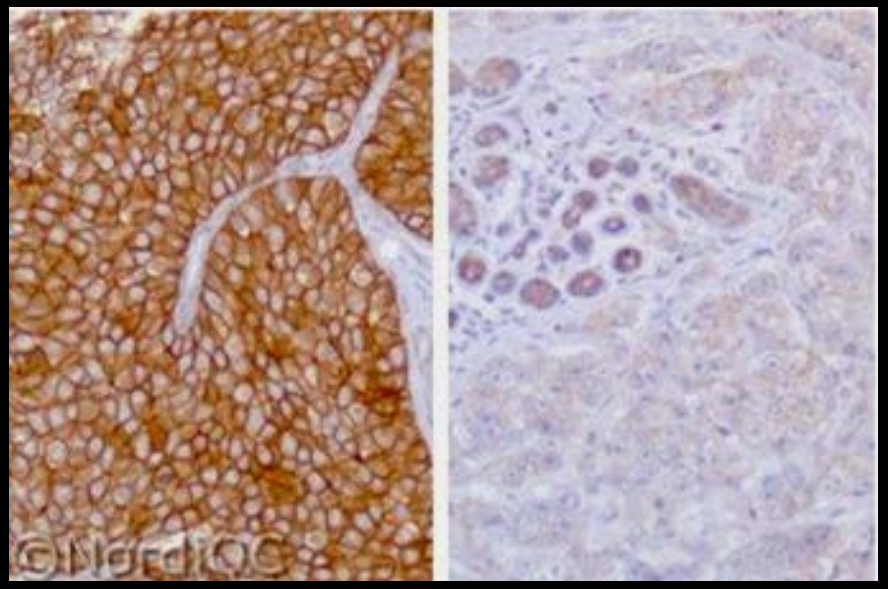


Unampl. 2+ Unampl. 0



Ampl. 3+ Ampl. 2+

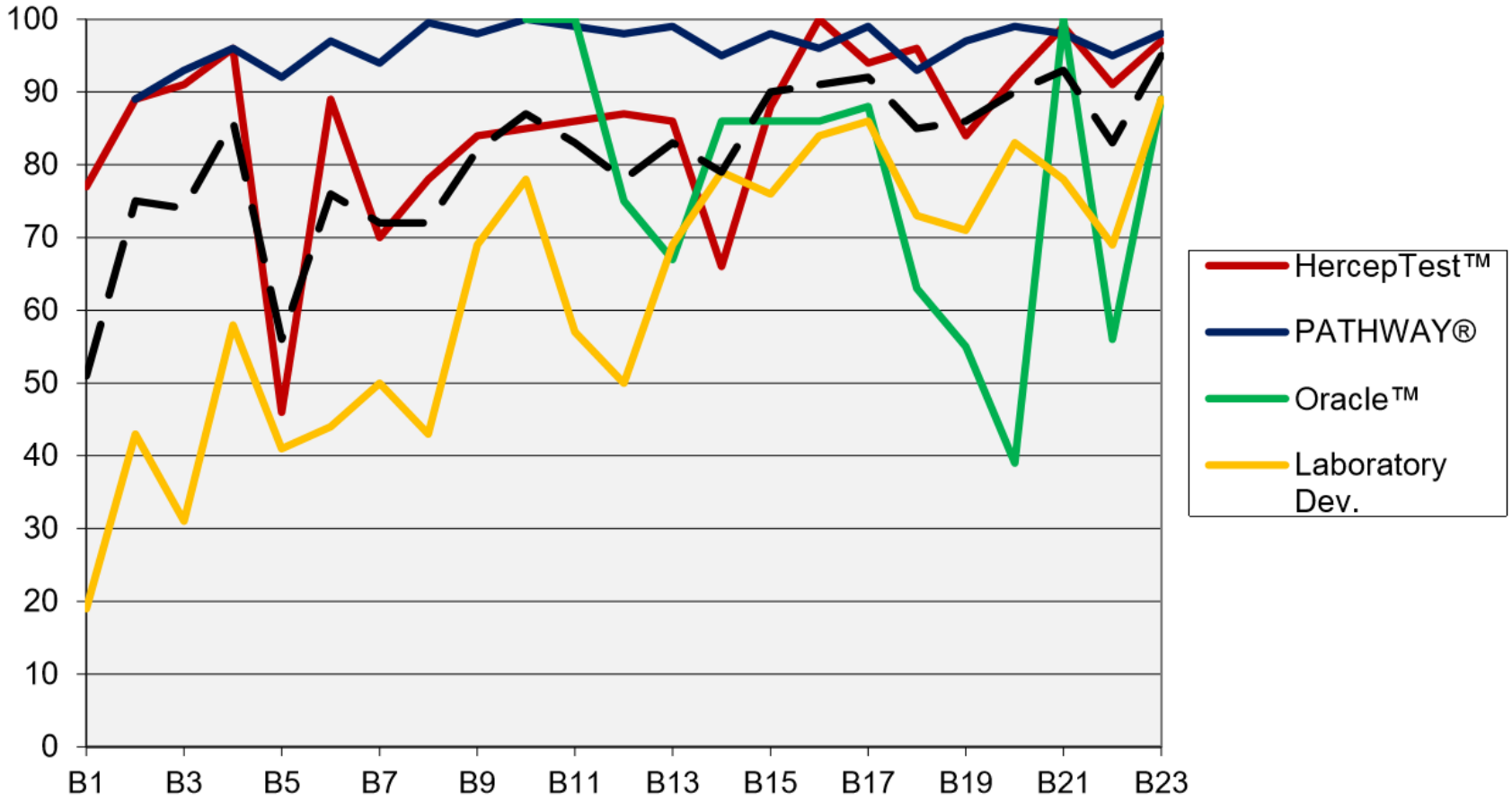
Poor



Unampl. 3+ Unampl. 1

IHC - Protocols and controls for Breast tumours

Figure 1. **Pass rates of 23 HER-2 IHC assessments in the NordiQC breast cancer module**



App 90 % of insuff. results are FN and seen both by FDA / CE-IVD kits and laboratory developed assays.

FP results have virtually only been seen by laboratory developed assays.

IHC - Protocols and controls for Breast tumours

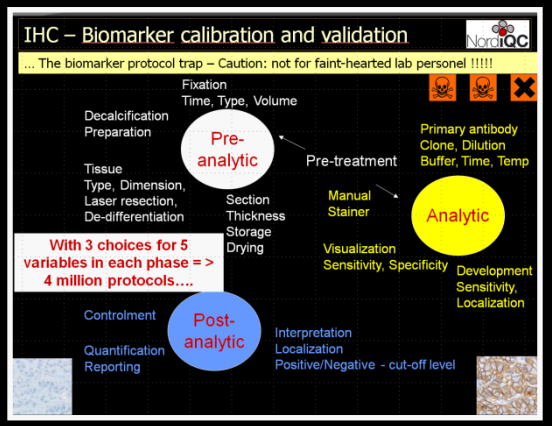
Typical causes for insufficient results in the NordiQC HER2 IHC breast module:

FDA / CE-IVD HER2 IHC kits:

PATHWAY®, Ventana:
Too short HIER (<24M) and/or too short incubation of primary Ab (<12M)

HercepTest™, Dako:
Too short HIER (<40M) and/or too short incubation of primary & secondary Ab (<30M)

Oracle™, Leica:
No single or combination of causes have been identified

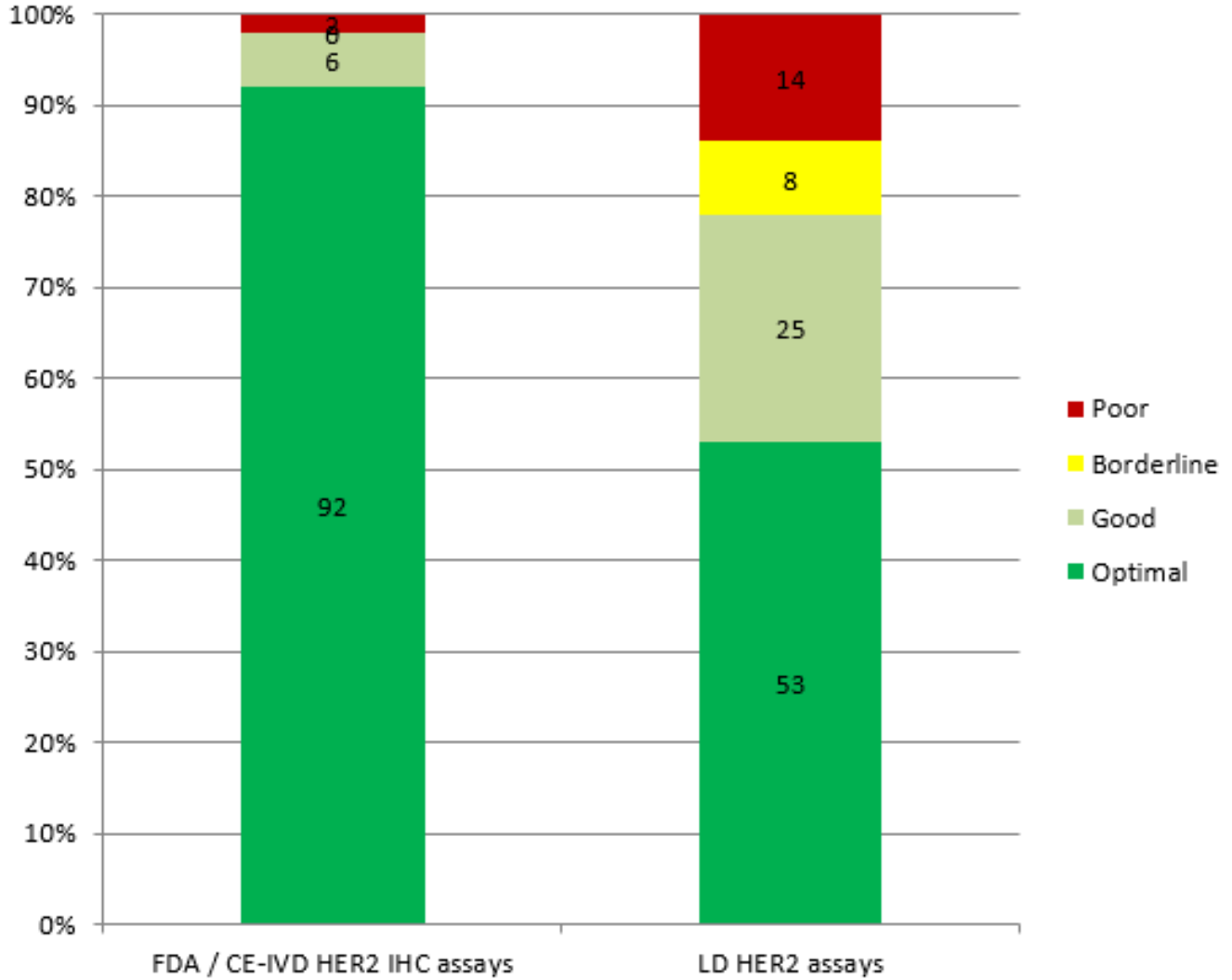


Laboratory developed assays:

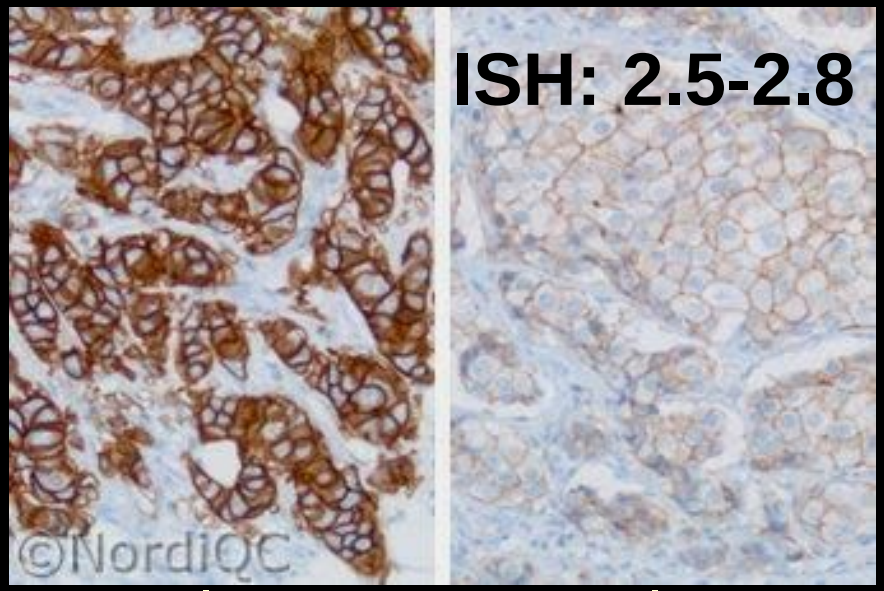
Inappropriate titre of primary Ab, less successful primary Ab, insufficient HIER, etc.....

IHC - Protocols and controls for Breast tumours

Figure 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**

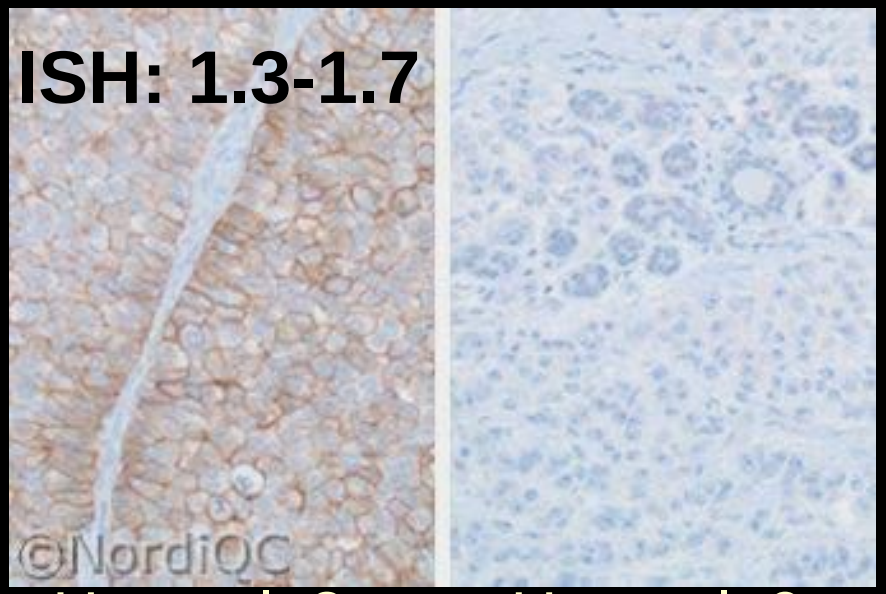


IHC - Protocols and controls for Breast tumours

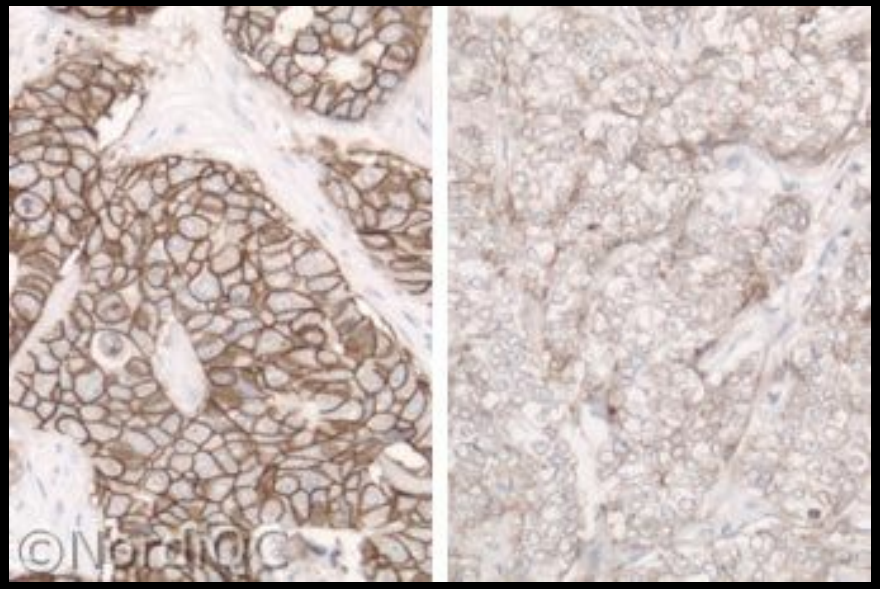


Ampl. 3+ Ampl. 2+

Optimal

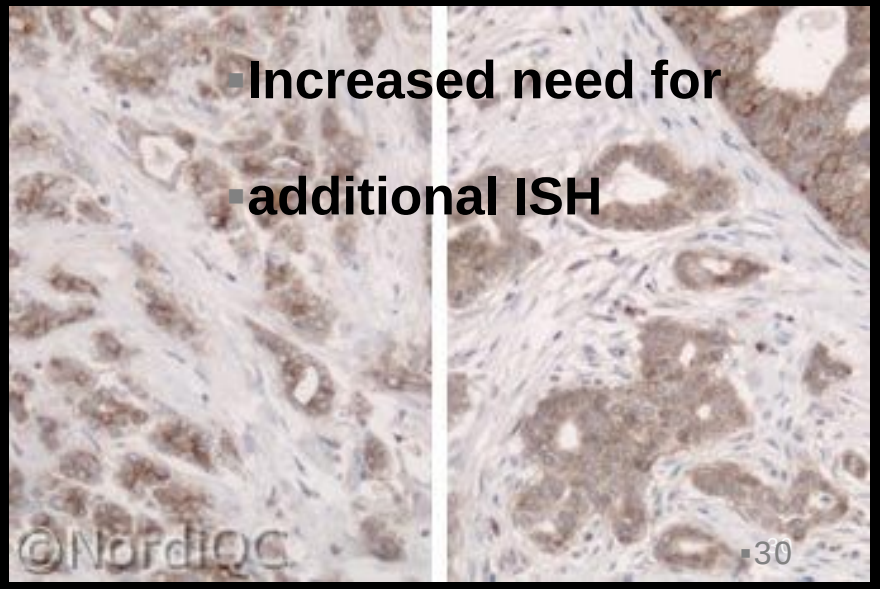


Unampl. 2+ Unampl. 0



Ampl. 3+ Ampl. 2+

Good



- **Increased need for**
- **additional ISH**

Unampl. 2+ Unampl. 2+

IHC - Protocols and controls for Breast tumours

Assessment Run H11 2017
HER-2 ISH

Material

Table 1. **Content of the multi-block used for the NordiQC HER-2 ISH assessment, run H11**

	HER-2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	HER-2/chr17 ratio¤	HER-2/chr17 ratio¤	HER-2 copies
1. Breast carcinoma	0	0.8	0.8 – 1.0	< 4
2. Breast carcinoma	2+	2.3	2.8 – 3.3	> 6
3. Breast carcinoma	3+	8.0	6.5 – 8.5	> 6
4. Breast carcinoma	2+	1.1	1.0 – 1.2	≥ 4 and < 6
5. Breast carcinoma	1+	1.5	1.2 – 1.5	< 4

* PATHWAY® (Ventana), data from two reference labs.
** Inform HER-2 Dual ISH kit (Ventana), range of data from one reference lab.
*** HER-2 FISH pharmDX™ Kit (Dako) and HER-2 FISH (Zytovision), range of data from one reference lab.
¤HER-2/chr17: HER-2 gene/chromosome 17 ratio

All tissues were fixed for 24 - 48 hours in 10% neutral buffered formalin according to the ASCO/CAP 2013 guidelines for tissue preparation of breast tissue for HER-2 ISH analysis.

HER-2 BRISH, Technical assessment
The main criteria for assessing a BRISH HER-2 analysis as technically **optimal** were the ability to interpret the signals and thus evaluate the HER-2/chr17 ratios in all five tissues.

Staining was assessed as **good**, if the HER-2/chr17 ratios could be evaluated in all five tissues, but the interpretation was slightly compromised e.g. due to excessive retrieval, weak or excessive counterstaining or focal negative areas.

Staining was assessed as **borderline** if one of the tissues could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

Staining was assessed as **poor** if two or more of the tissue cores could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

HER-2 BRISH and FISH interpretation
For both BRISH and FISH, participating laboratories were asked to submit a scoring sheet with their interpretation of the HER-2/chr17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyse scoring consensus.

Consensus scores from the NordiQC FISH reference laboratories

- Breast ductal carcinomas, no. 1 and 5: non-amplified
- Breast ductal carcinomas, no. 4: non-amplified or equivocal
- Breast ductal carcinoma no. 2 and 3: amplified

The ASCO/CAP 2013 guidelines were applied for the interpretation of the HER-2 status

Unamplified: HER-2/chr17 ratio < 2.0 using a dual probe assay or an average < 4 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Equivocal: HER-2/chr17 ratio of < 2.0 using a dual probe assay with an average of ≥ 4 and < 6 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Amplified: HER-2/chr17 ratio ≥ 2.0 using a dual probe assay or an average ≥ 4 HER-2 copies per cell/nucleus. Using a single probe assay an average of ≥ 6 HER-2 copies per cell/nucleus.

IHC - Protocols and controls for Breast tumours

Participation

Number of laboratories registered for HER-2 BRISH	124
Number of laboratories returning slides	121 (98%)
Number of laboratories returning scoring sheet	112 (90%)
Number of laboratories registered for HER-2 FISH	64
Number of laboratories returning scoring sheet	54 (84%)

Results BRISH, technical assessment

In total, 121 laboratories participated in this assessment. 73 laboratories (60%) achieved a sufficient mark (optimal or good). Results are summarized in table 2.

Table 2. **HER-2 BRISH systems and assessment marks for BRISH HER-2 run H11.**

Two colour HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	91	Ventana	32	24	15	20	62%	65%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER-2 IHC**	14	Ventana	3	4	6	1	50%	58%
DuoCISH pharmDx™ **SK109**	3	Dako	1	1	1	0	-	-
ZytoDot® 2C **C-3022 / C-3032**	5	ZytoVision	2	2	1	0	80%	100%
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	3	Ventana	0	2	0	1	-	-
ZytoDot® **C-3003**	5	ZytoVision	1	1	1	2	40%	75%
Total	121		39	34	24	24	60%	-
Proportion			32%	28%	20%	20%		

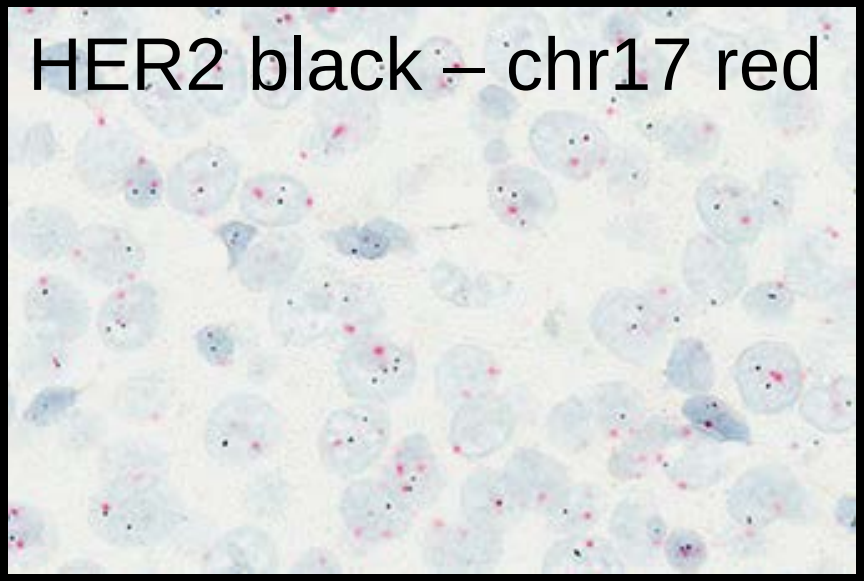
1) Proportion of sufficient stains.
2) Proportion of sufficient stains with optimal protocol settings only, see below.

IHC - Protocols and controls for Breast tumours

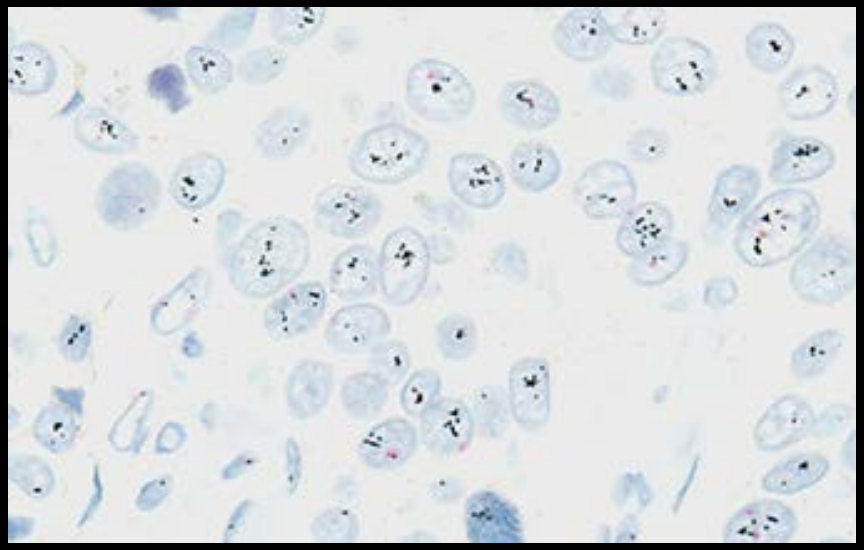
Technically optimal results in the NordiQC HER2 ISH breast module:

INFORM™ HER2 Dual ISH, Ventana

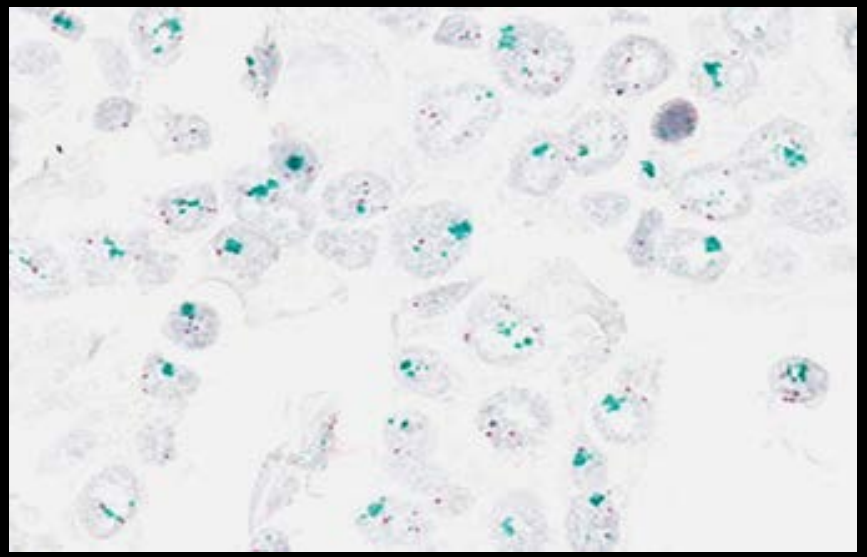
ZytoDot® 2C, ZytoVision



U



A



IHC - Protocols and controls for Breast tumours

Typical causes for insufficient results in the NordiQC HER2 ISH breast module:

FDA / CE-IVD HER2 BRISH (CISH/DDISH/etc) kits:

INFORM™ HER2 Dual ISH, Ventana:
Excessive proteolysis (>16M), HIER in CC1.

DuoCISH™ pharmDx™, Dako:
Insufficient proteolysis, inappropriate handling of chromogen.

Zyto*Dot*® 2C, ZytoVision:
Excessive proteolysis.

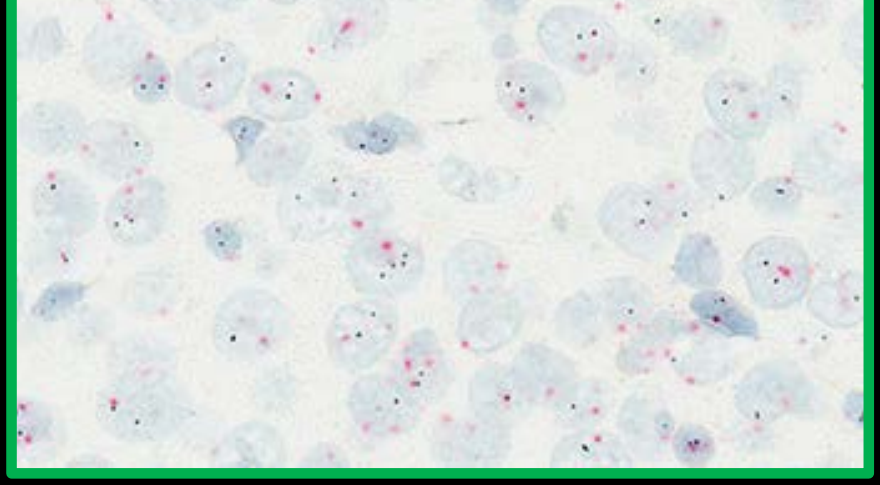
In 90% of insufficient results, no single or combination of causes could be identified

IHC - Protocols and controls for Breast tumours

Technically insufficient results in the NordiQC HER2 ISH breast module:

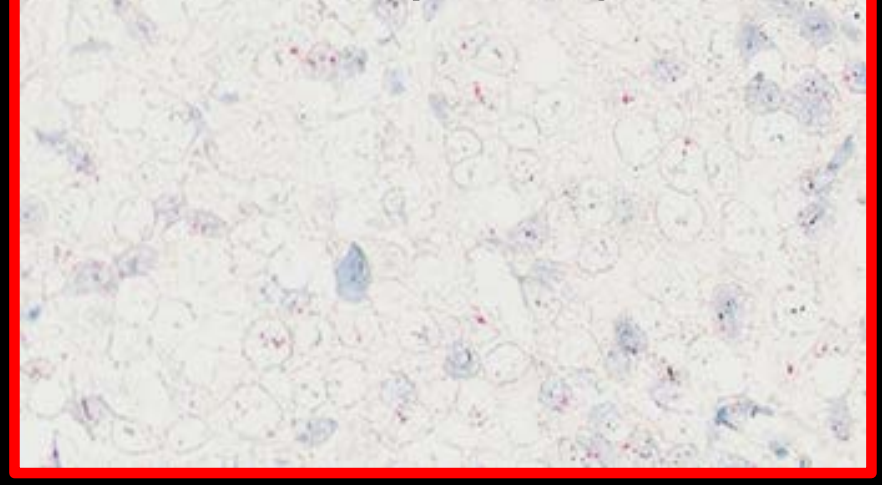
INFORM™ HER2 Dual ISH, Ventana

HER2 black – chr17 red

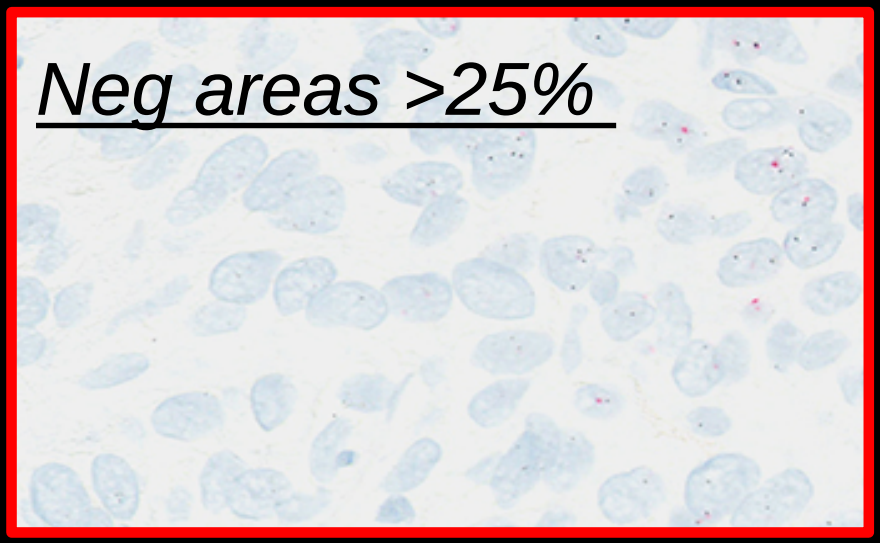


U

Excessive protelysis

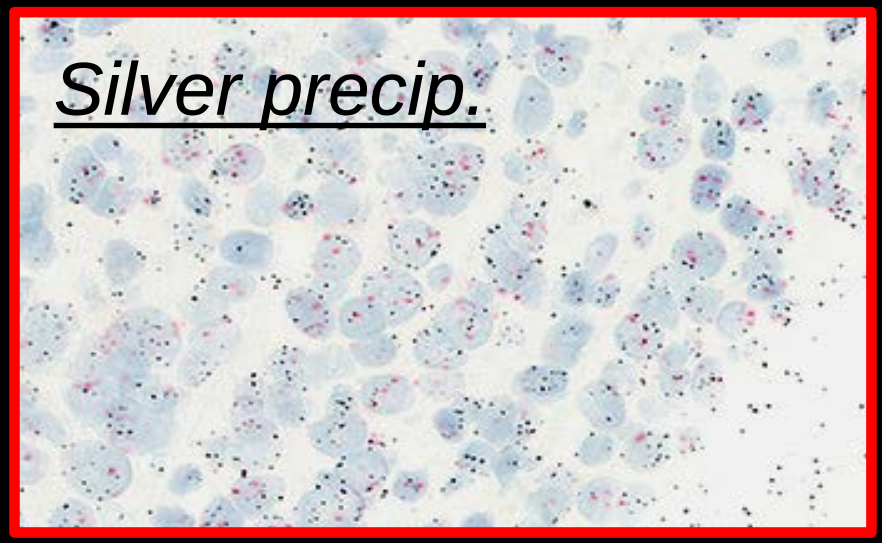


Neg areas >25%



U

Silver precip.

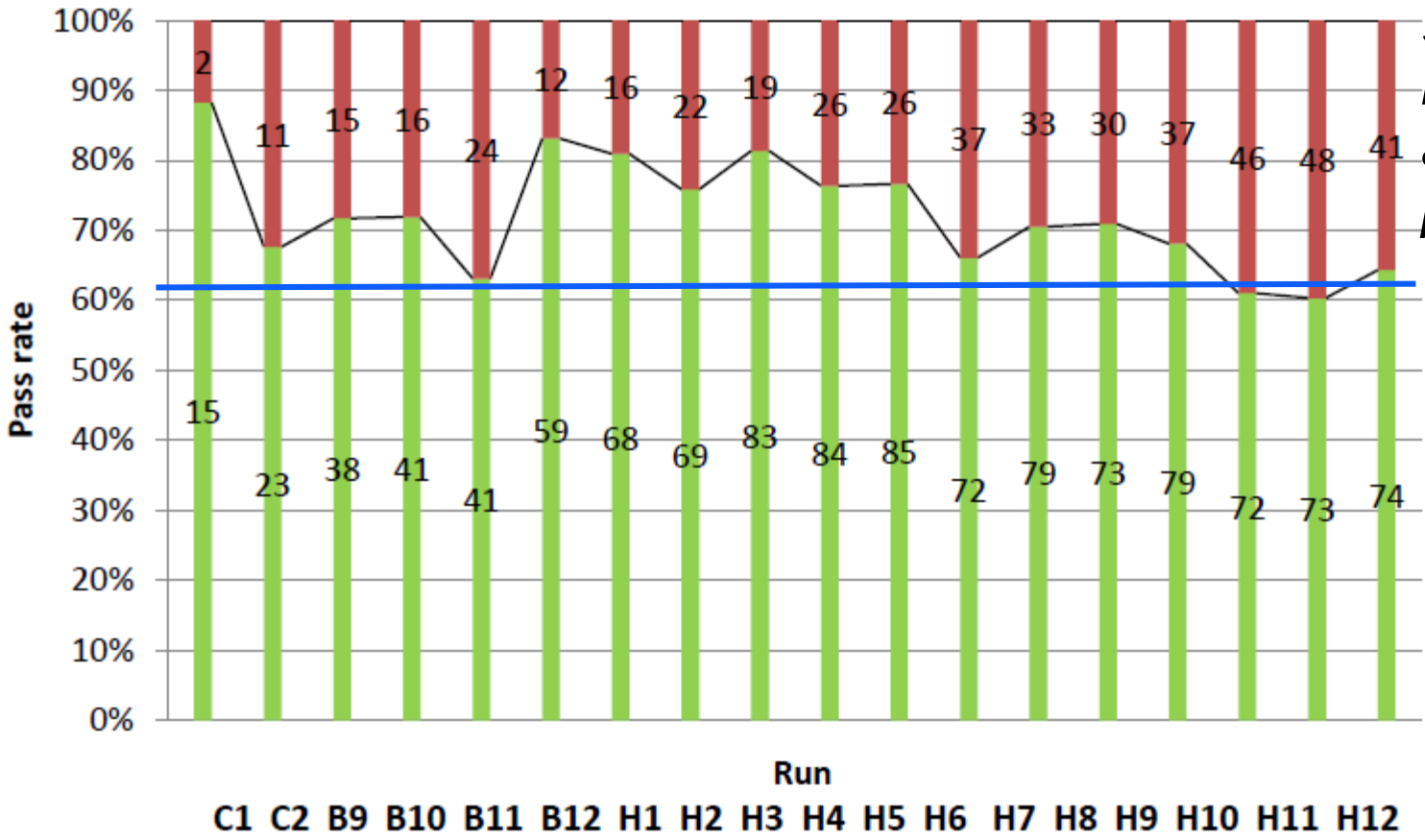


IHC - Protocols and controls for Breast tumours

HER-2 BRISH, Technical assessment

The main criteria for assessing a BRISH HER-2 analysis as technically **optimal** were the ability to interpret the signals and thus evaluate the HER2/chr17 ratios in all five tissues.

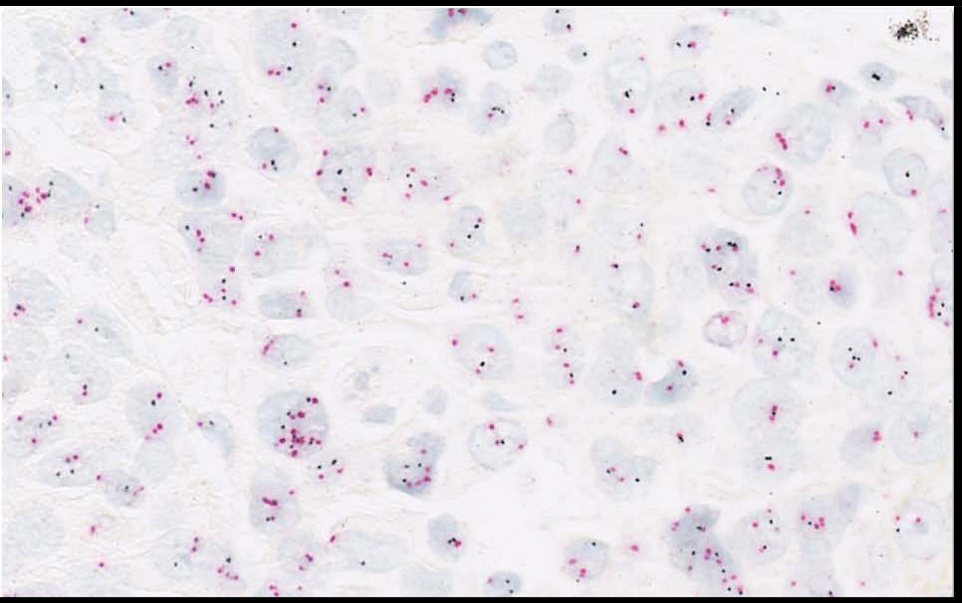
Graph 1. **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessment**

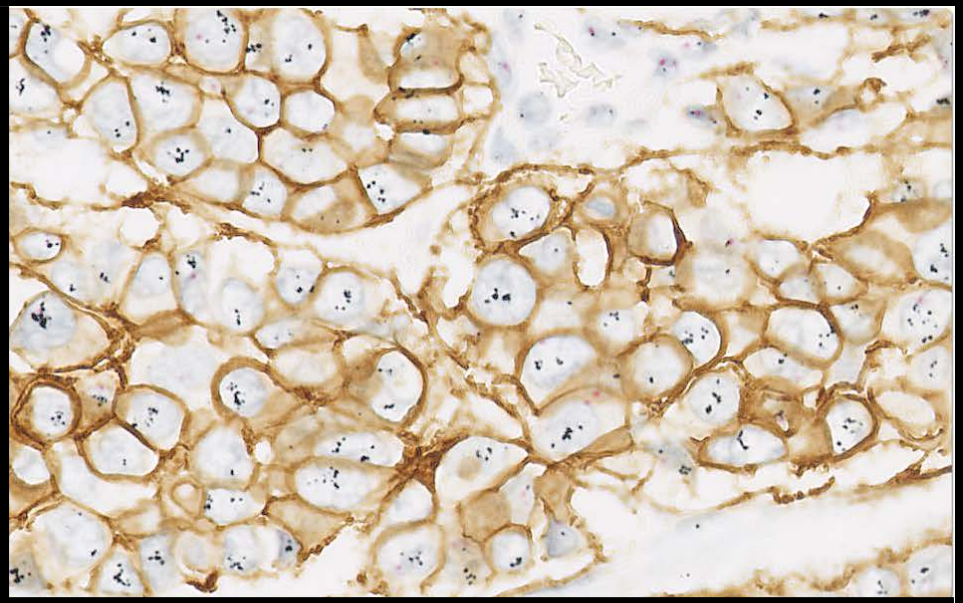


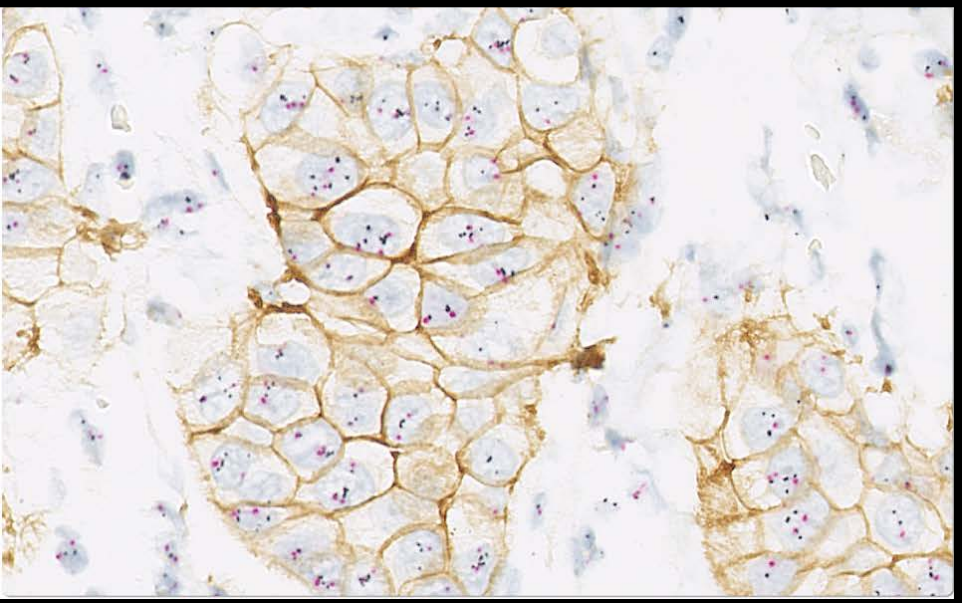
90% of ins. results used appropriate protocol.

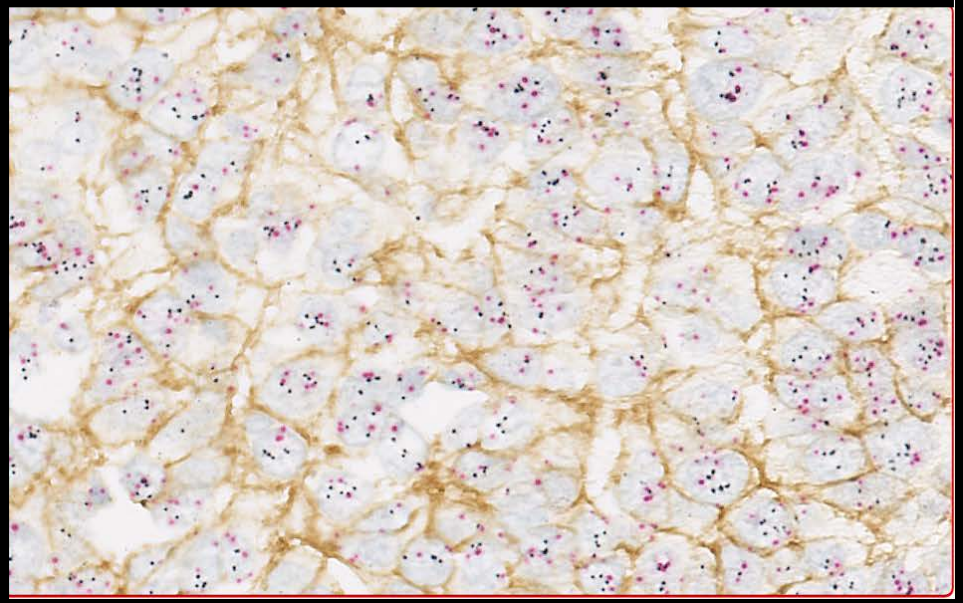
IHC - Protocols and controls for Breast tumours

HER2 Gene-Protein-Assay (Roche): HER2 IHC + DDISH – lim. EQA data









IHC - Protocols and controls for Breast tumours

Participation

Number of laboratories registered for HER-2 BRISH	124
Number of laboratories returning slides	115 (93%)
Number of laboratories returning scoring sheet	105 (92%)
Number of laboratories registered for HER-2 FISH	60
Number of laboratories returning scoring sheet	56 (93%)

Latest Run

Results BRISH, technical assessment

In total, 115 laboratories participated in this assessment. 73 laboratories (64%) achieved a sufficient mark (optimal or good). Results are summarized in Table 2.

Table 2. **HER-2 BRISH systems and assessment marks for BRISH HER-2 run H12.**

Two colour HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	83	Ventana	28	23	19	13	61%	64%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	17	Ventana	11	5	1	0	94%	100%
Zyto*Dot*® 2C **C-3022 / C-3032**	6	ZytoVision	2	1	1	2	50%	50%
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	4	Ventana	1	0	0	3	25%	-
Zyto*Dot*® **C-3003**	5	ZytoVision	1	2	1	1	60%	60%
Total	115		43	31	22	19	64%	-
Proportion			37%	27%	19%	17%		

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

HER2 – NordiQC perspective

Central issues to adress for control material of HER2 IHC test

1. External control – no useful internal HER2 expression

2. Must be processed under similar conditions as patient material – otherwise documented that identical results are generated by other methods (fixative, duration, etc)

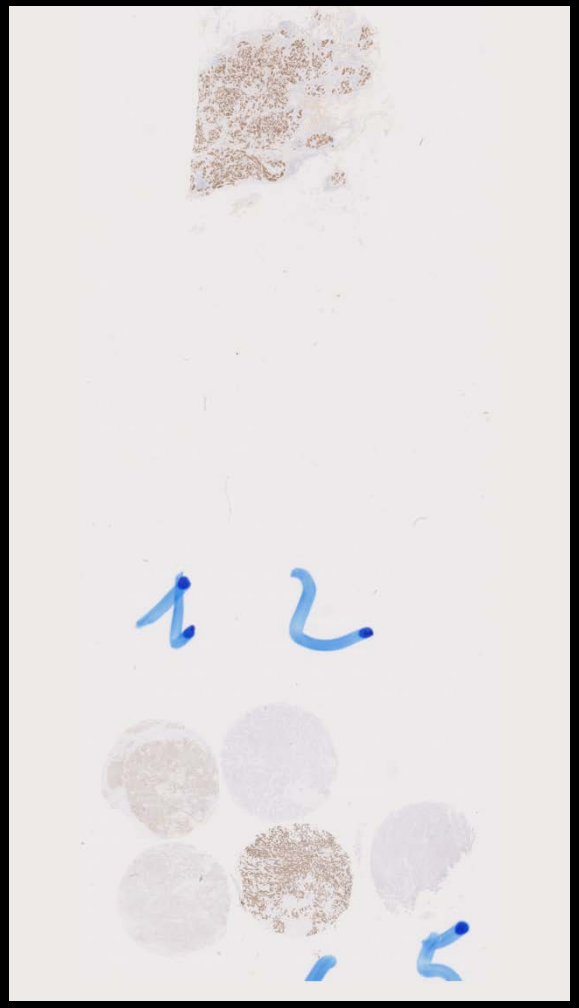
3. Must reflect the range of HER2 expression seen for diagnostics

4. Consistent and stable HER2 expression throughout the control material

HER2 – NordiQC perspective

Central issues to adress for control material of HER2 IHC test

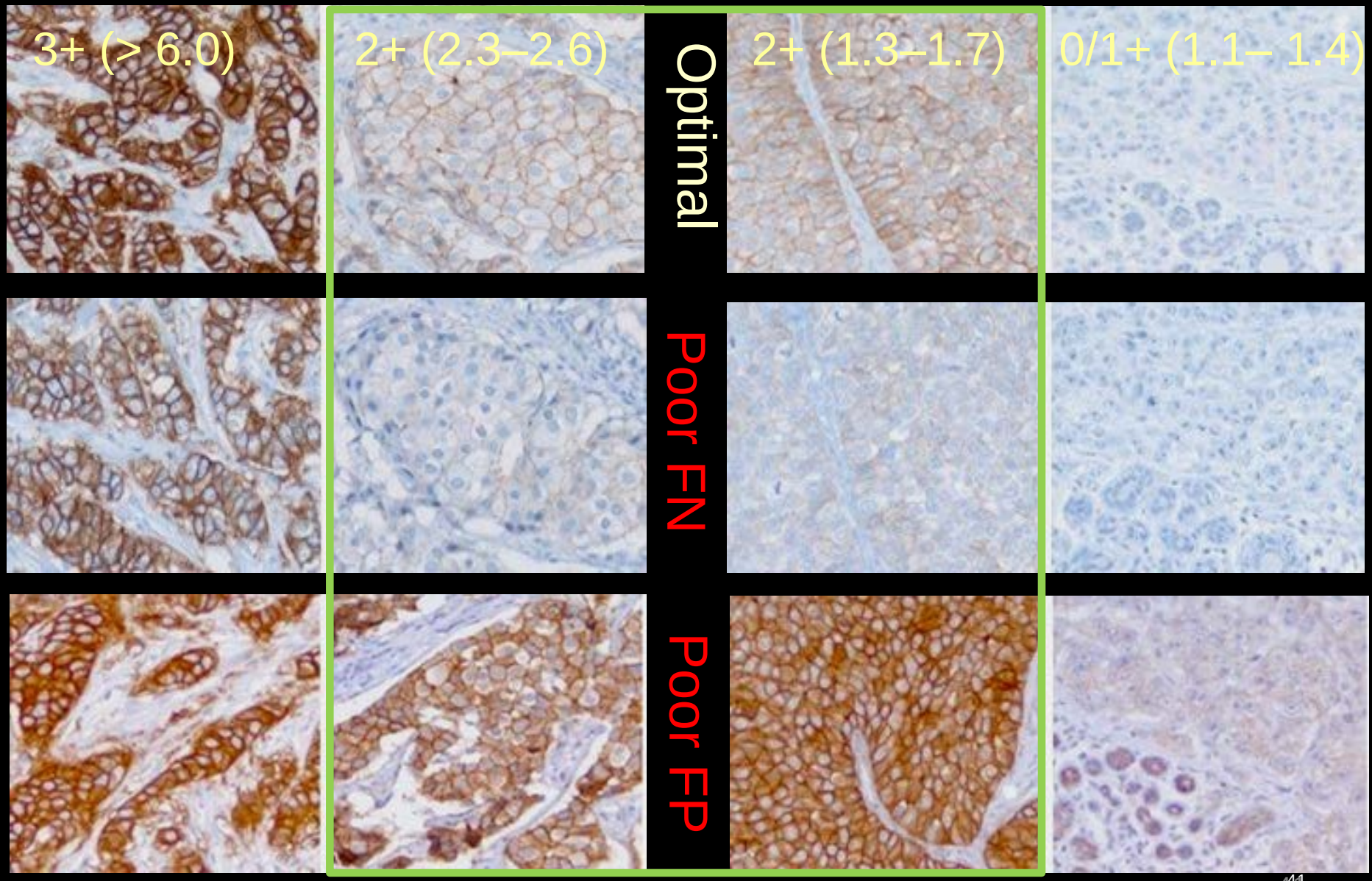
In NordiQC app. 60-70% of laboratories use a 3+ tumour as routine positive control for HER2 IHC



Question:

Is this reliable to monitor a consistent level of HER2 assay ?

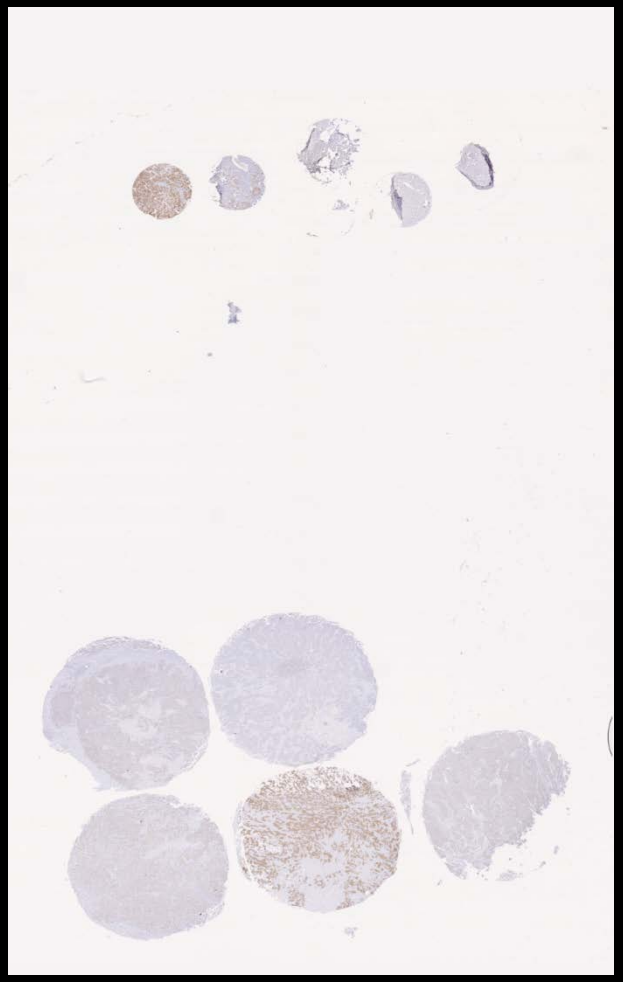
HER2 – NordiQC perspective



HER2 – NordiQC perspective

Central issues to adress for control material of HER2 IHC test

In NordiQC app. 60-70% of laboratories use a 3+ tumour as positive control for HER2 IHC



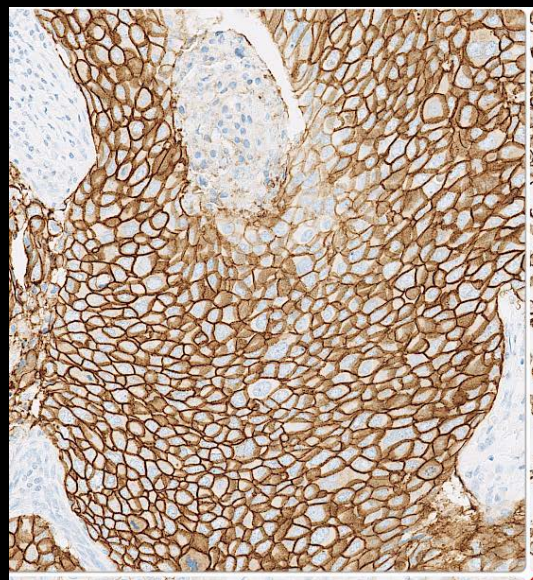
Optimally:

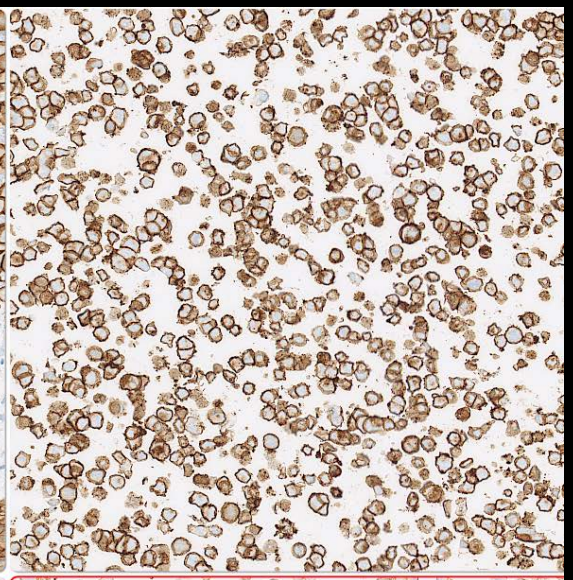
Use small TMA with 1+, 2+ & 3+ mounted on same slide as pt material for daily control of HER2 IHC assay

IHC - Protocols and controls for Breast tumours

Control material for HER2 IHC: performace control / consistency

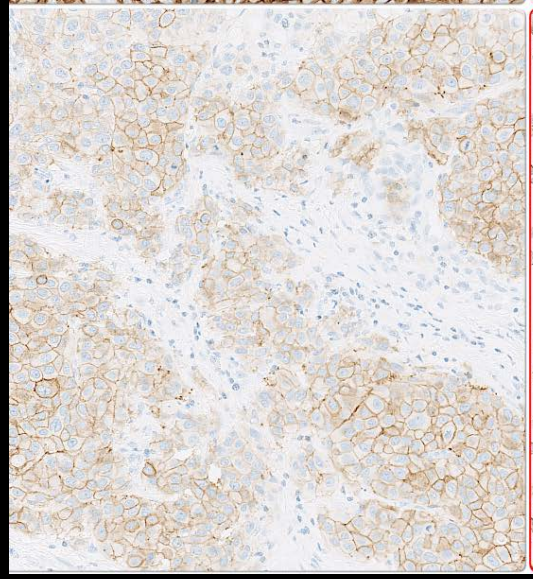
Histology:
3+ tumour

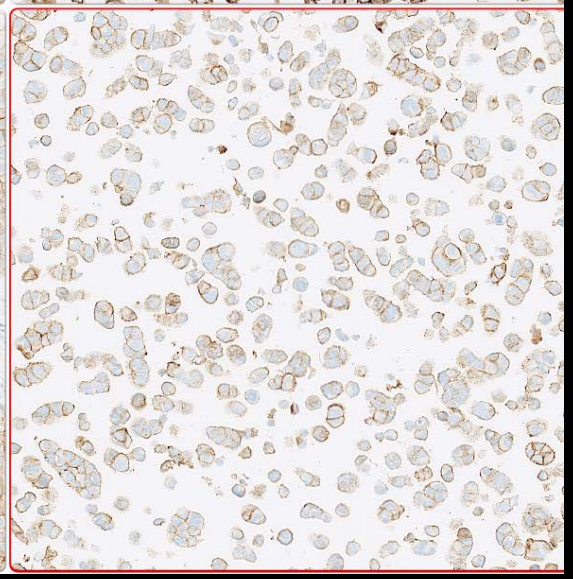




Cell lines:
3+

<u>2+ tumour</u>





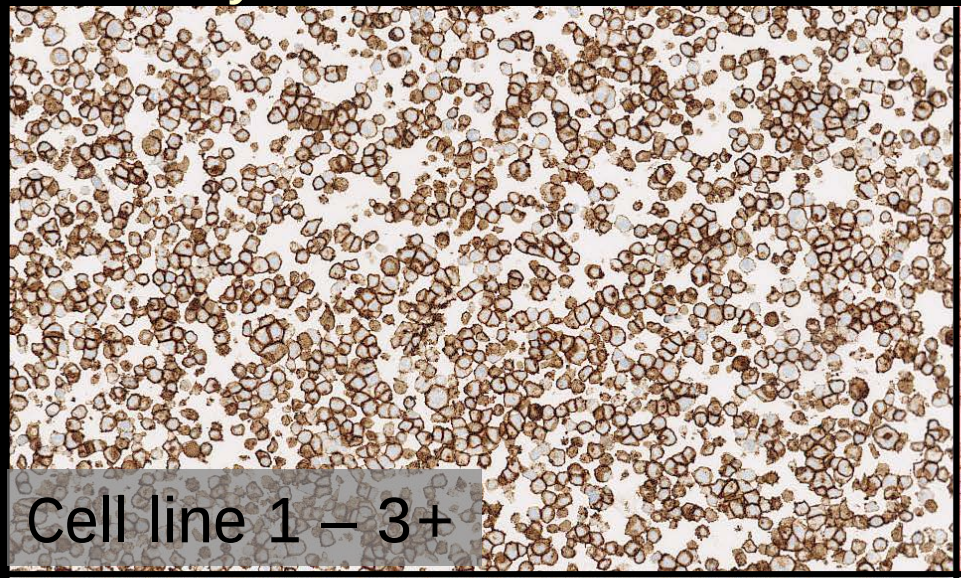
<u>2+</u>

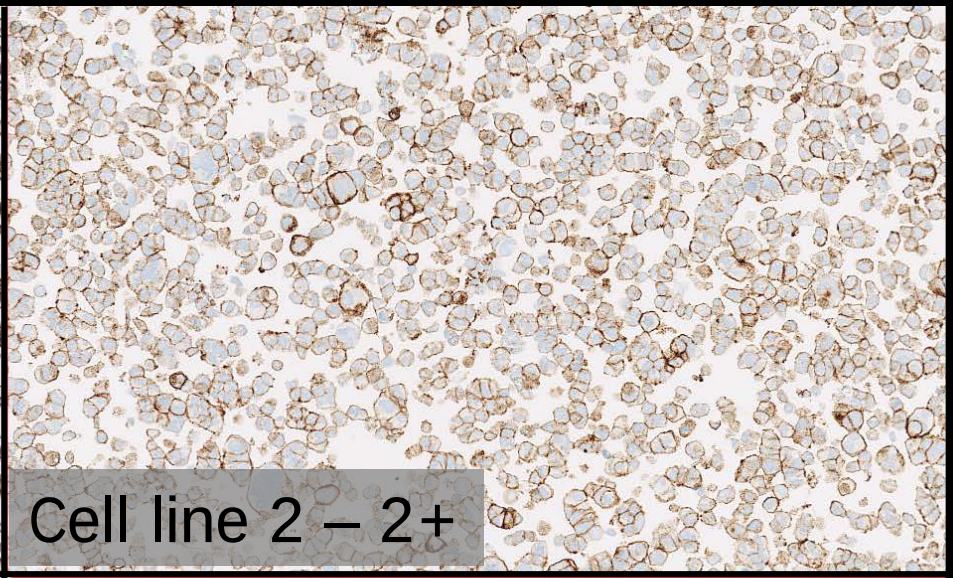
Applicable for DIA & ref data comparing run-to-run

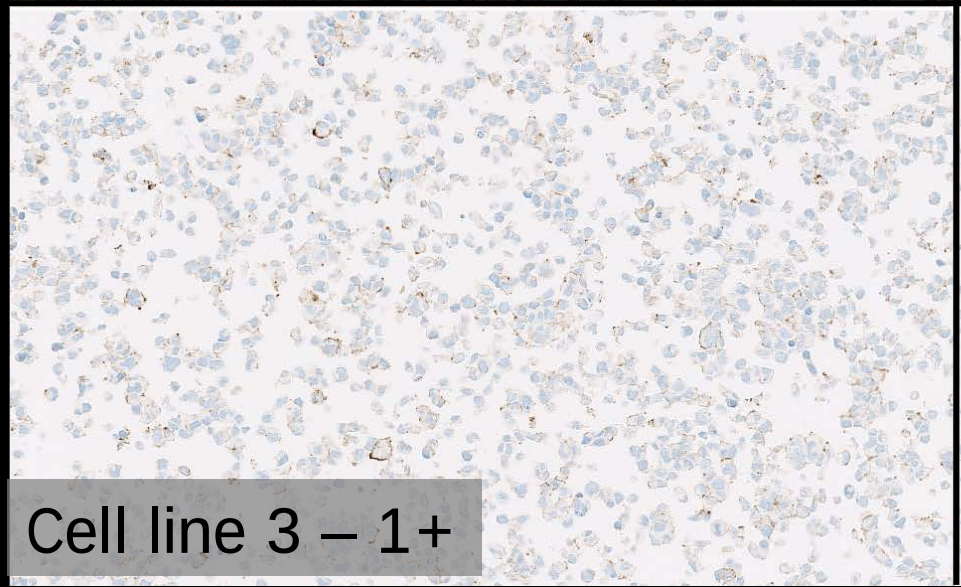
IHC - Protocols and controls for Breast tumours

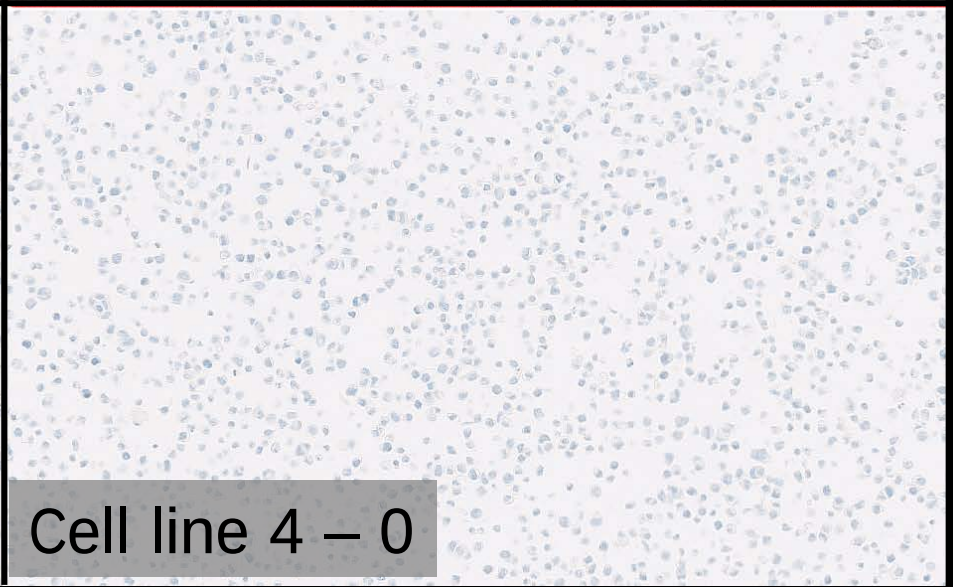
Control material for HER2 IHC: performace control / consistency

Histocyte cell lines HER2: PATHWAY IHC





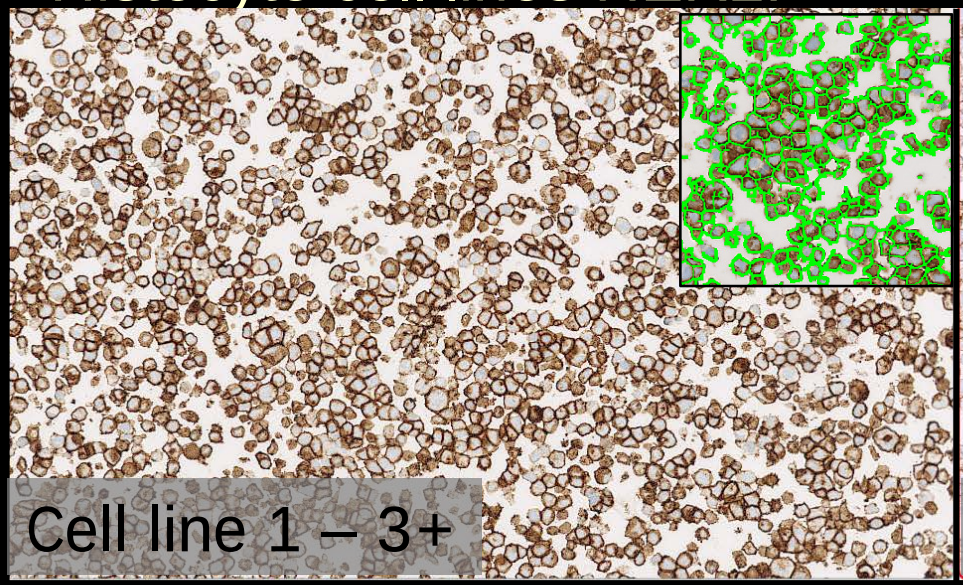




IHC - Protocols and controls for Breast tumours

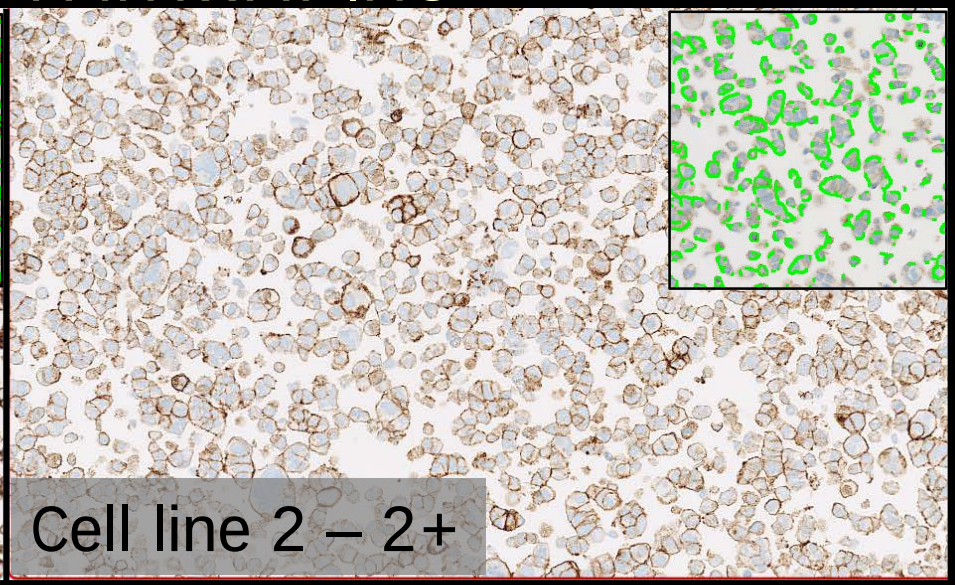
Control material for HER2 IHC: performace control / consistency

Histocyte cell lines HER2:

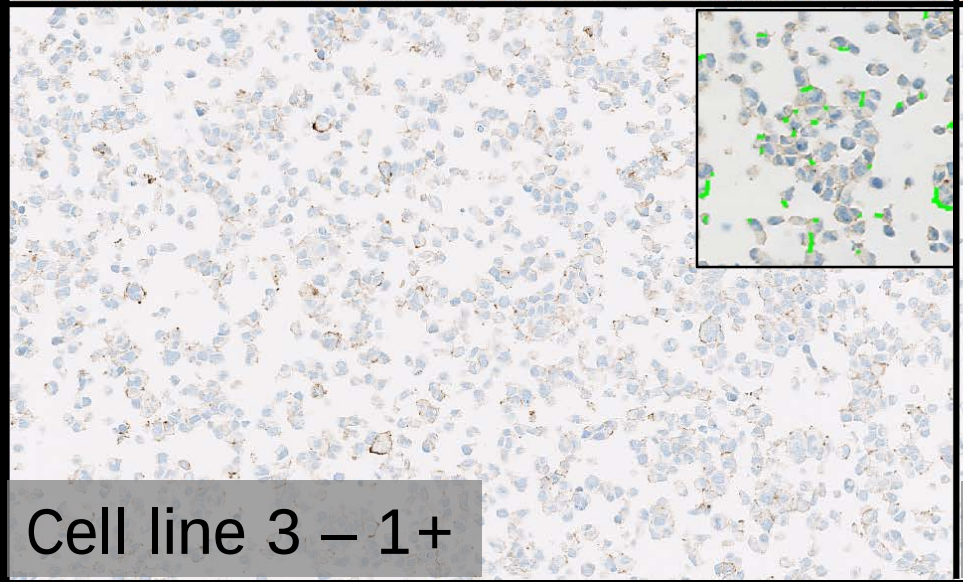


Cell line 1 – 3+

PATHWAY IHC



Cell line 2 – 2+



Cell line 3 – 1+

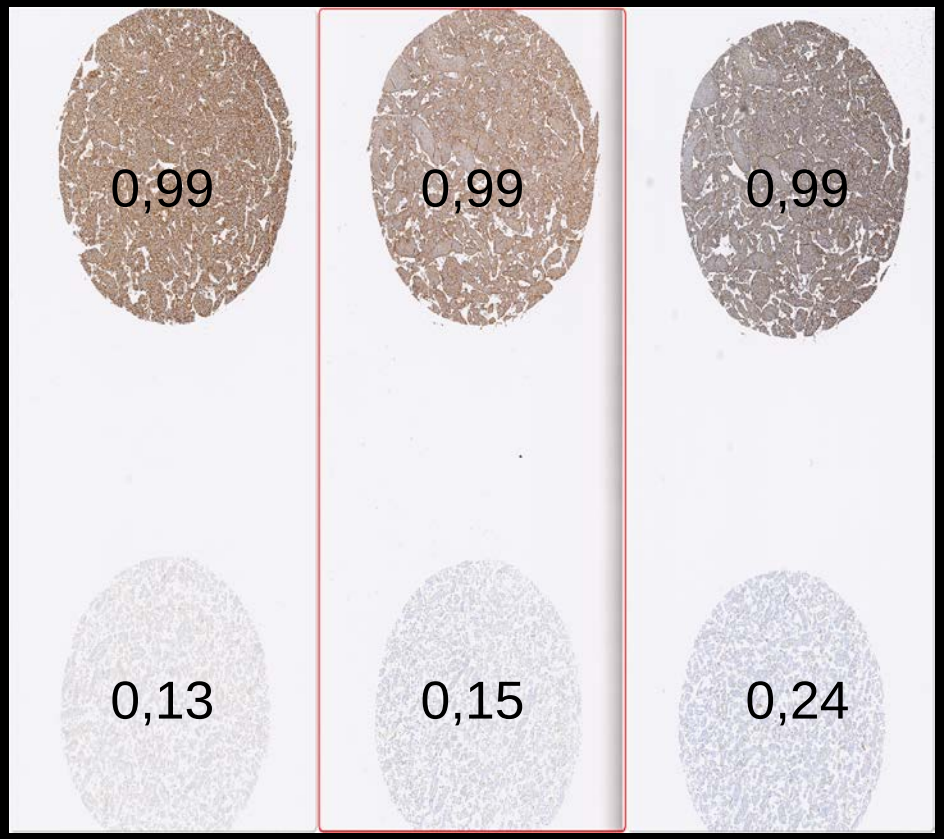
Connectivity	DIA score
≤ 0.40	0/1+
> 0.40 - ≤ 0.64	2+
> 0.64	3+

Cell line 4 – 0

IHC - Protocols and controls for Breast tumours

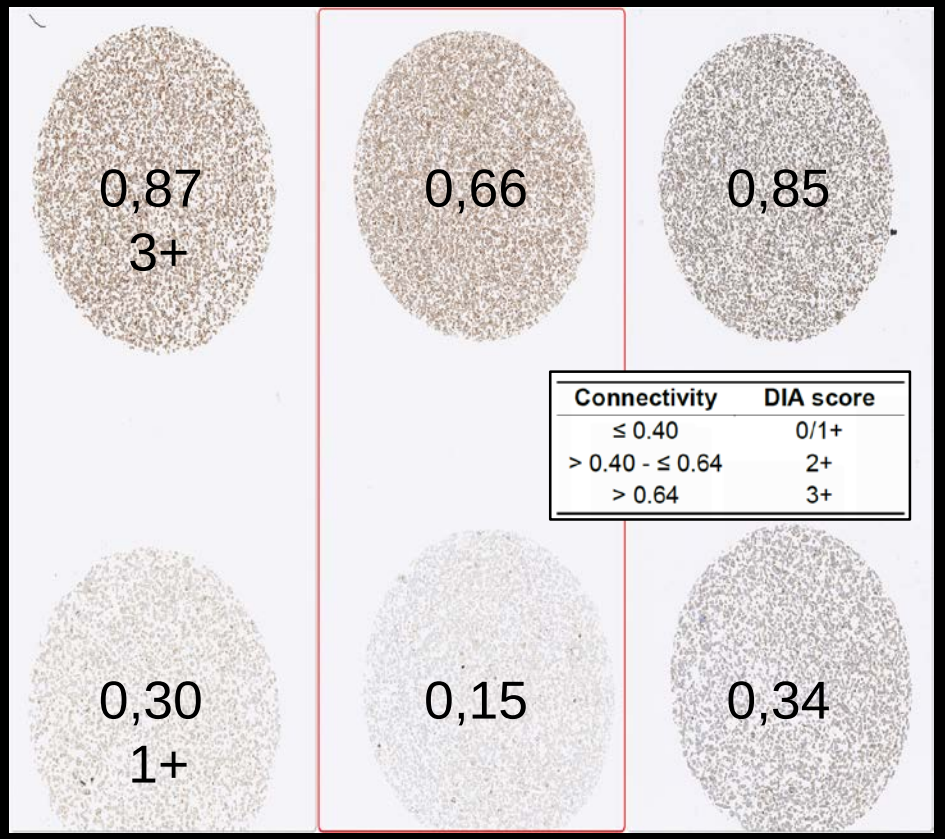
Control material for HER2 IHC: performance control / consistency

Histocyte cell lines HER2:



Pathway Oracle Herceptest

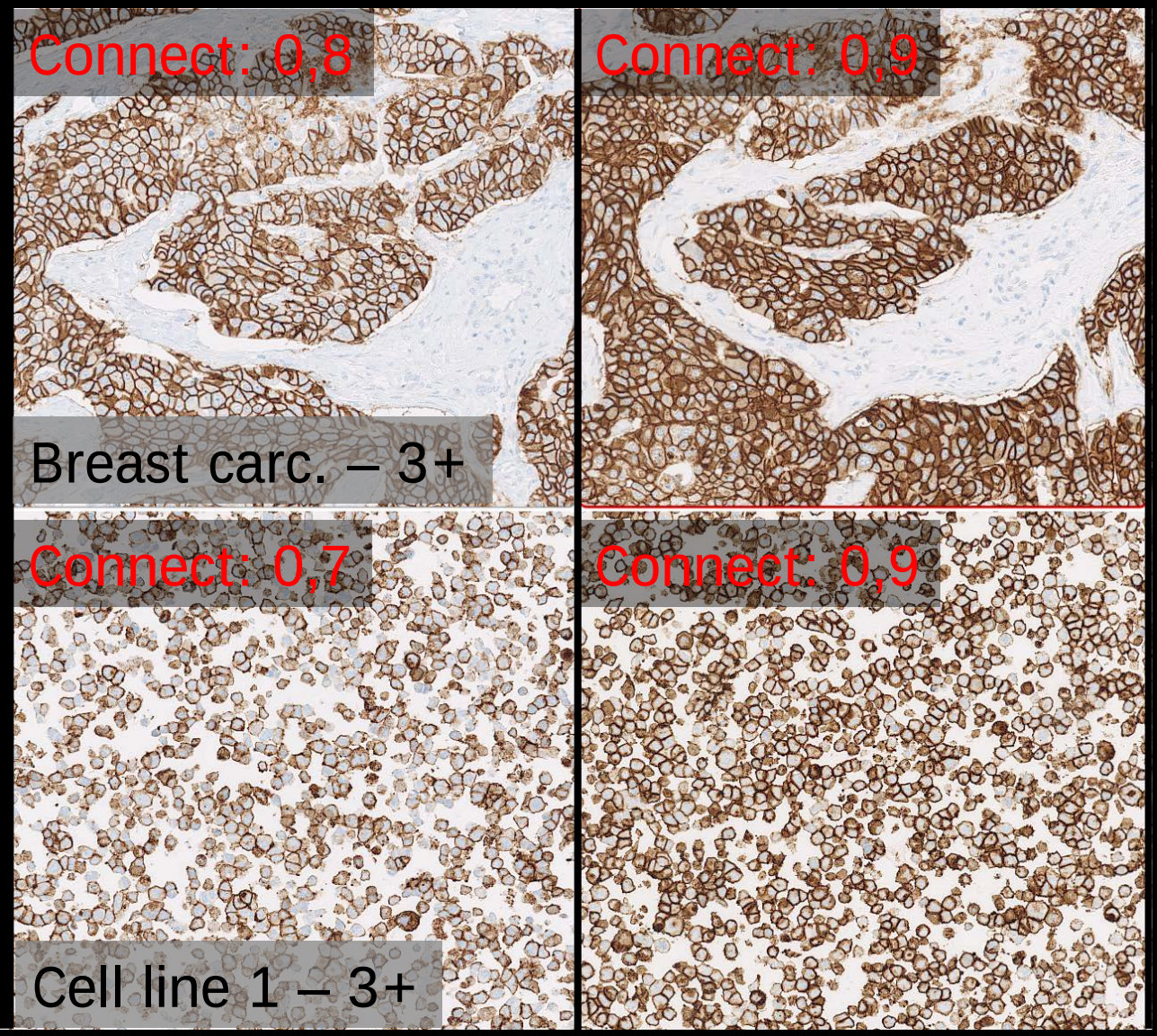
Horizon cell lines HER2



Pathway Oracle HercepTest

IHC - Protocols and controls for Breas

Connectivity	DIA score
≤ 0.40	0/1+
> 0.40 - ≤ 0.64	2+
> 0.64	3+



PATHWAY Run 1 – Opt

PATHWAY Run. 2 – Opt.

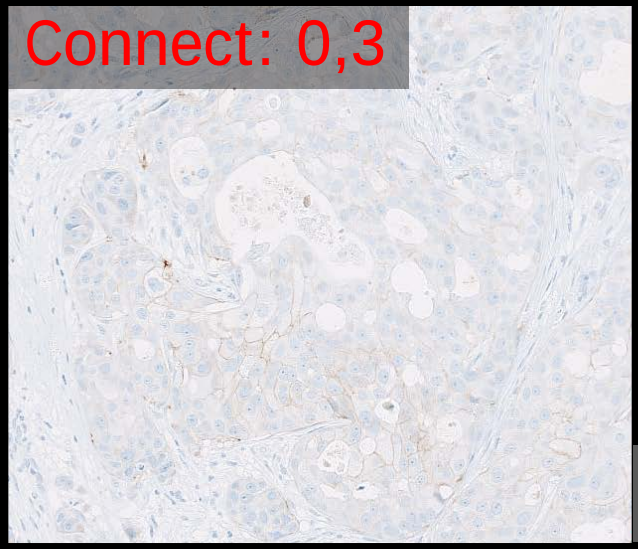
Reference values

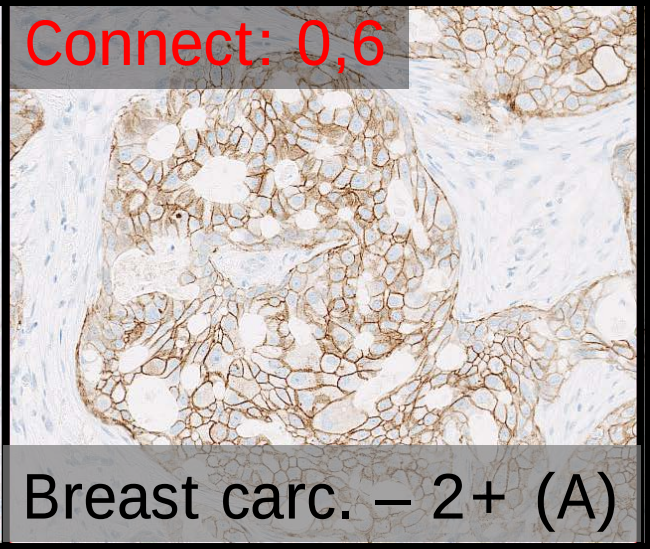
Tolerance criteria

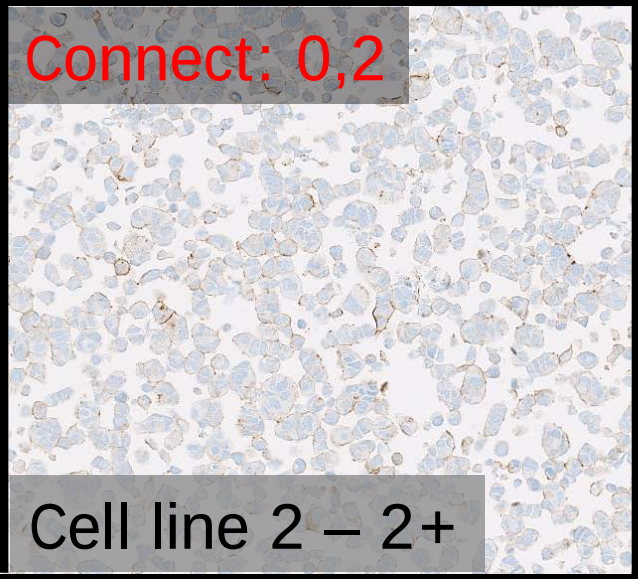
If ok in cell line – Tumour can ce scored

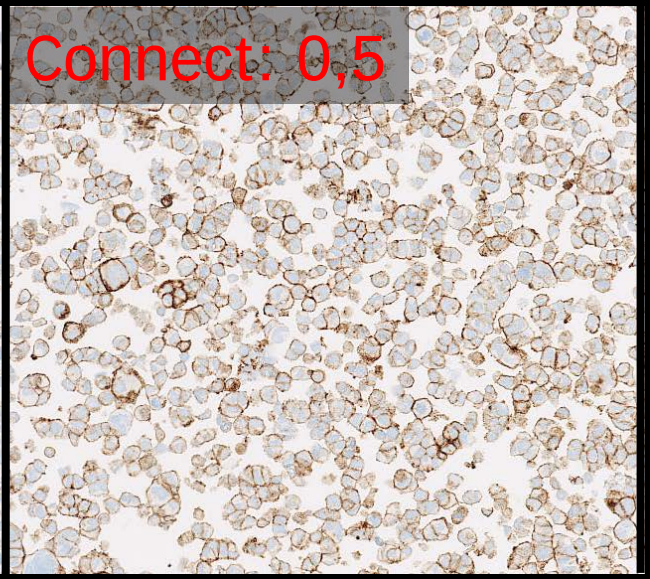
IHC - Protocols and controls for Breas

Connectivity	DIA score
≤ 0.40	0/1+
> 0.40 - ≤ 0.64	2+
> 0.64	3+









Reference values

Tolerance criteria

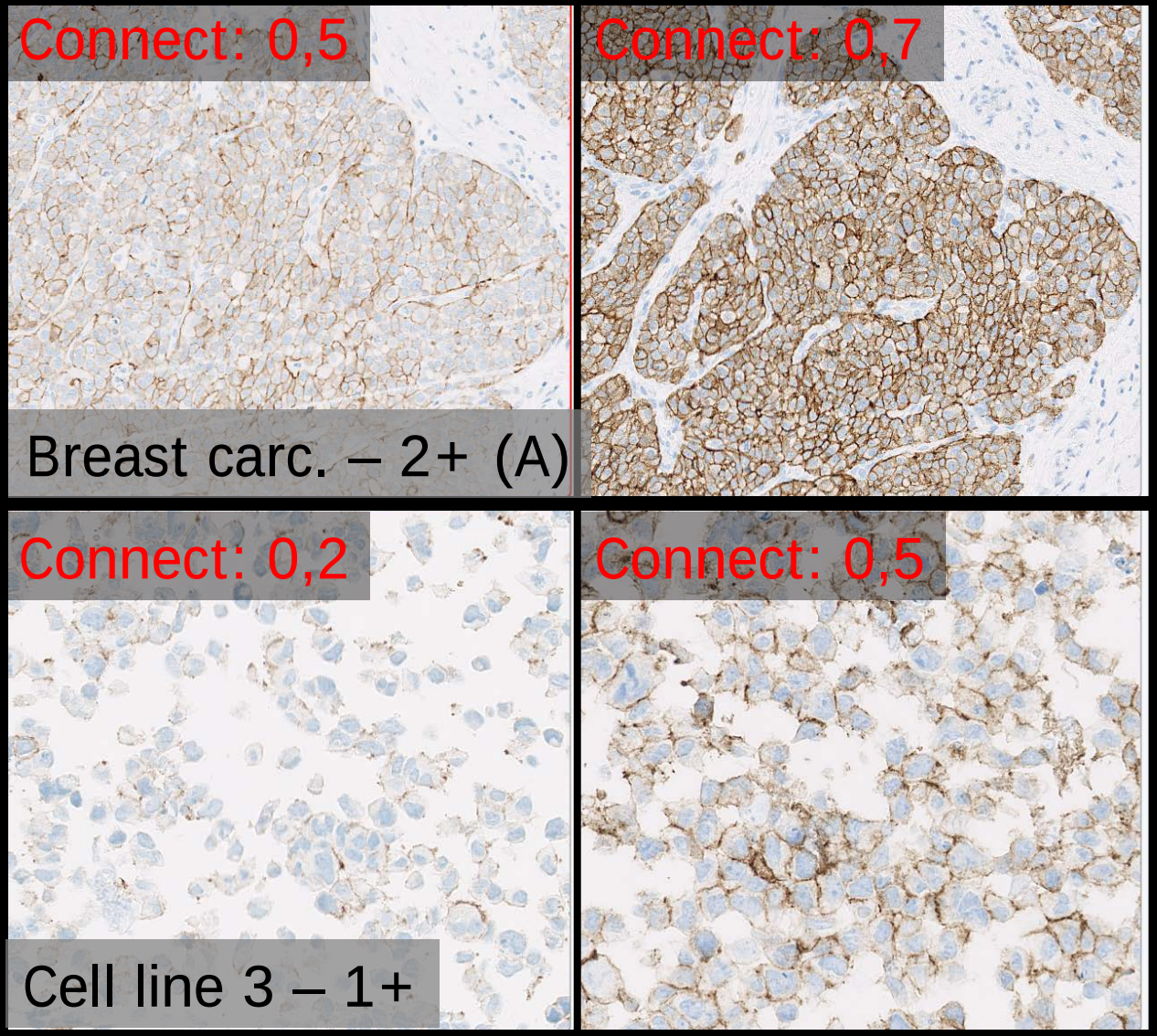
If ok in cell line – Tumour can ce scored

PATHWAY Run 1 – FN

PATHWAY Run 2 – Opt.

IHC - Protocols and controls for Breas

Connectivity	DIA score
≤ 0.40	0/1+
> 0.40 - ≤ 0.64	2+
> 0.64	3+



PATHWAY Run 1 – Opt

PATHWAY Run 2 – FP

Reference values

Tolerance criteria

If ok in cell line – Tumour can ce scored

IHC - Protocols and controls for Breast tumours

The combination of cell lines and DIA might be useful for;

IHC assay reproducibility / consistency
Run-to-Run

Implementation of IHC assay (kits) for verification

Require reference values for sufficient versus insufficient test

Potential for EQA for IHC assays (kits) – LDTS to be confirmed

IHC - Protocols and controls for Breast tumours

Conclusions:

1. Pass rates for ER, PR and HER2 IHC are improved.

   Robust clones, high quality IHC systems.

2. CE-IVD labelled RTU assays / systems have shown superior performance compared to laboratory developed assays.

3. HER2 BRISH (DDISH/SISH/CISH) results have not been improved.

IHC - Protocols and controls for Breast tumours