NordiQC

Workshop in Diagnostic Immunohistochemistry
Aalborg University Hospital, September 19th – 21st 2016

Optimization of antibodies, selection, protocols and controls

Breast tumours

Søren Nielsen
Project coordinator & Scheme Manager NordiQC
Aalborg University Hospital, Denmark

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

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- Mammaglobin
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- (p63)
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- **ER**
- **PR**
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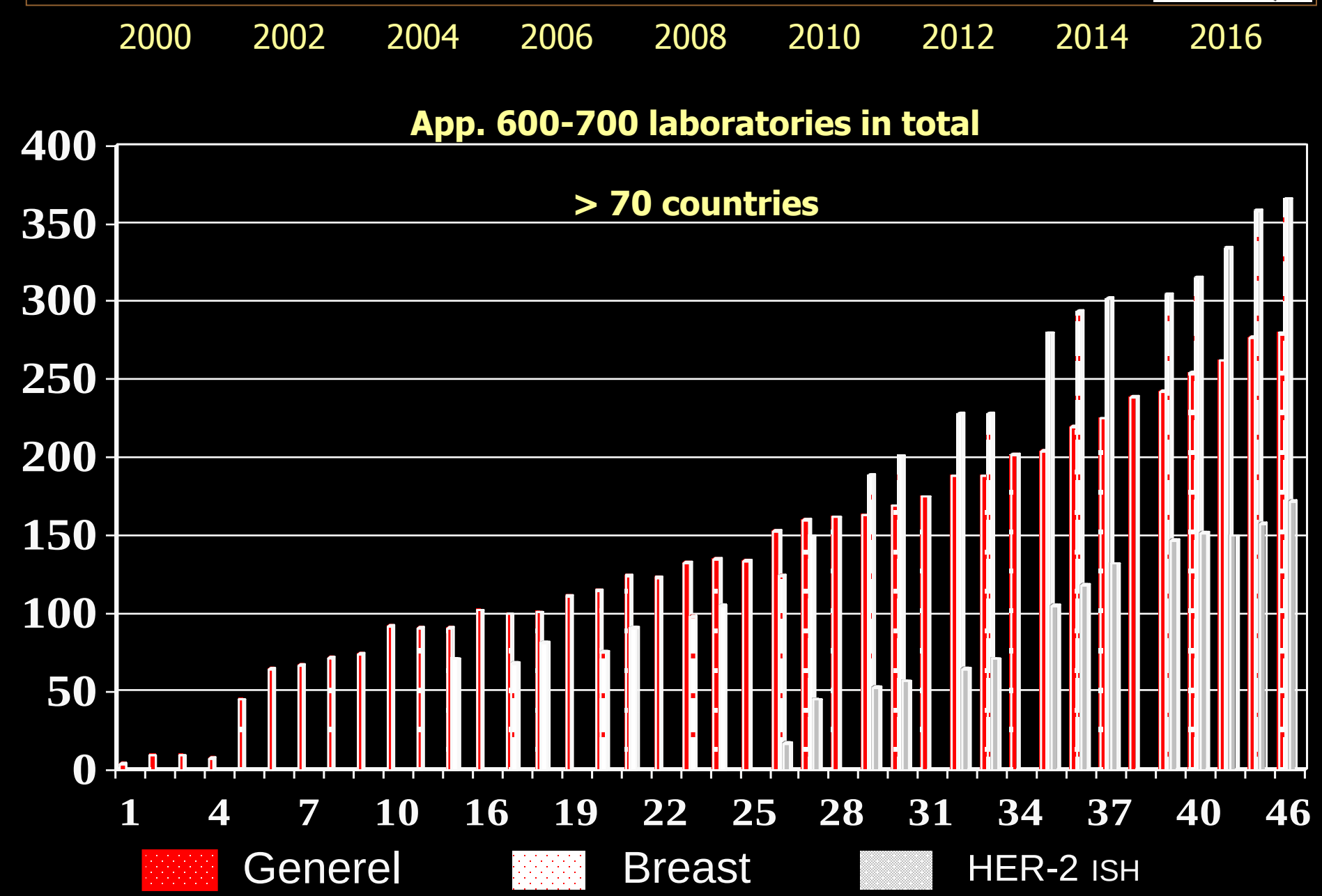

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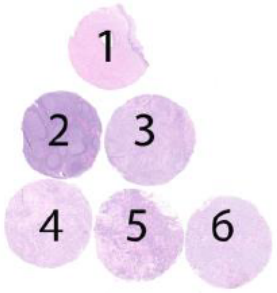
Assessment Run B21 2016

Estrogen receptor (ER)

Material

The slide to be stained for ER comprised:

No.	Tissue	ER-positivity*	ER-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Tonsil	1-5%	Weak to strong
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	40-60%	Weak to moderate
5.	Breast carcinoma	60-80%	Weak to strong
6.	Breast carcinoma	80-100%	Moderate to strong



*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

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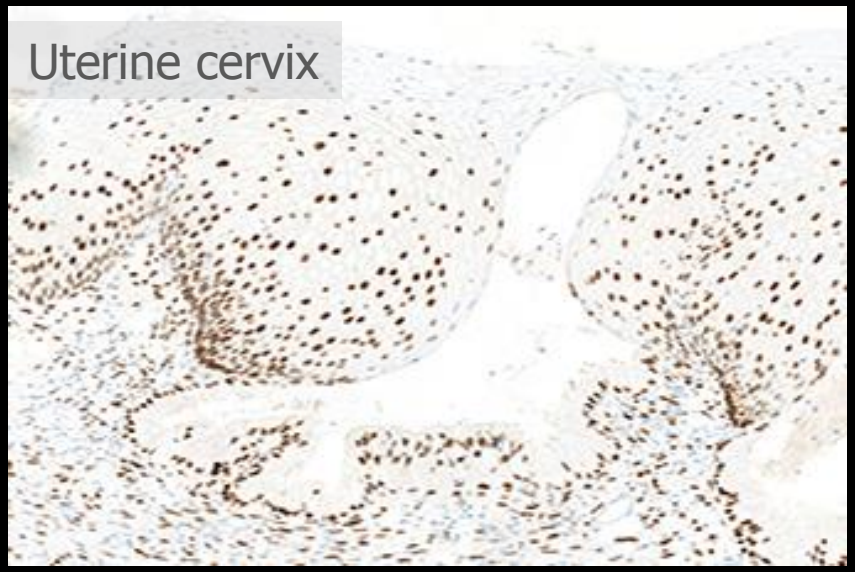

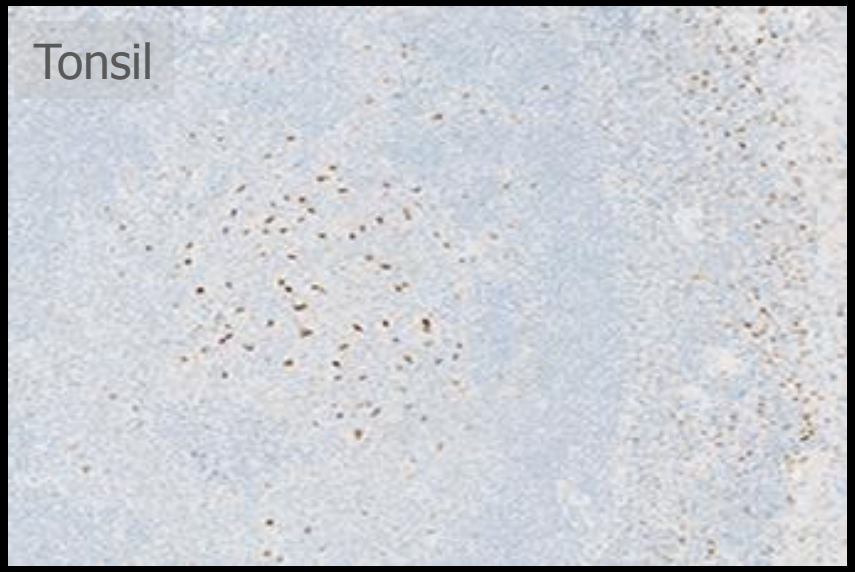

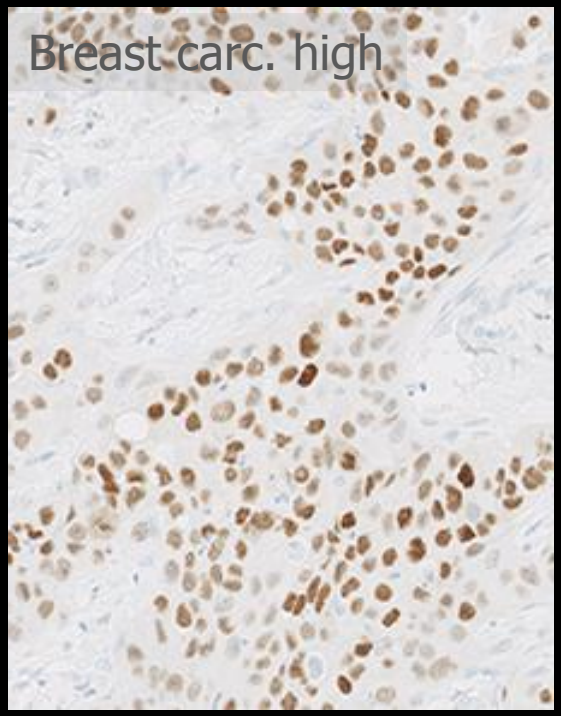

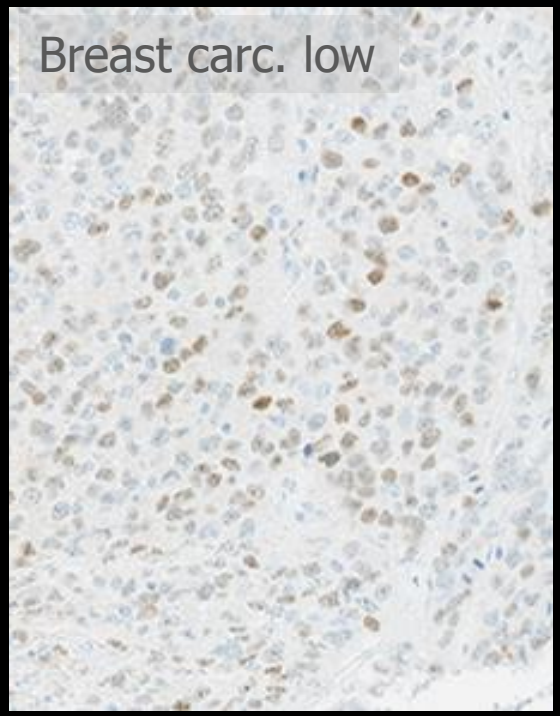

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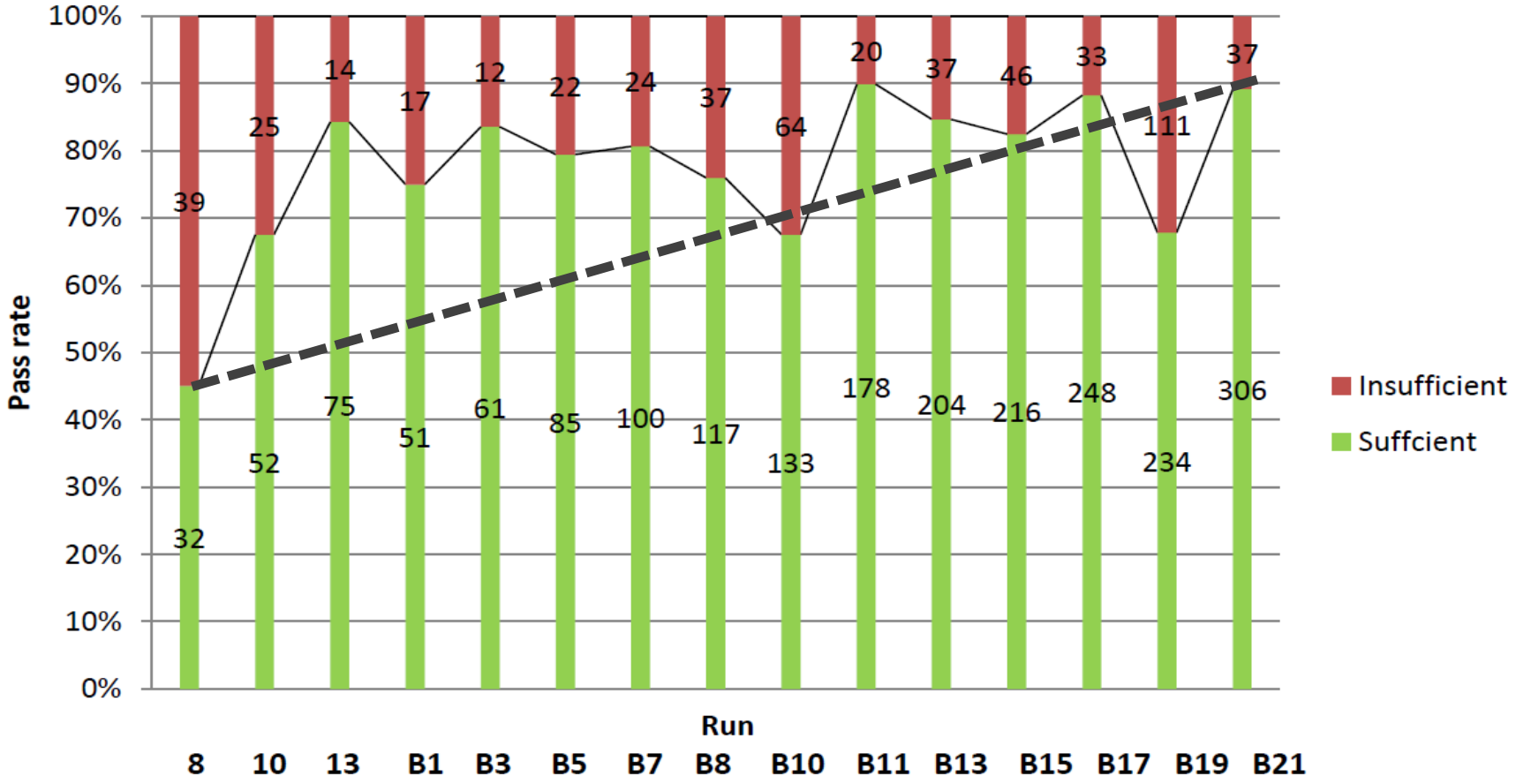
Performance history

This was the 15th NordiQC assessment of ER. The proportion of sufficient results was increased compared to the last run and now back at a comparable level as seen from run B11 and onward. (Figure 1).

Fig. 1. **Participant numbers and pass rates for ER during 15 runs**



2003 2005 2007 2009 2011 2013 2015 2016

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Estrogen receptor;

Pass rate influenced by participation

Performance history
This was the 15th NordiQC assessment of ER. The proportion of sufficient results was increased compared to the last run and now back at a comparable level as seen from run B11 and onward. (Figure 1).

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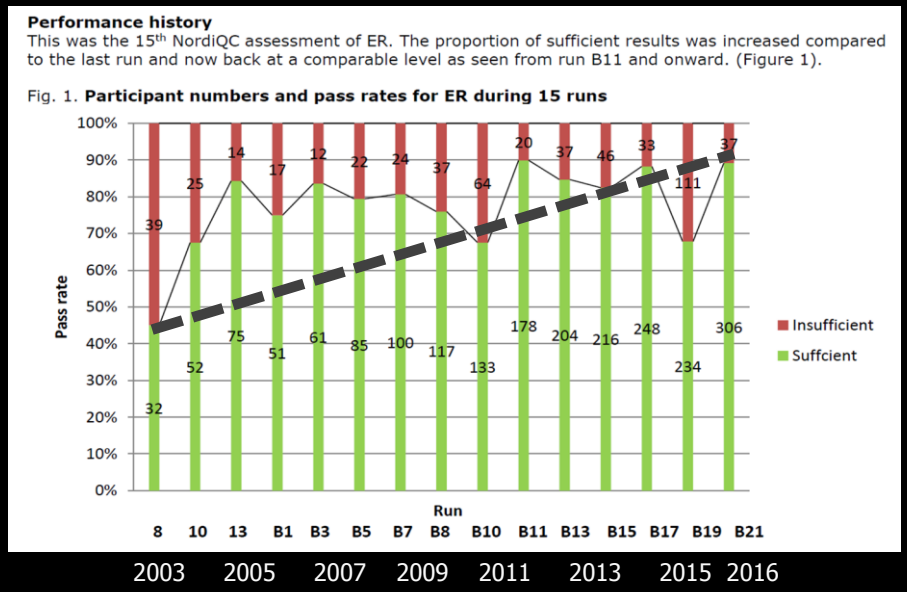

	New participants	”Old” participants
Run B10, 2004	57% (n=61)	71% (n=134)
Run B15, 2010	70% (n=54)	86% (n=208)
Run B19, 2015	51% (n=86)	73% (n=259)

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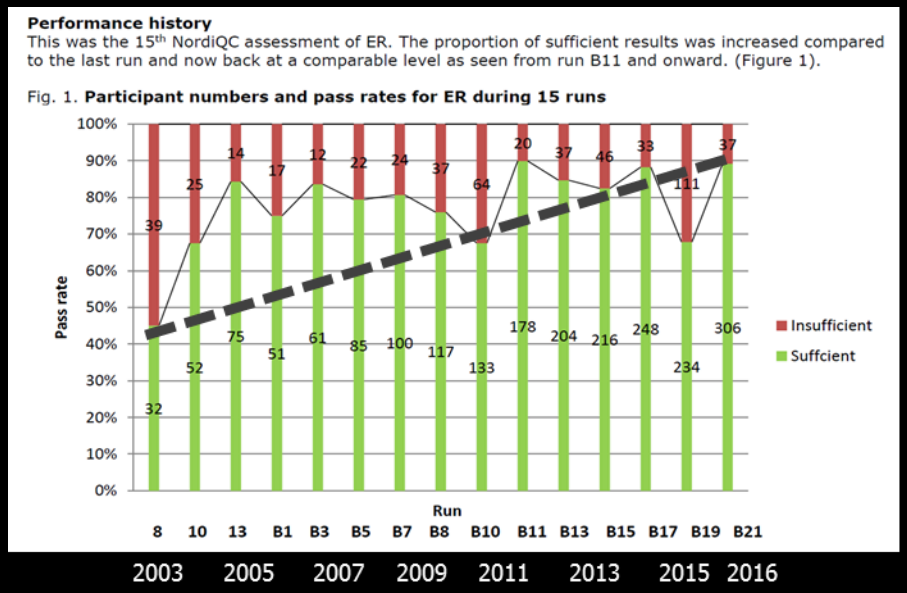
Estrogen receptor;

Pass rate influenced by protocol harmonization



	2003 B8	2013 B15
Titre range / average titre	1:10-1.000 / 1:125	1:10-400 / 1:90
HIER by in-house buffer	88%	6%
HIER by high pH	70%	94%
Polymer/multimer kit	56%	93%
Fully automated system	6%	59%

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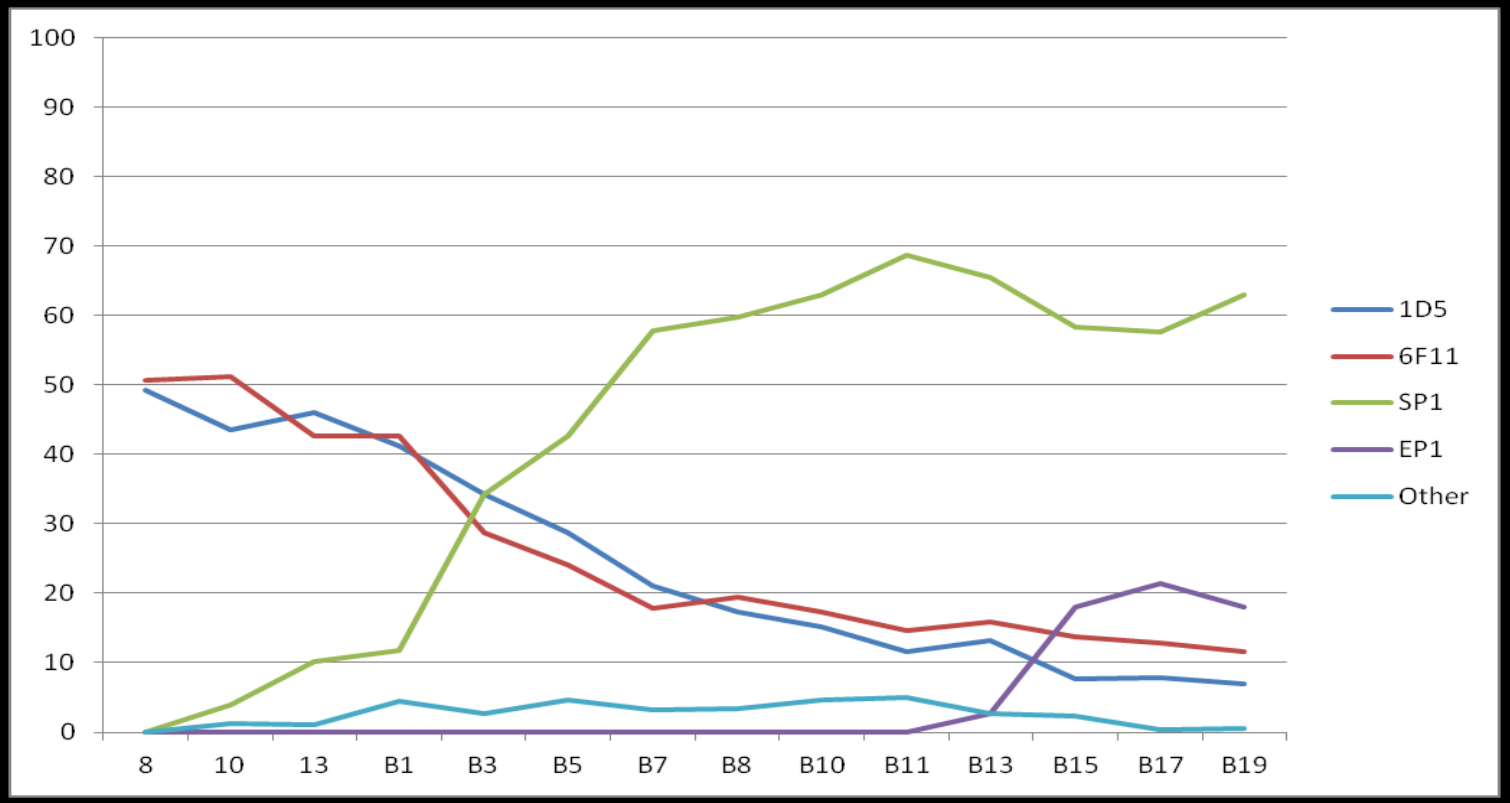


2003 2005 2007 2009 2011 2013 2015

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

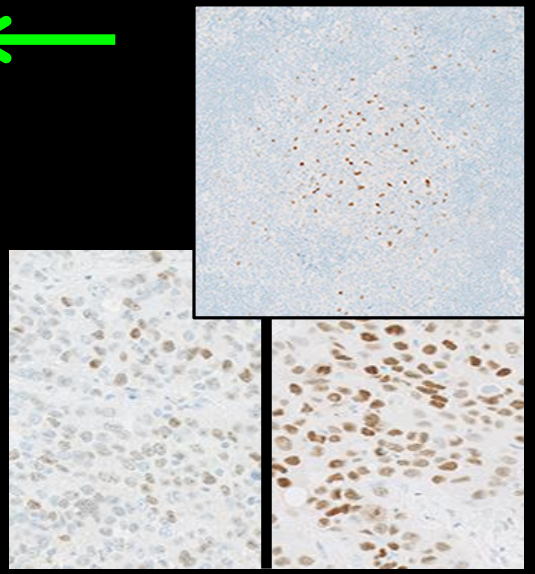
Table 1. **Antibodies and assessment marks for ER, run B21**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1D5**	3 1	Dako/Agilent Zytomed	0	1	1	2	-	-
mAb clone **6F11**	27	Leica/Novocastra	9	14	4	0	85%	91%
rmAb clone **EP1**	18 1	Dako/Agilent Cell Marque	8	7	4	0	79%	77%
rmAb clone **SP1**	28 4 3 2	Thermo/Neomarkers Spring Bioscience Immunologic Cell Marque	28	8	1	0	97%	97%
Ready-To-Use antibodies								
mAb clone **1D5 IR/IS657**	7	Dako/Agilent	3	2	1	1	71%	100%
mAb clones **1D5 + ER-2-123 K4071/SK310**	2	Dako/Agilent	0	1	1	0	-	-
mAb clone **6F11 PA0151**	7	Leica/Novocastra	1	4	2	0	71%	100%
rmAb **EP1 IR/IS084**	61	Dako/Agilent	28	17	8	8	74%	87%
rmAb clone **SP1 790-4324/5**	173	Ventana/Roche	129	40	4	0	98%	98%
rmAb clone **SP1 MAD-000306QD**	2	Master Diagnostica	0	2	0	0	-	-
rmAb clone **SP1 ILM30142-R25**	1	Immunologic	1	0	0	0	-	-
rmAb clone **SP1 KIT-0012**	1	Maixin	1	0	0	0	-	-
rmAb clone **SP1 RM-9101-R7**	1	Thermo/Neomarkers	1	0	0	0	-	-
rmAb + mAb clone cocktail **EP1+6F11 IPI3150**	1	Biocare	1	0	0	0	-	-
Total	343		210	96	26	11	-	
Proportion			61%	28%	8%	3%	89%	

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



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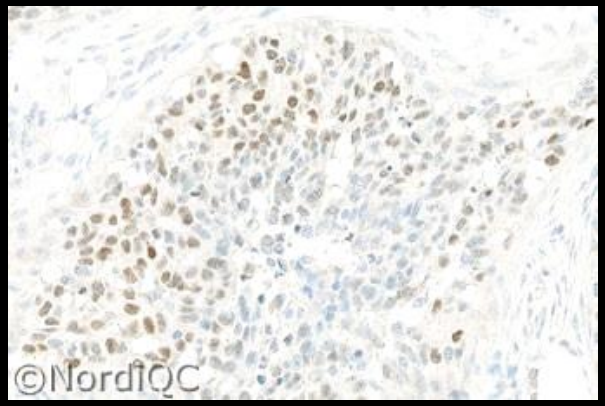
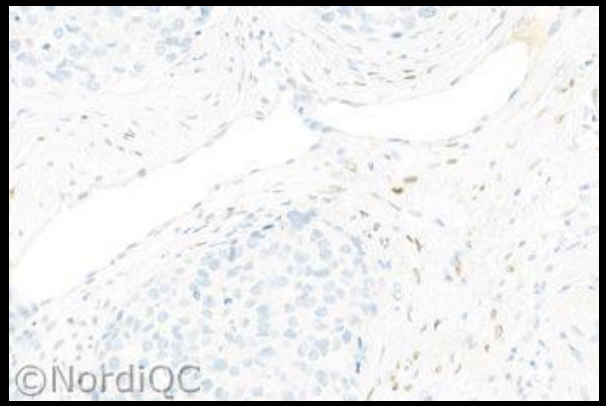
Estrogen receptor;

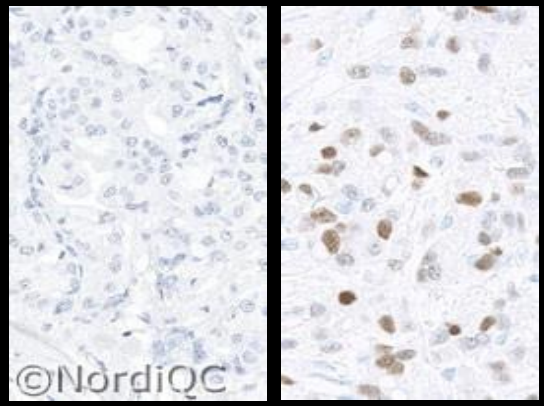
85% Weak / False negative

10% False positive

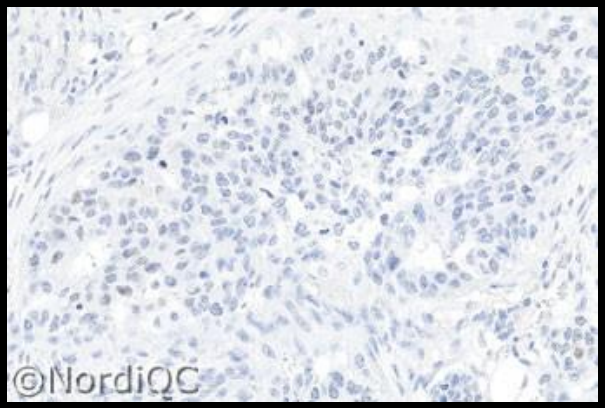
5% Impaired morphology, etc

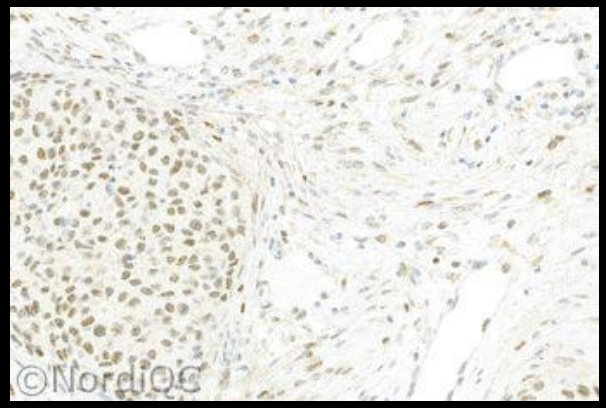


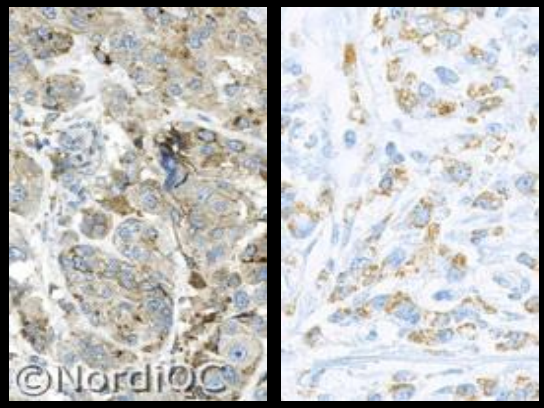




Suf.







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

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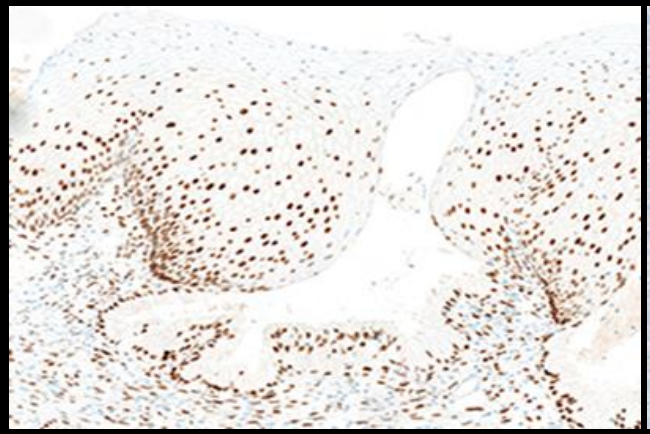
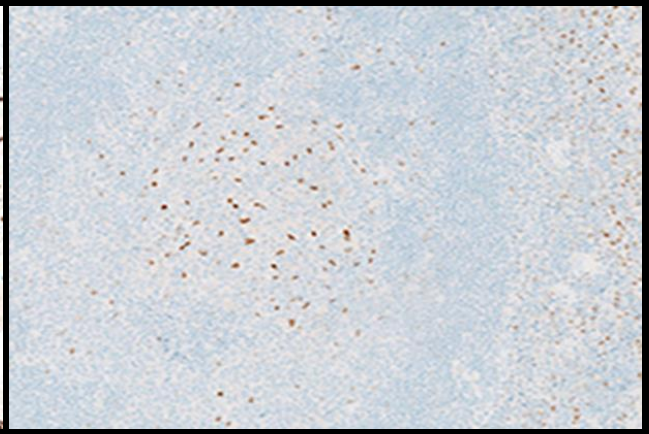
Breast panel: Estrogen Receptor
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 1D5	*HIER High*	*1:25-50*	*2- & 3-step*	*Dako*	*2- & 3-step*
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;

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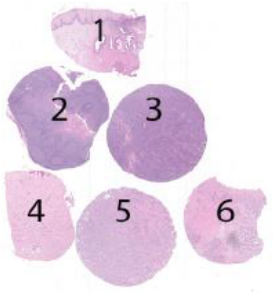
Assessment Run B20 2015
Progesterone Receptor (PR)

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

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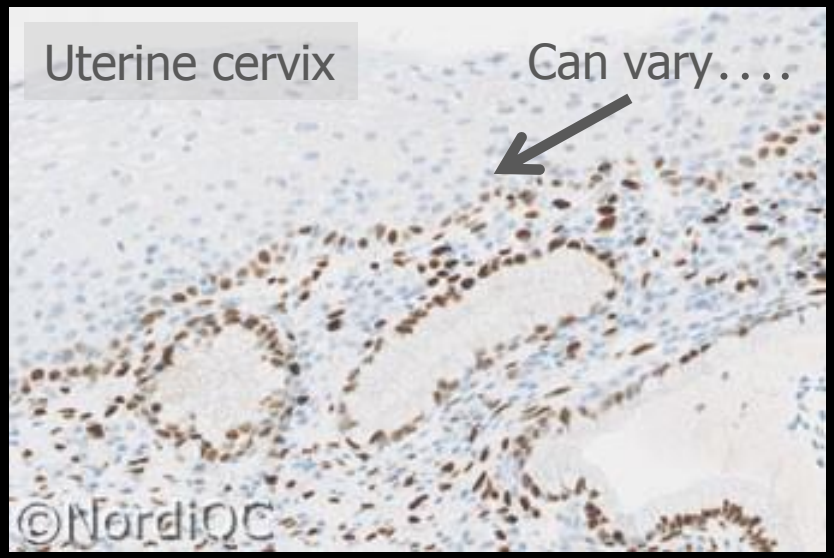

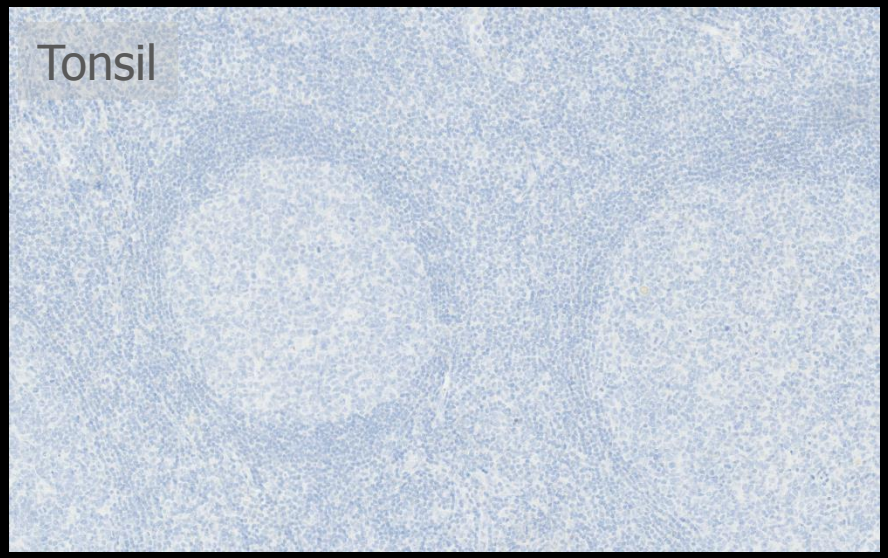

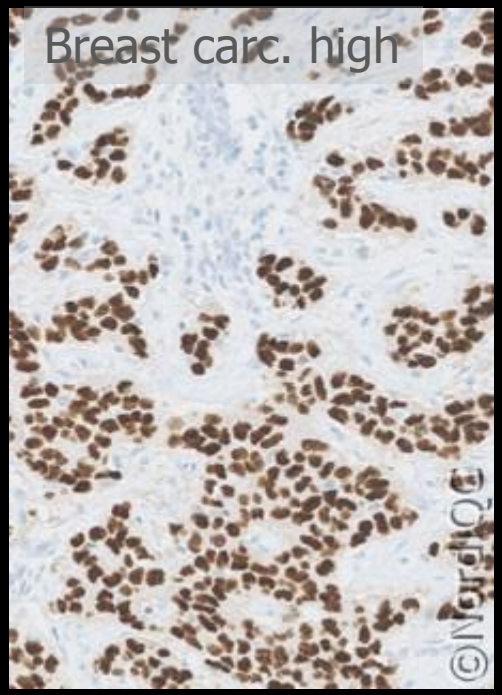

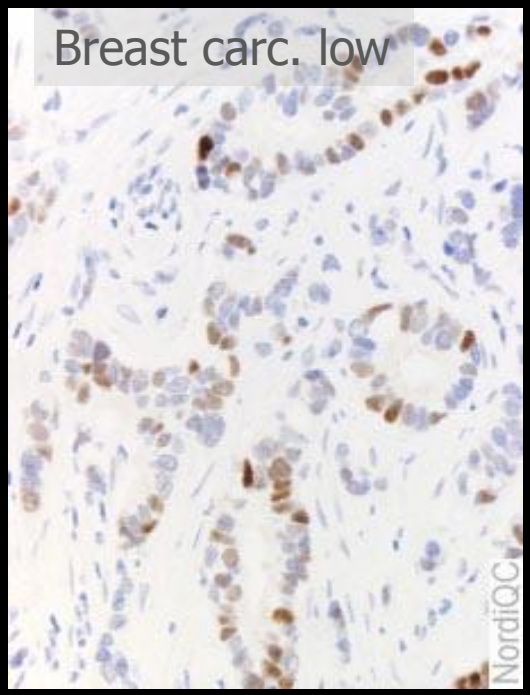

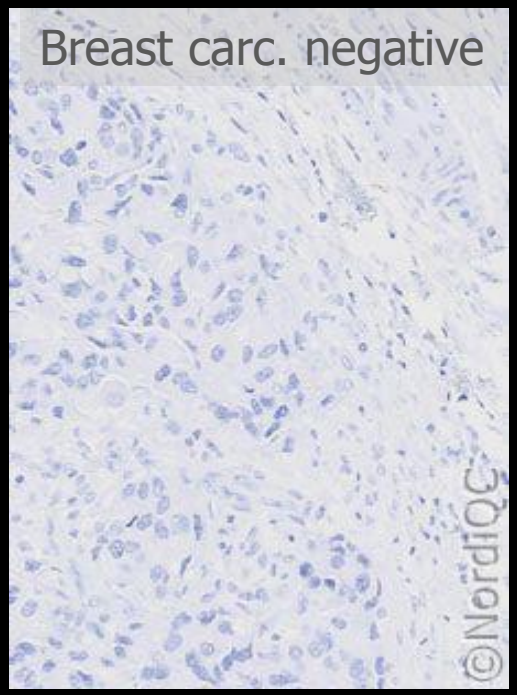

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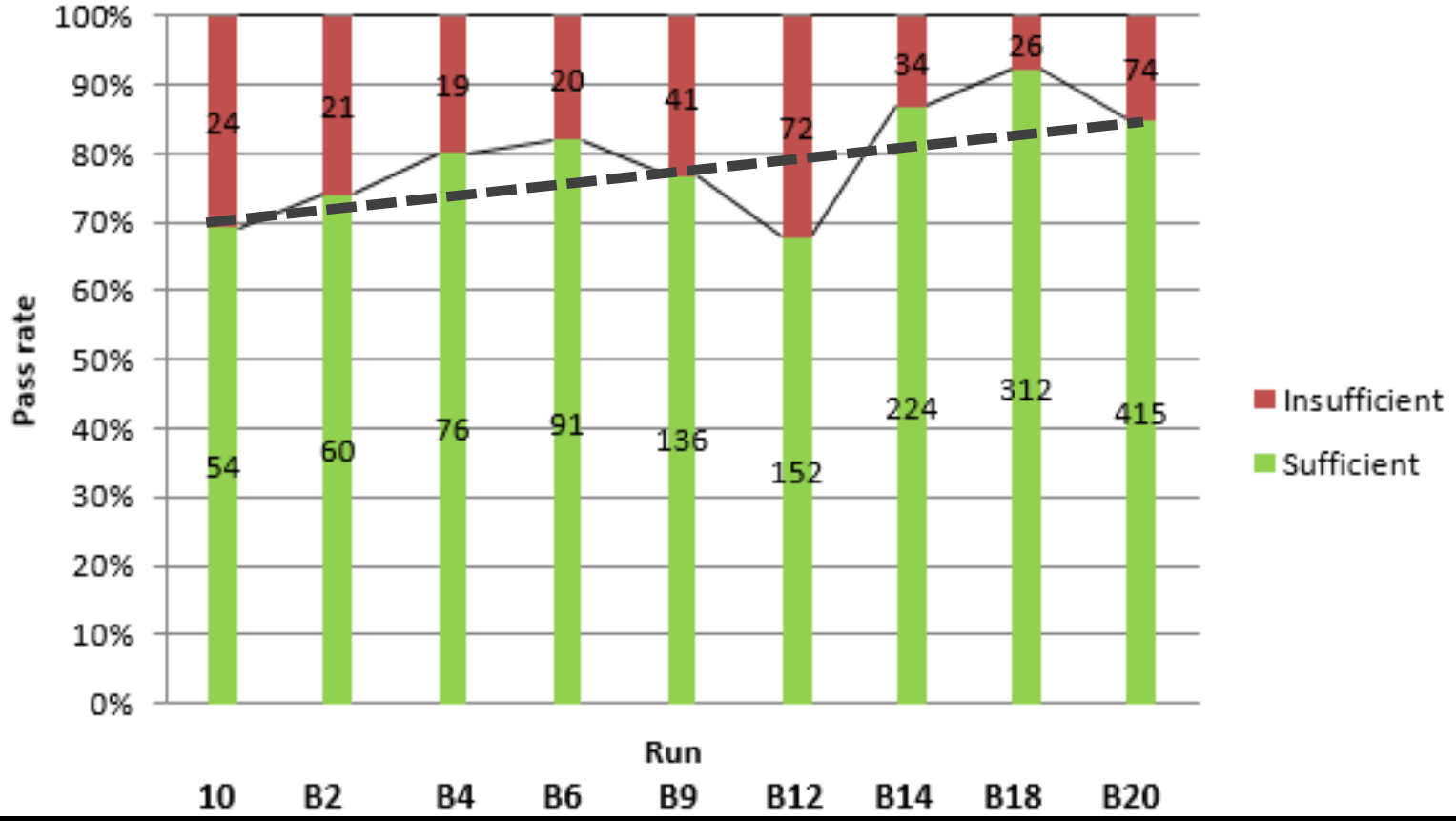
Performance history

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



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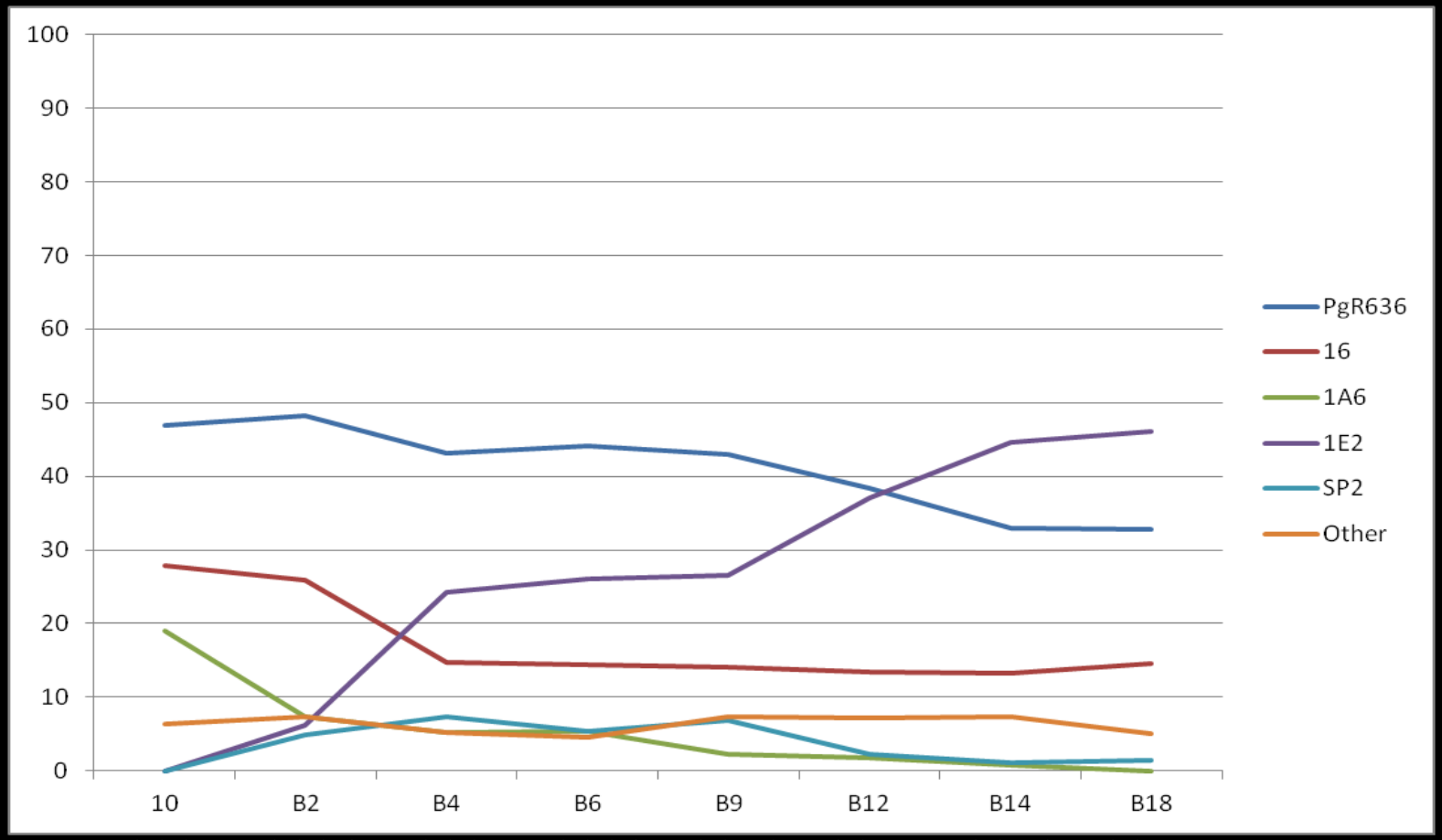


Comparison of different antibodies for detection of progesterone receptor in breast cancer

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,[3] Bharat Jasani, PhD,[4] and Keith Miller, MSc[1]

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

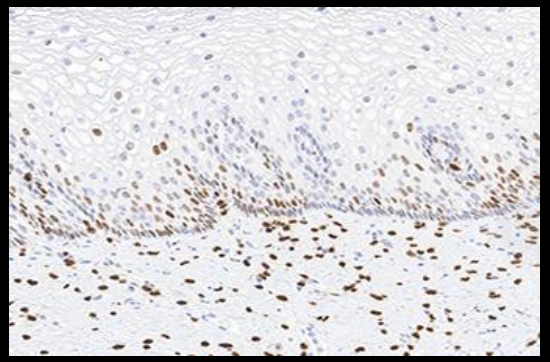
IHC - Protocols and controls for Breast tumours

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

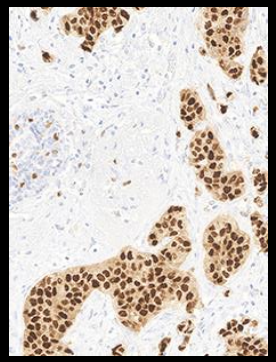
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	48 1 1	Leica/Novocastra Biocare Vector	39	7	3	1	92%	96%
mAb clone cocktail **16 + SAN27**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **1A6**	5	Leica/Novocastra	2	1	0	2	-	-
mAb clone **PgR 636**	68	Dako	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%	

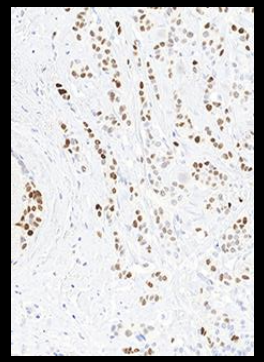
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
*discontinued product

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).





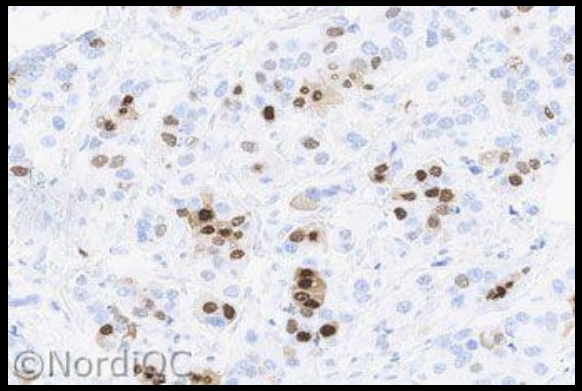
IHC - Protocols and controls for Breast tumours

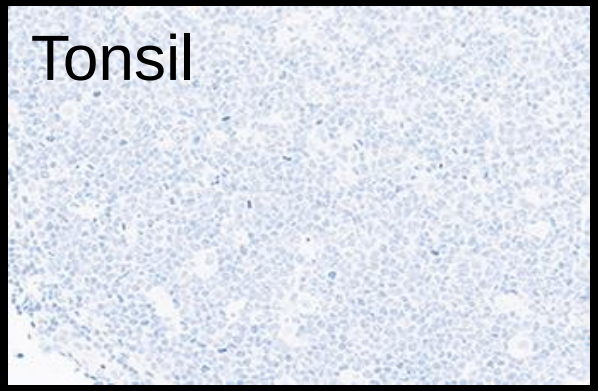
Progesterone receptor;

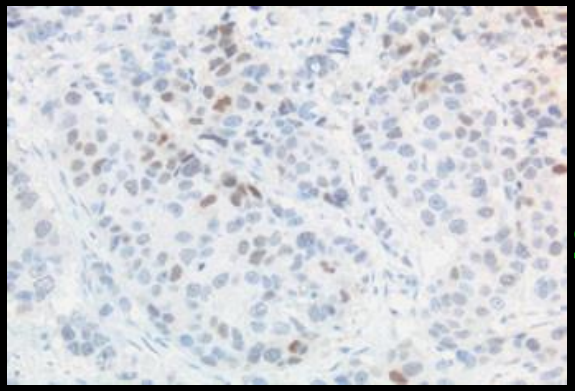
75% Weak / False negative

20% False positive

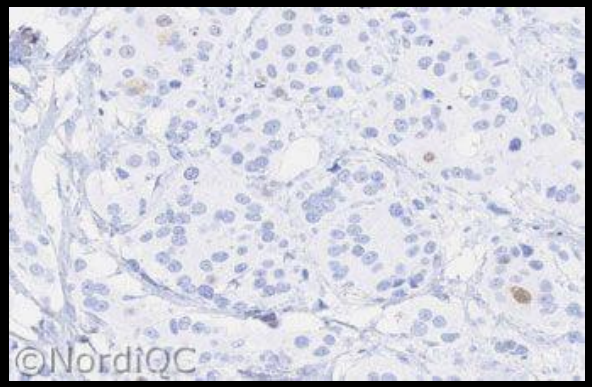
5% Impaired morphology, etc

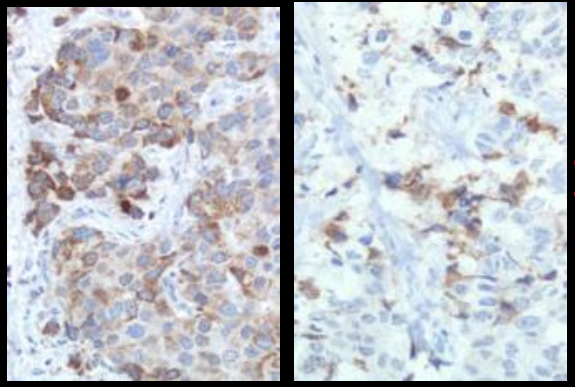






Suf.





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

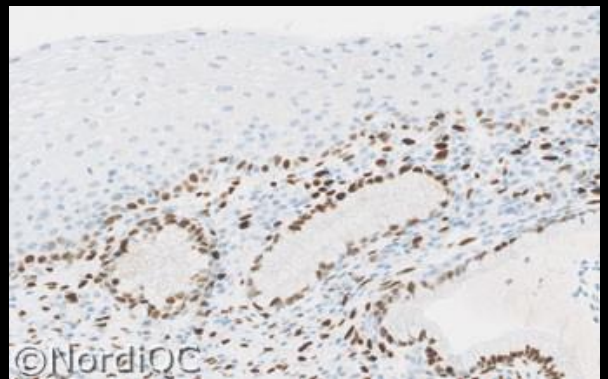
IHC – Protocols and controls for Breast tumours

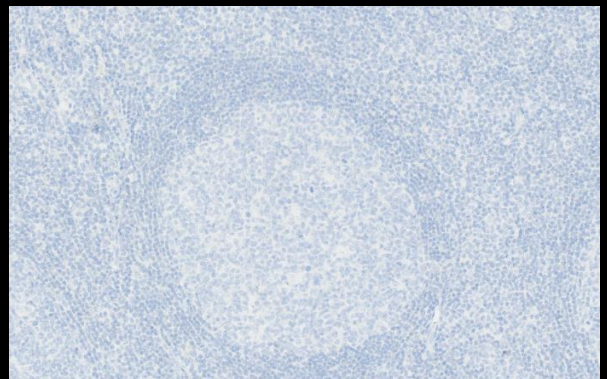
Breast panel: Progesterone Receptor
Basic protocol settings for an optimal staining result (NQC)

	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

Assessment Run B21 2016

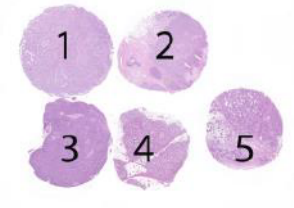
HER-2 IHC

Recommended HER2 assays Recommended HER2 control tissue

Material

The slide to be stained for HER-2 comprised the following 5 tissues:

	IHC: HER-2 Score* (0, 1+, 2+, 3+)	FISH: HER-2 gene/chr 17 ratio**
1.Breast carcinoma	3+	> 6.0 (clusters) (amplified)
2.Breast carcinoma	2+	2.4 – 2.9 (amplified)
3.Breast carcinoma	1-2+	1.2 – 1.7 (unamplified)
4.Breast carcinoma	0-1+	1.1 – 1.4 (unamplified)
5.Breast carcinoma	0-1+	0.9 – 1.2 (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 gene/chromosome 17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)

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

Focus:

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

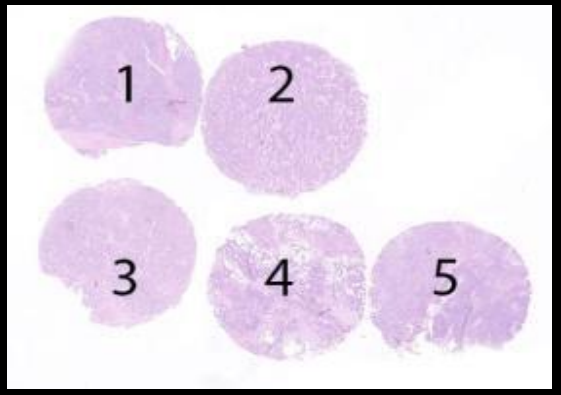
Appropriate analytical sensitivity and specificity – indicated by concordance to FISH status and IHC level established by reference data in all the included tumours.

IHC - Protocols and controls for Breast tumours

Table 1. **Assessment marks for IHC assays run B21, 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**	119	Ventana/Roche	110	6	1	2	97%	97%
CONFIRM™, rmAb clone **4B5, 790-4493**	64	Ventana/Roche	62	1	0	1	98%	100%
CONFIRM™, rmAb clone **4B5, 800-2996**	2	Ventana/Roche	2	0	0	0	-	-
HercepTest™ **SK001**	42	Dako/Agilent	39	1	0	2	95%	98%
HercepTest™ **K5207**	8	Dako/Agilent	7	1	0	0	100%	100%
HercepTest™ **K5204**	5	Dako/Agilent	2	3	0	0	100%	100%
Oracle™ mAb clone **CB11, TA9145**	6	Leica	4	2	0	0	100%	100%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **10A7**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **CB11**	6 1 1	Leica/Novocastra Biogenex Klinipath	3	2	2	1	62%	100%
rmAb clone **EP1045Y**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb clone **EP3**	1 1 1	Biocare Bio SB PathnSitu	2	1	0	0	-	-
rmAb clone **SP3**	17 2 2 1 1 1	Thermo/NeoMarkers Thermo/Pierce Zytomed Cell Marque Immunologic Spring Bioscience	13	7	1	3	83%	85%
pAb clone **A0485**	33	Dako	20	6	3	4	79%	82%
Unknown	1	Unknown	0	0	0	1	-	-
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostics	0	0	0	1	-	-
Ab clone **MXR001, RMA-0701**	1	Maixin	1	0	0	0	-	-
Total	319		265	32	7	15	-	-
Proportion			83%	10%	2%	5%	93%	-

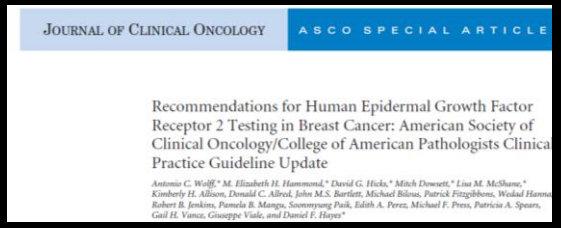
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.



	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)

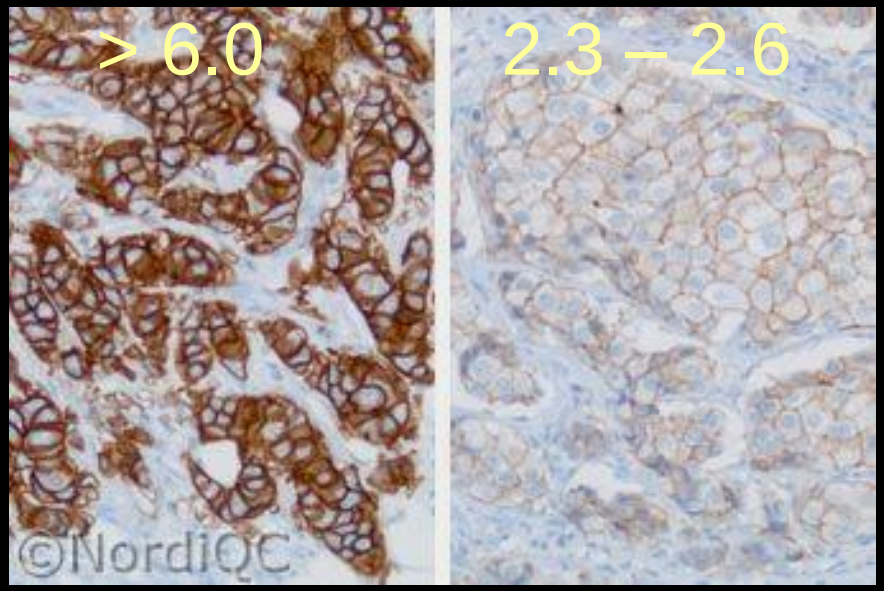
Material processed according to ASCO/CAP


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

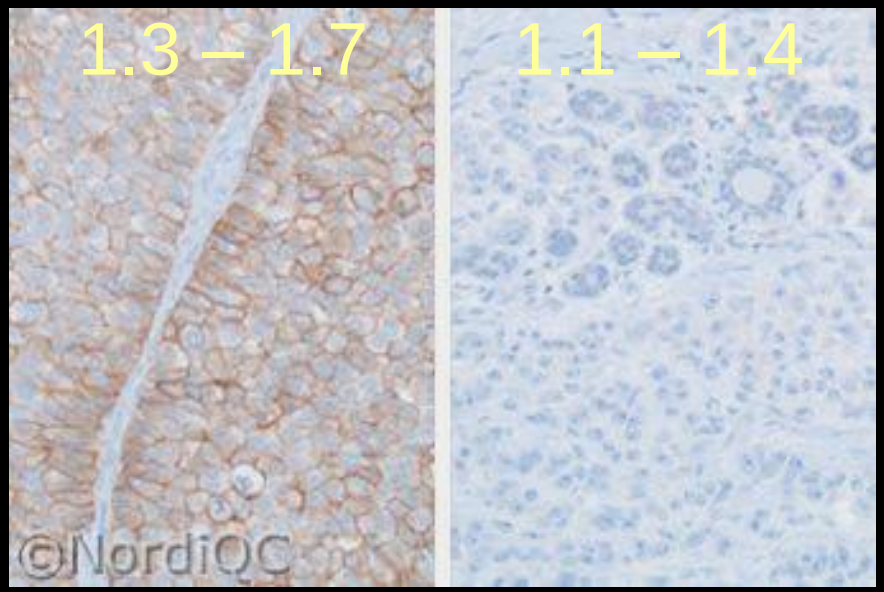
Antonio C. Wolff, M. Elizabeth H. Hammond, David G. Hicks, Mitch Dowsett, Lisa M. McShane, Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears,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

IHC - Protocols and controls for Breast tumours

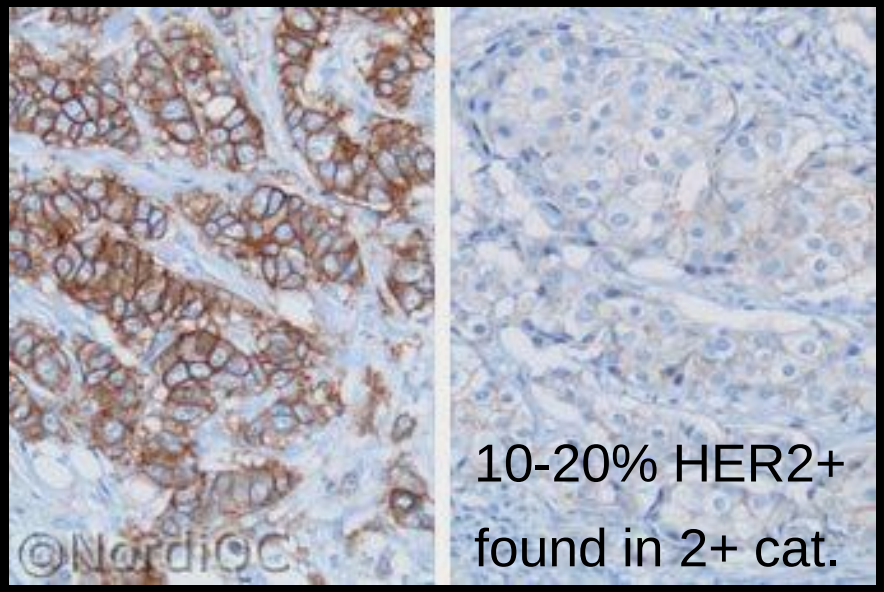


Ampl. 3+ | Ampl. 2+

Optimal

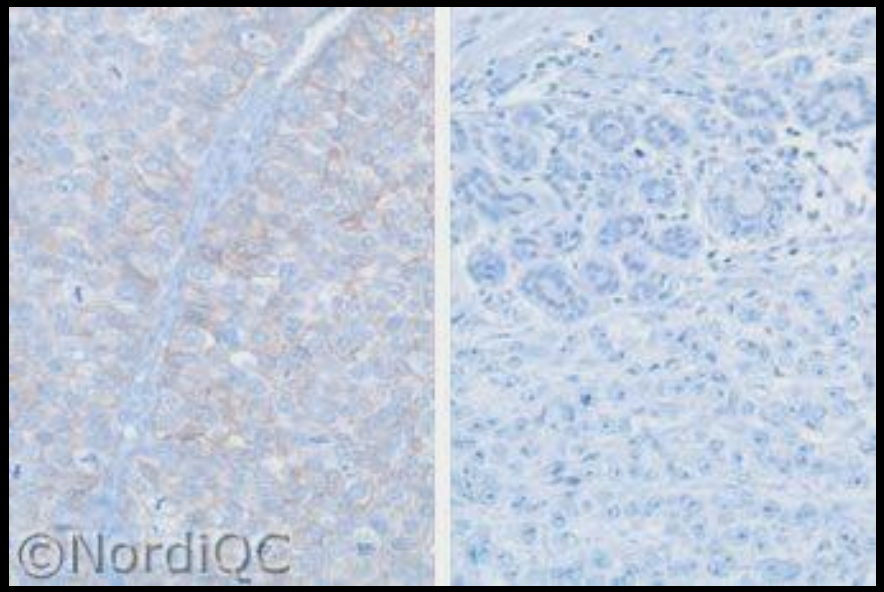


Unampl. 2+ | Unampl. 0



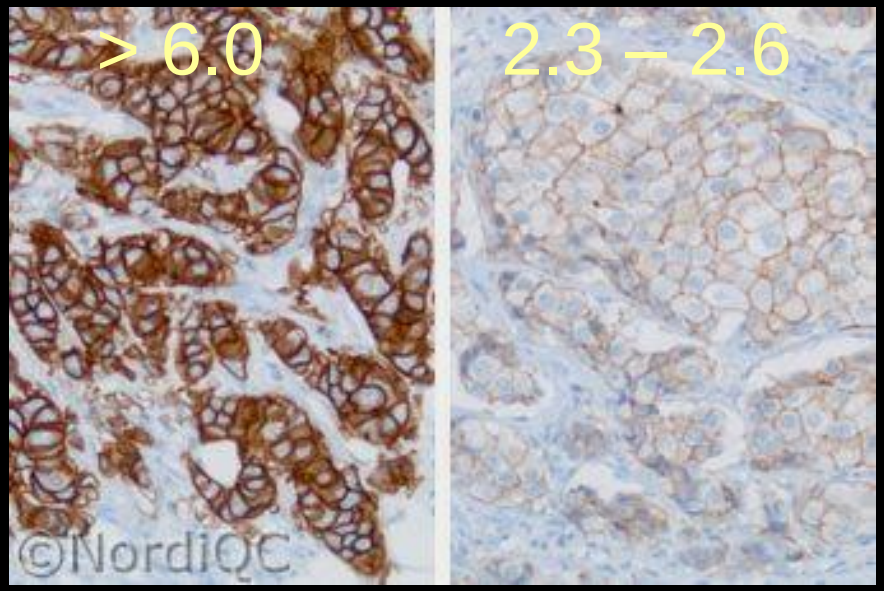
Ampl. 3+ | Ampl. 1+

Poor



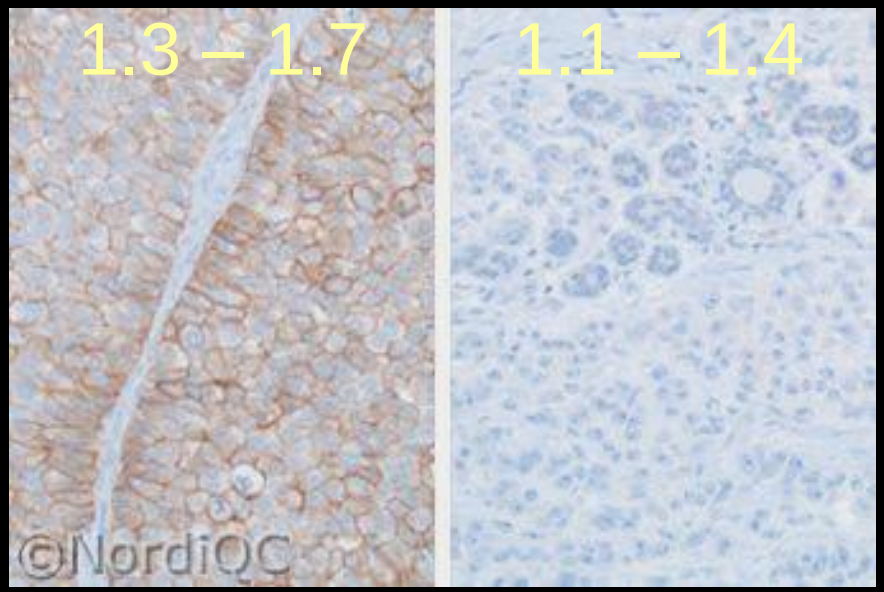
Unampl. 1+ | Unampl. 0

IHC - Protocols and controls for Breast tumours

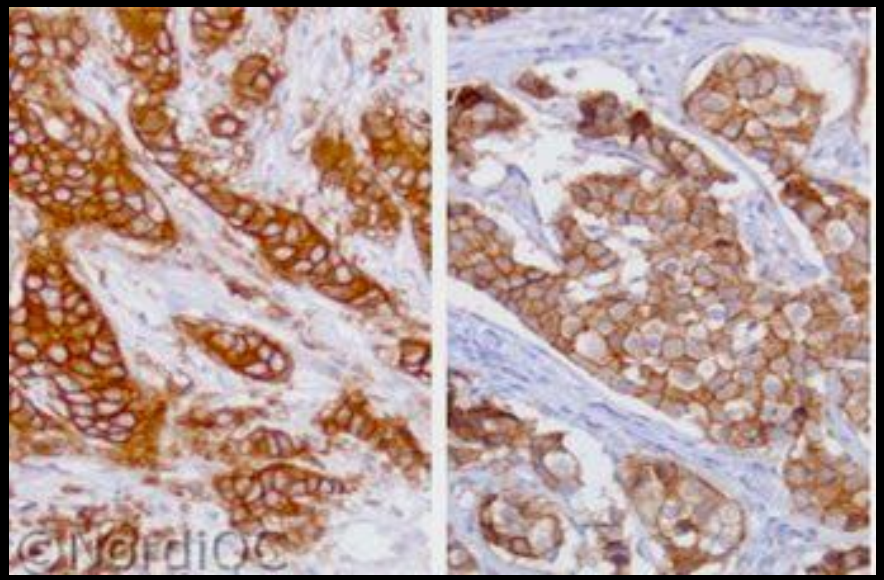


Ampl. 3+ Ampl. 2+

Optimal

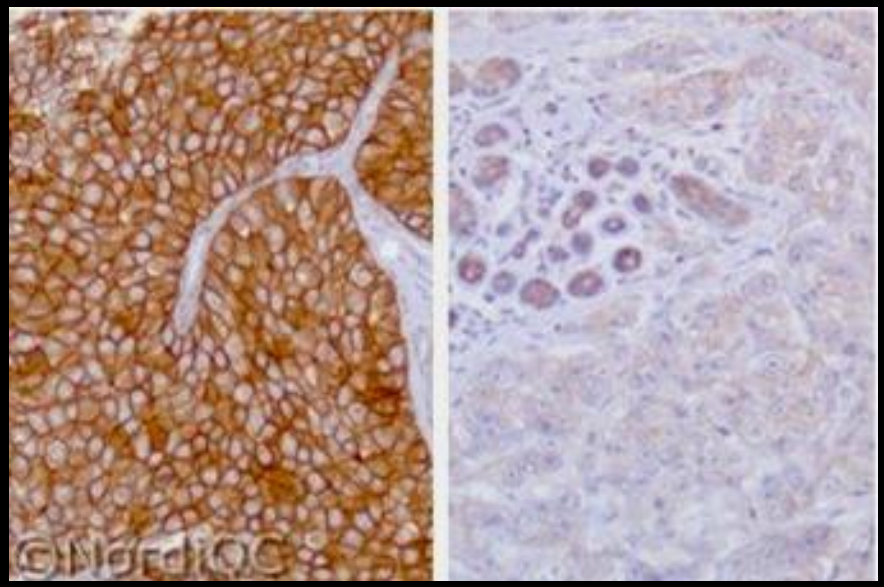


Unampl. 2+ Unampl. 0



Ampl. 3+ Ampl. 2+

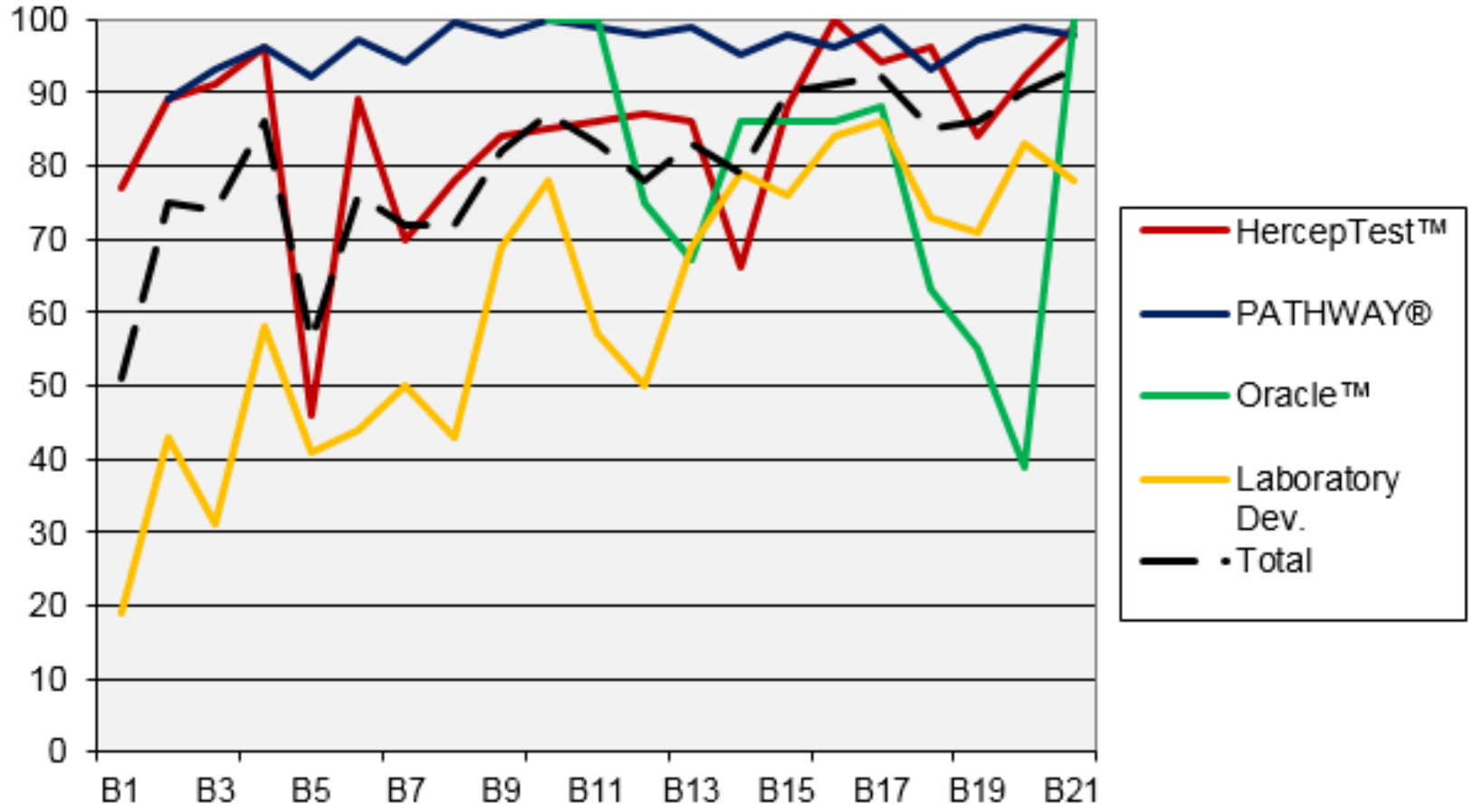
Poor



Unampl. 3+ Unampl. 1

IHC - Protocols and controls for Breast tumours

Figure 1. **Pass rates of 21 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

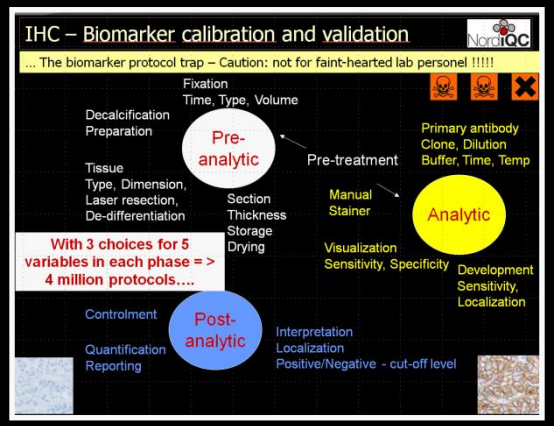
Typical causes for insufficient results in the NordiQC HER2 IHC breast module:

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



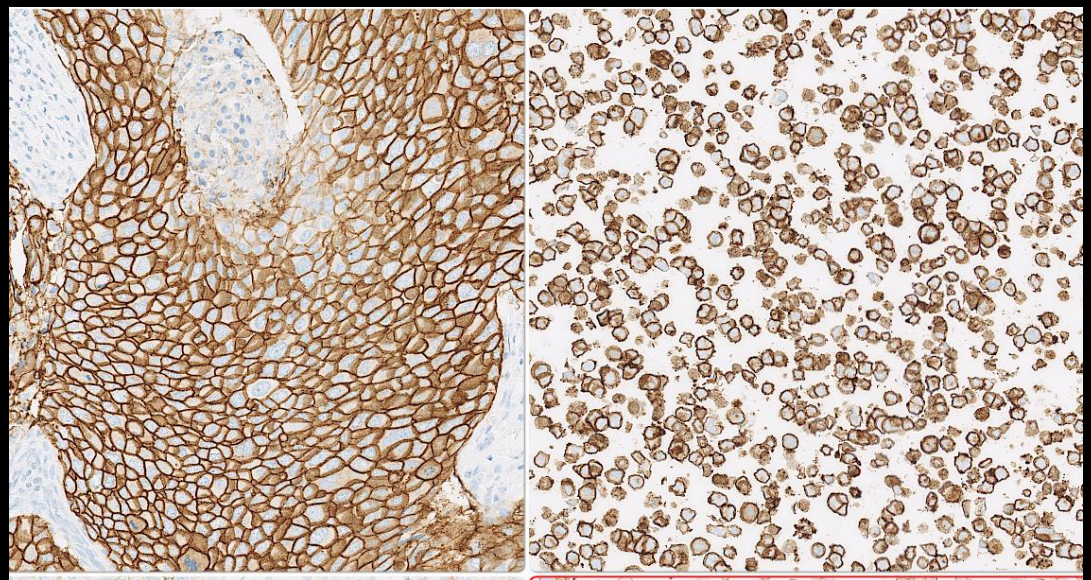
Laboratory developed assays:

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

IHC - Protocols and controls for Breast tumours

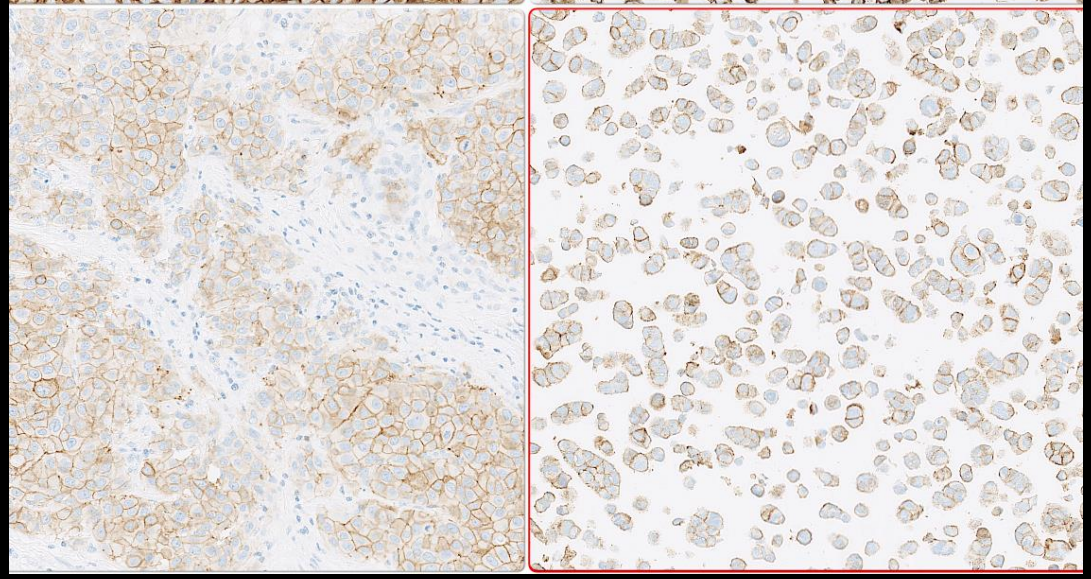
Control material for HER2 IHC: performace control / consistency

Histology:
3+ tumour



Cell lines:
3+

2+ tumour



2+

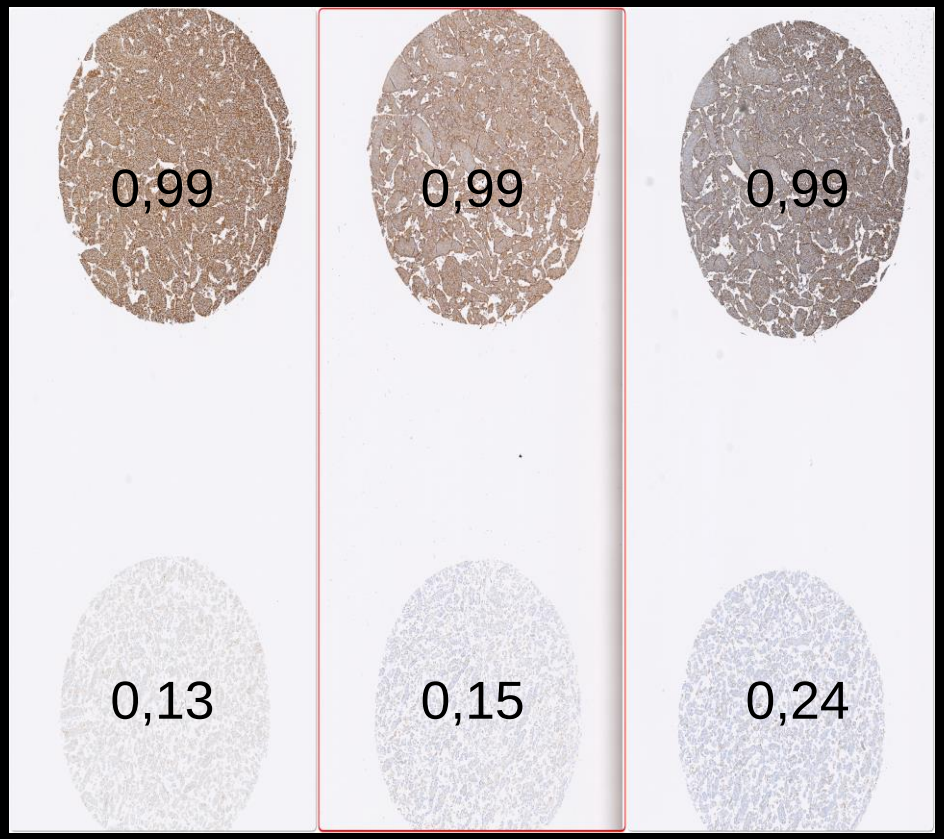
Applicable for DIA & ref data comparing run-to-run

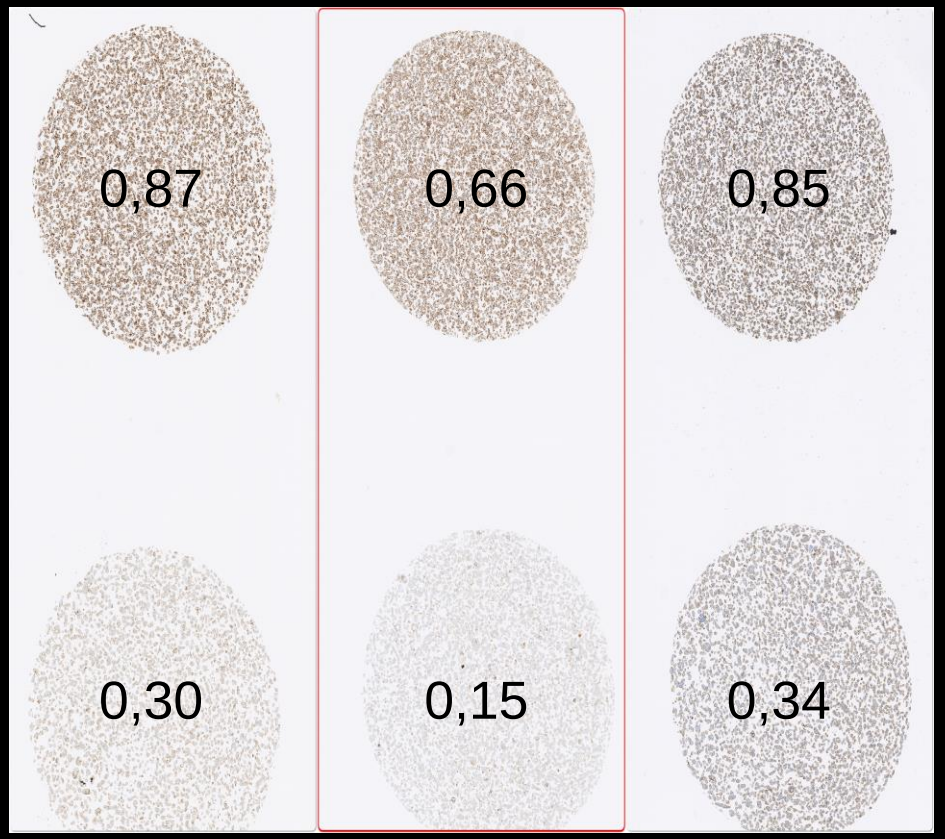
IHC - Protocols and controls for Breast tumours

Control material for HER2 IHC: performace control / consistency

Histocyte cell lines HER2:

Horizon cell lines HER2

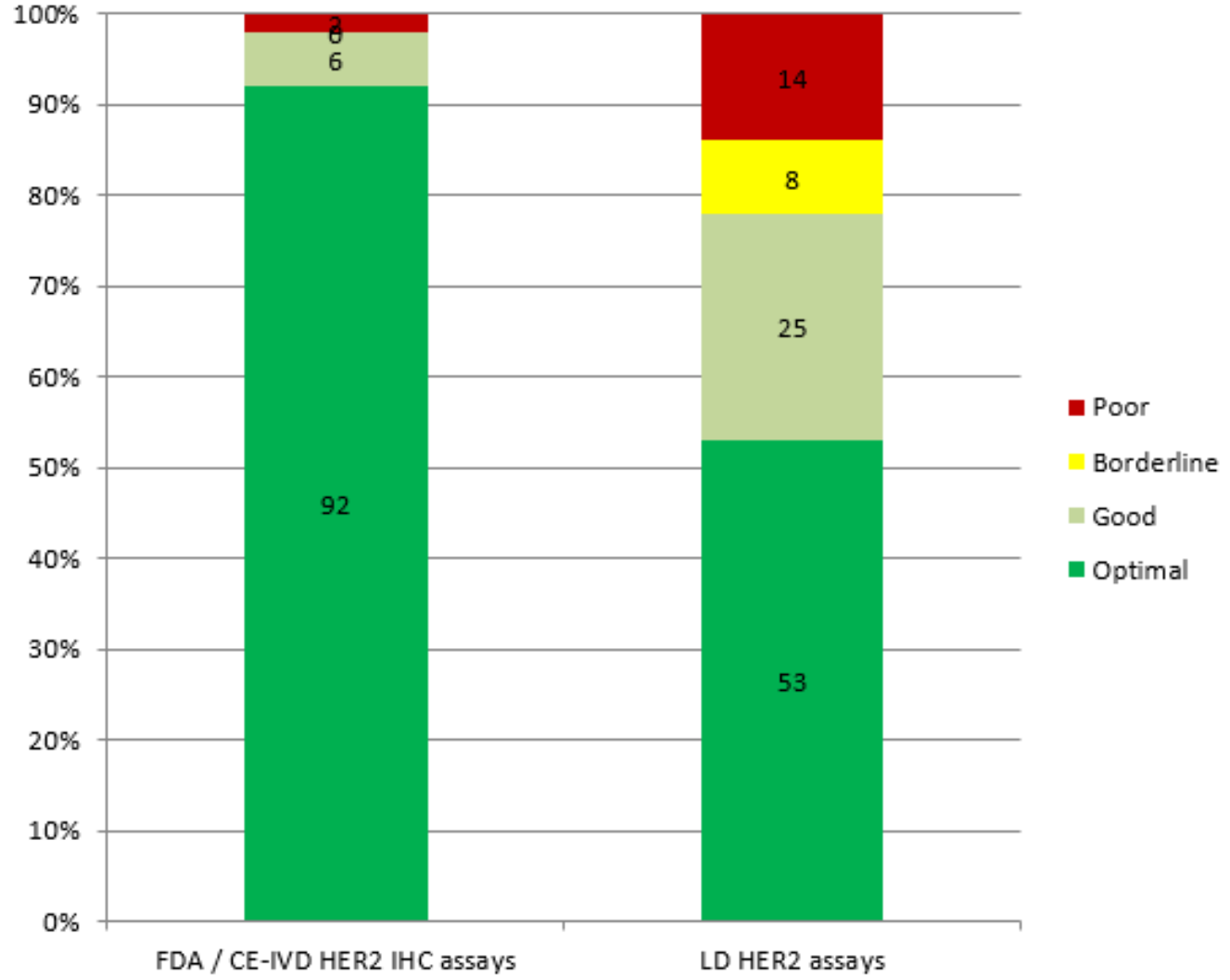




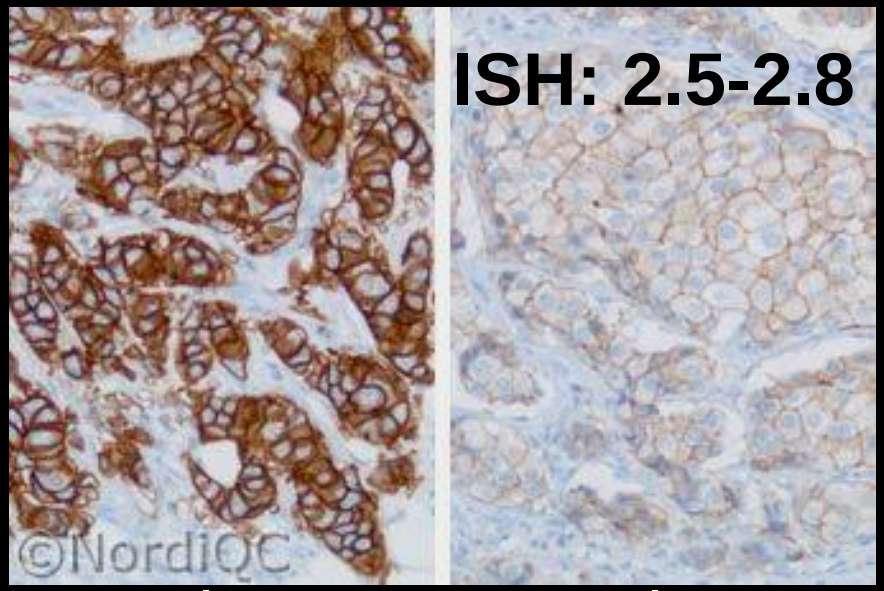
Pathway Oracle Herceptest Pathway Oracle HercepTest

IHC - Protocols and controls for Breast tumours

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

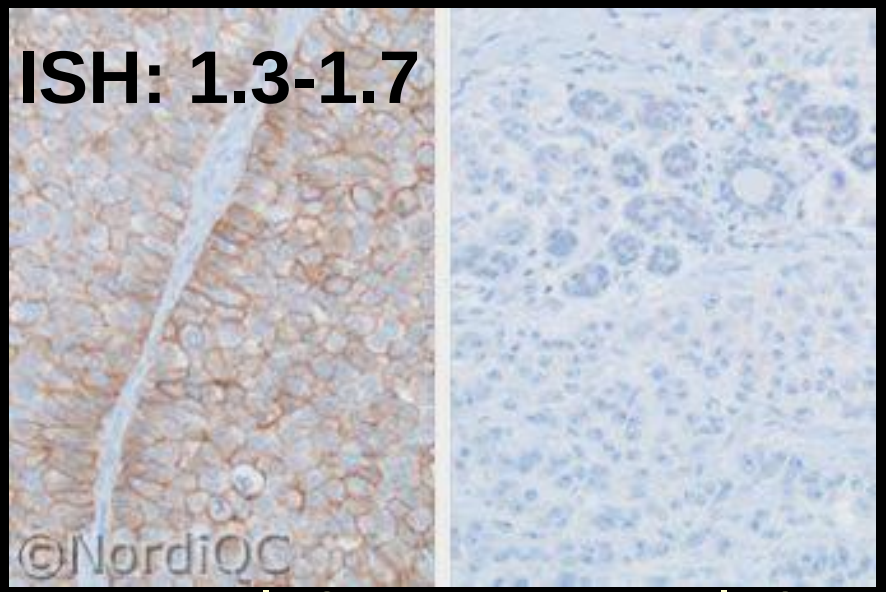


IHC - Protocols and controls for Breast tumours

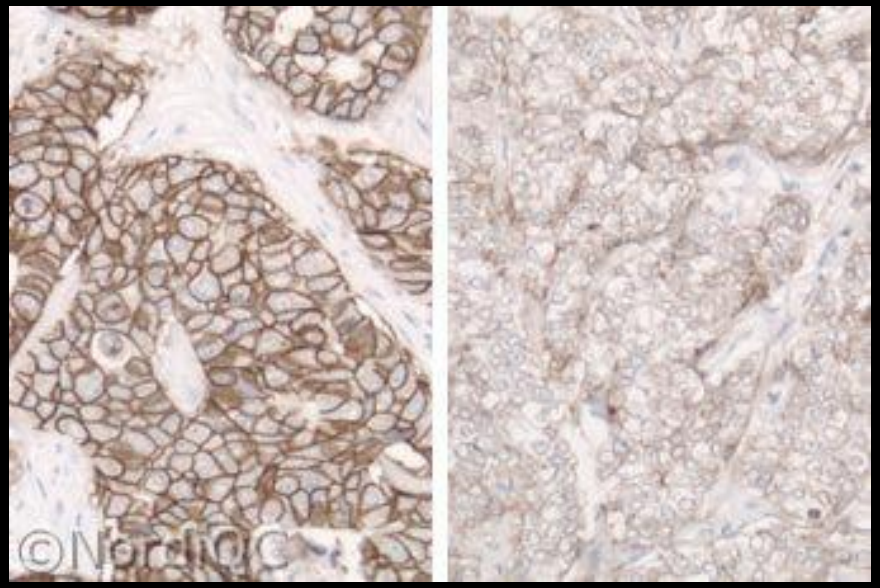


Ampl. 3+ Ampl. 2+

Optimal

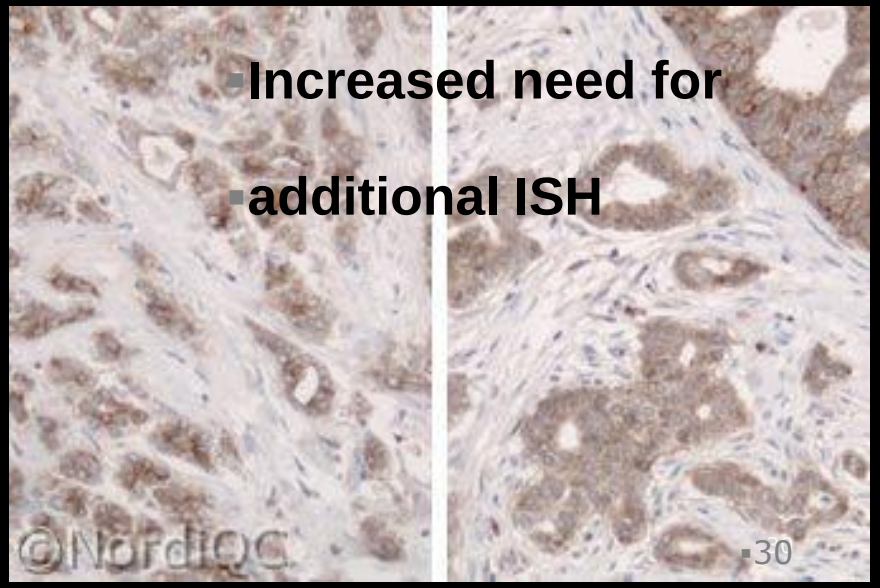


Unampl. 2+ Unampl. 0



Ampl. 3+ Ampl. 2+

Good



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

Unampl. 2+ Unampl. 2+

IHC - Protocols and controls for Breast tumours

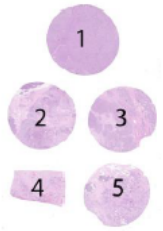
Assessment Run H9 2016

HER-2 ISH

Material

Table 1. **Content of the multi-tissue block used for the NordiQC HER-2 ISH assessment, run H9**

	HER-2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	HER2/chr17 ratio¤	HER2/chr17 ratio¤	HER2 copies
1. Breast carcinoma	0	0.8	0.9 – 1.0	< 4
2. Breast carcinoma	2+	1.1	1.0 – 1.3	< 4, ≥ 4 < 6
3. Breast carcinoma	2+	1.5	1.3 – 1.9	< 4, ≥ 4 < 6
4. Breast carcinoma	2+	2.3	2.2 – 2.9	> 6
5. Breast carcinoma	3+	7.0	4.2 – 6.4	> 6



* PATHWAY® (Ventana), data from two reference labs.
** Inform HER-2 Dual ISH kit (Ventana), data from one reference lab.
*** HER2 FISH pharmDX™ Kit (Dako) and HER2 FISH (Zytovision), range of data from one reference lab.
¤HER2/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 HER2/chr17 ratios in all five tissues.

The staining reaction was assessed as **good**, if the HER2/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.

The staining reaction 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.

The staining reaction 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 HER2/chr17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyze scoring consensus.

Consensus scores from the NordiQC FISH reference laboratories

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

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

Unamplified: HER2/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: HER2/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)

IHC - Protocols and controls for Breast tumours

Participation

Number of laboratories registered for HER-2 BRISH	126
Number of laboratories returning slides	116 (90%)
Number of laboratories returning scoring sheet	104 (92%)
Number of laboratories registered for HER-2 FISH	61
Number of laboratories returning scoring sheet	54 (89%)

5% didn't return slides for BRISH due to technical errors.

Results BRISH, technical assessment

In total, 116 laboratories participated in this assessment. 79 laboratories (68%) achieved a sufficient mark (optimal or good). Results are summarized in Table 2.

Table 2. **HER-2 BRISH assays and assessment marks for BRISH HER-2 run H9.**

Two colour HER-2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	86	Ventana	32	22	17	15	63%	73%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	7	Ventana	5	1	1	0	86%	86%
DuoCISH pharmDx™ **SK109**	6	Dako	3	3	0	0	100%	100%
Zyto*Dot*® 2C **C-3022 / C-3032**	6	ZytoVision	4	1	0	1	83%	100%
One colour HER-2 assays								
INFORM™ HER-2 SISH **780-4332**	5	Ventana	3	2	0	0	100%	100%
Zyto*Dot*® **C-3003**	6	ZytoVision	3	0	2	1	50%	75%
Total	116		50	29	20	17	68%	-
Proportion								

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:

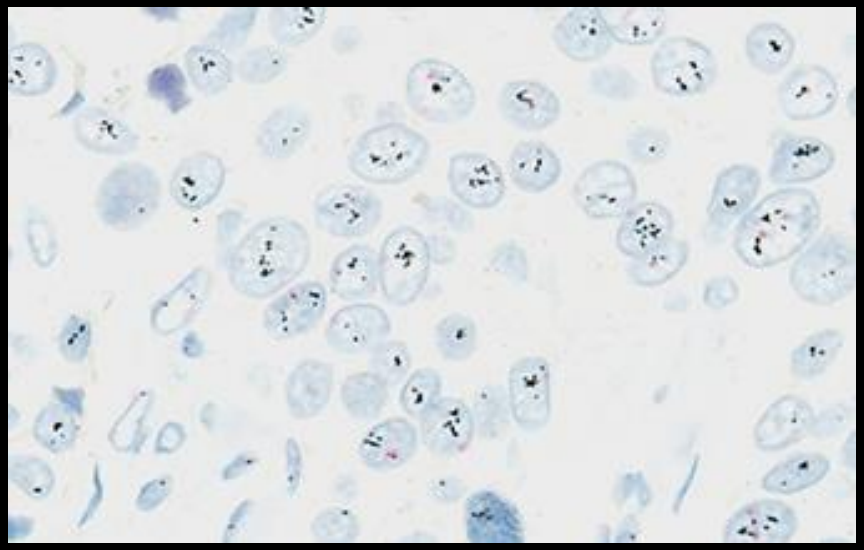
INFORM™ HER2 Dual ISH, Ventana

ZytoDot® 2C, ZytoVision

HER2 black – chr17 red

U

HER2 green – chr17 red



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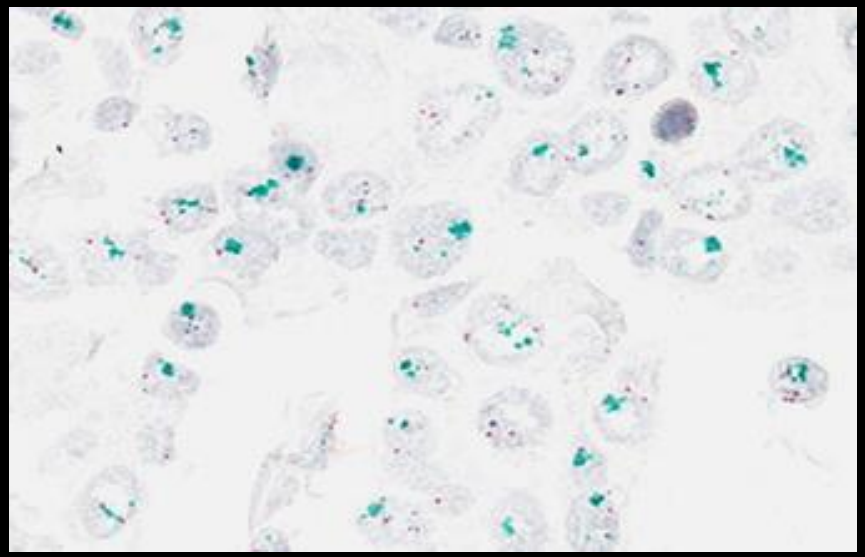


IHC - Protocols and controls for Breast tumours

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

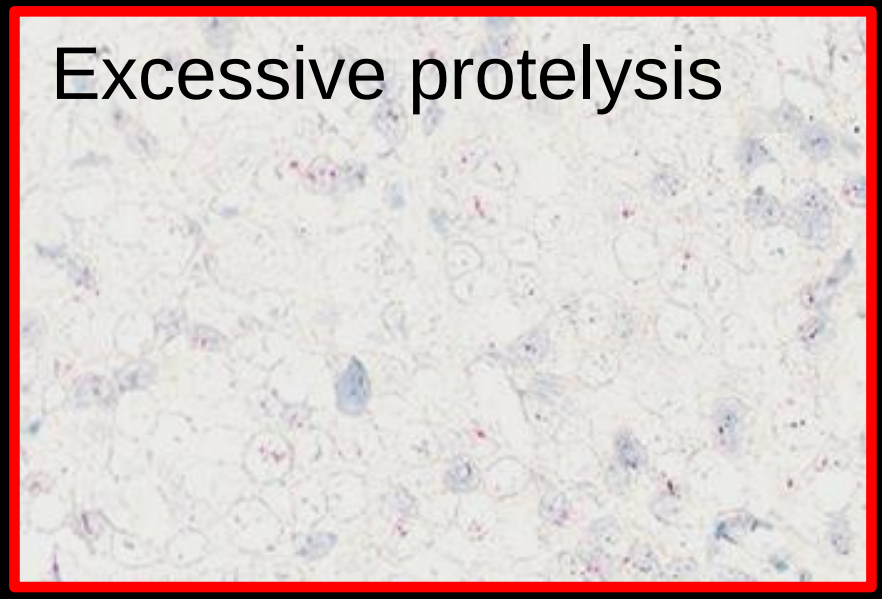
Technically insufficient results in the NordiQC HER2 ISH breast module:

INFORM™ HER2 Dual ISH, Ventana

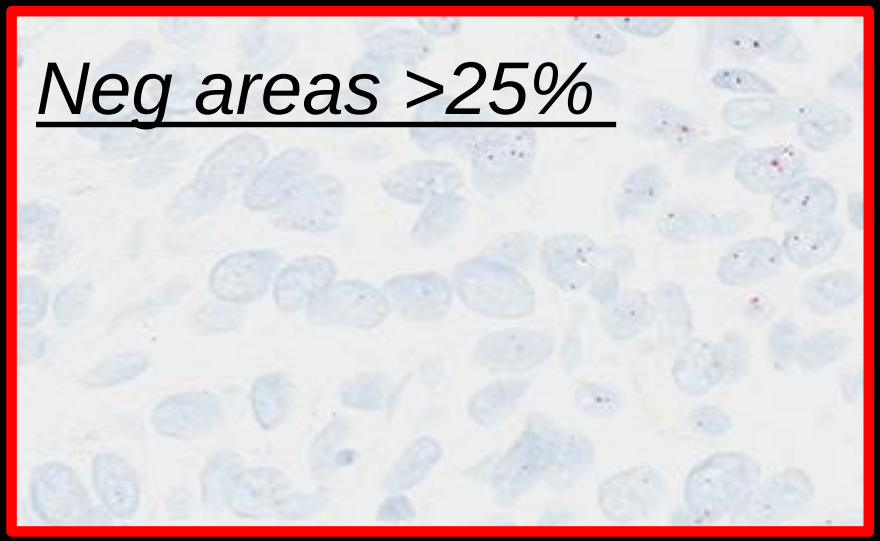
HER2 black – chr17 red

U

Excessive protelysis

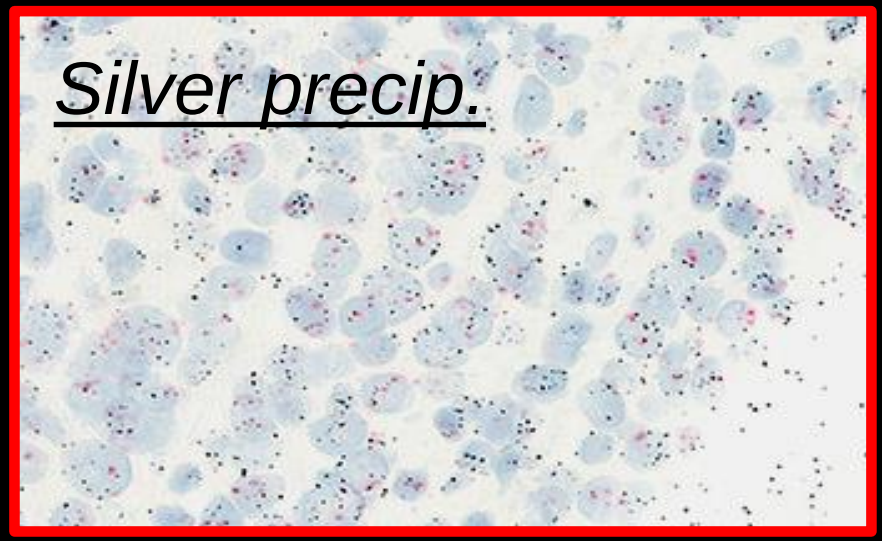


Neg areas >25%



U

Silver precip.



IHC - Protocols and controls for Breast tumours

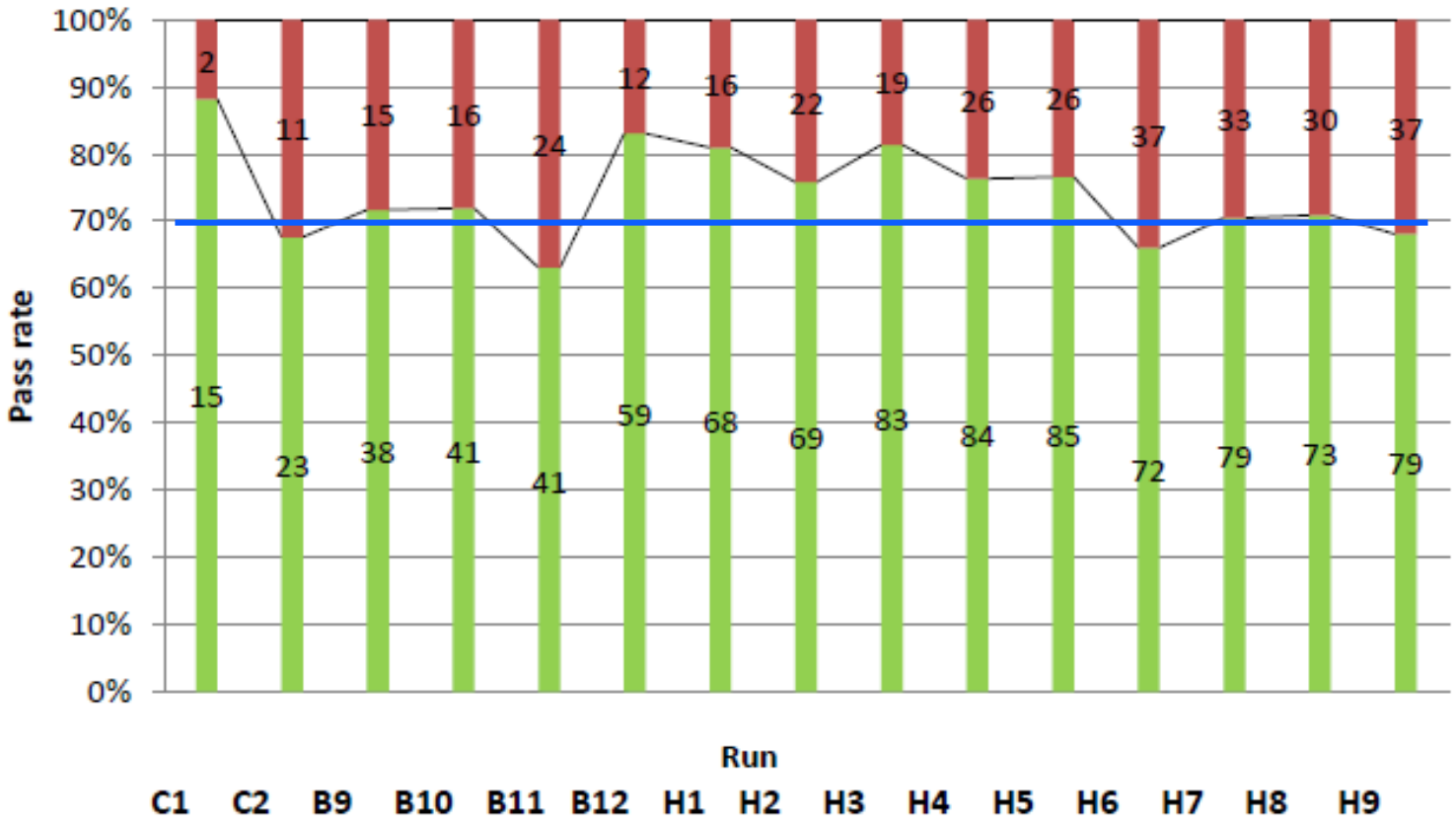
HER-2 BRISH, Technical assessment

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.

Performance history

This was the 15th assessment of HER-2 BRISH in NordiQC and a relatively consistent pass rate level has been observed in the latest runs. Data is shown in Fig 1.

Fig. 1: **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**

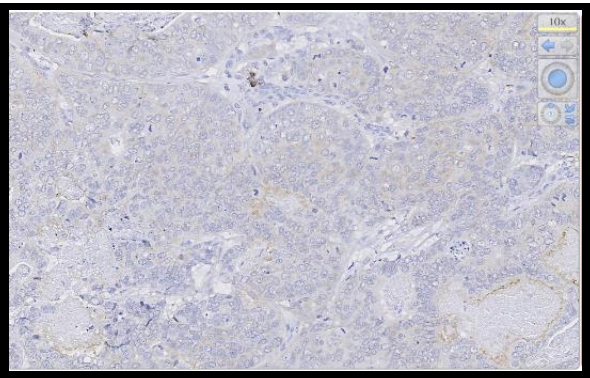


90% of ins. results used approriate protocol.

IHC - Protocols and controls for Breast tumours

IHC scoring system according to the 2013 ASCO/CAP guidelines

Score 0	No staining is observed or incomplete membrane staining is observed in ≤ 10% of the tumour cells.
Score 1+	A faint perceptible and incomplete membrane staining is observed in more than 10% of the tumour cells.
Score 2+	A weak to moderate circumferential incomplete membrane staining is observed in more than 10% of the tumour cells or an intense circumferential complete membranous staining in ≤ 10% of the tumour cells.
Score 3+	An intense circumferential complete membrane staining is observed in more than 10% of the tumour cells.



- What is faint ? Visible at 40X ?
- What is weak ? Visible at 10X ?

Up to 20-40% HER2 IHC tests are reflexed to ISH due to expanded criteria for 2+ (internal data)

IHC - Protocols and controls for Breast tumours

Performance history

This was the 15th assessment of HER-2 BRISH in NordiQC and a relatively consistent pass rate level has been observed in the latest runs. Data is shown in Fig 1.

Fig. 1: **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**

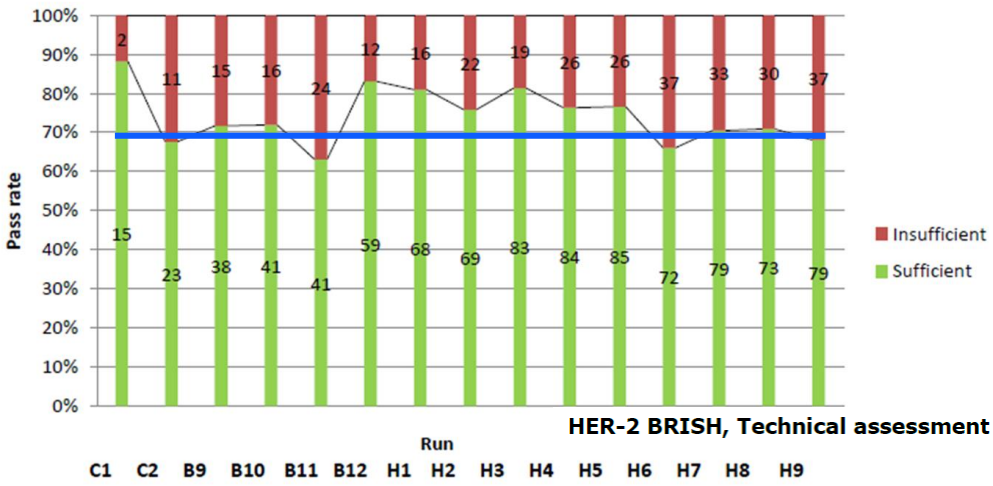
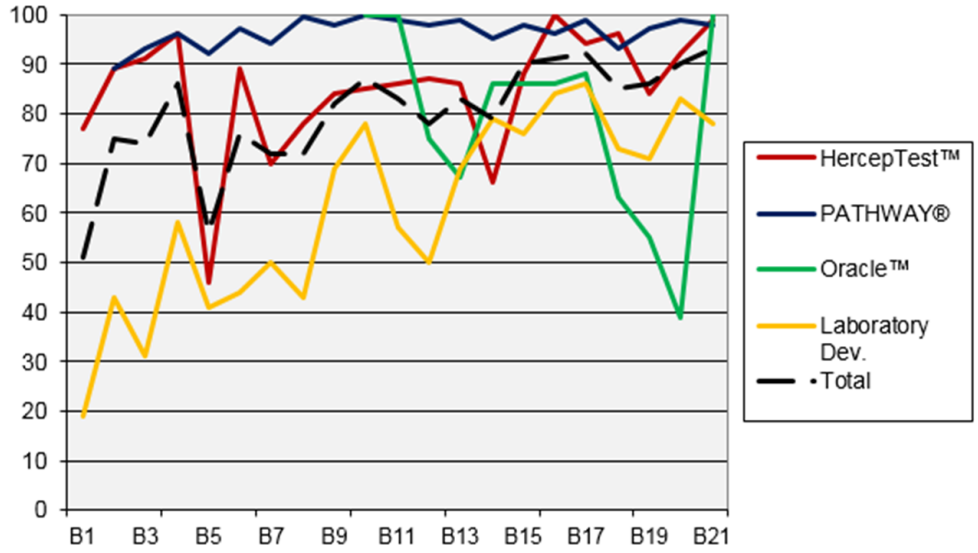


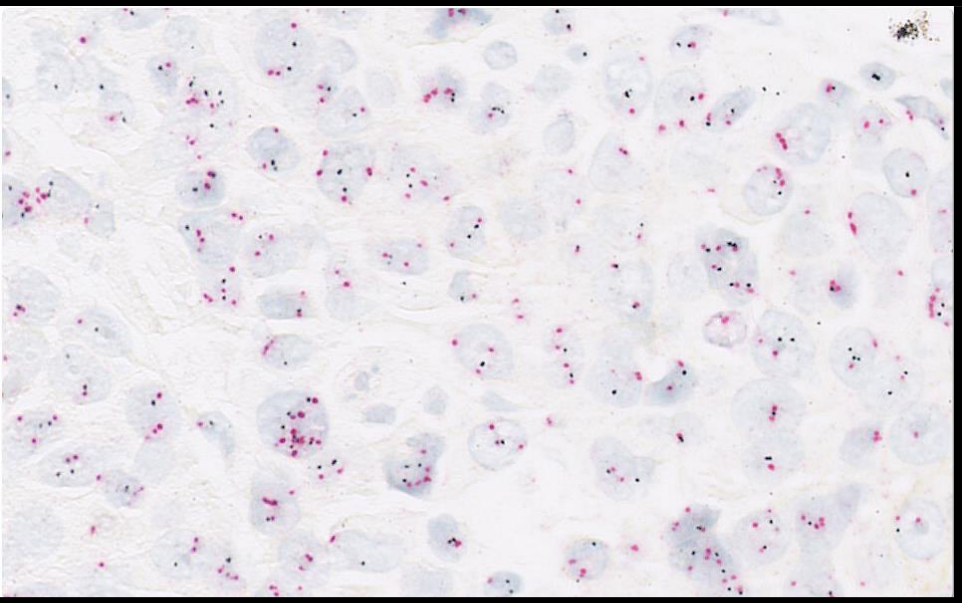
Figure 1. **Pass rates of 21 HER-2 IHC assessments in the NordiQC breast cancer module**

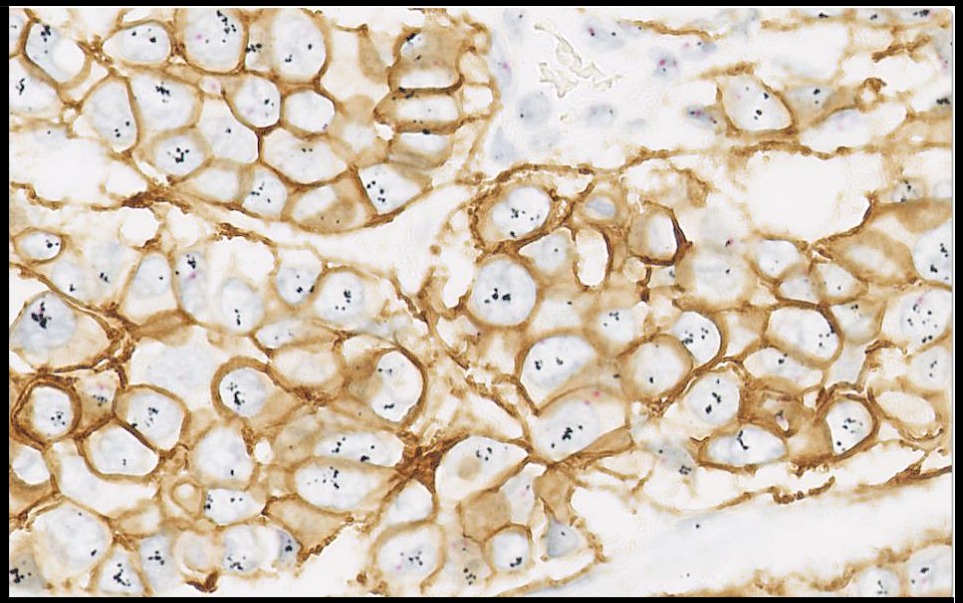


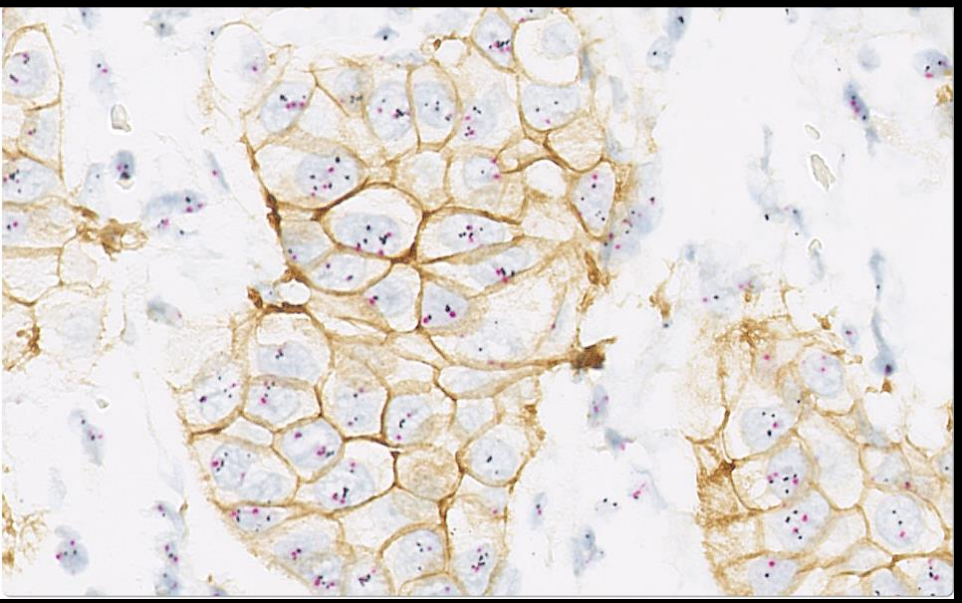
From a technical point of view, it might be critical to change from a robust and relatively simple IHC assay to a less robust and complex test and simultanously more expensive test……

IHC - Protocols and controls for Breast tumours

HER2 Gene-Protein-Assay (Roche): HER2 IHC + DDISH – no EQA data yet.

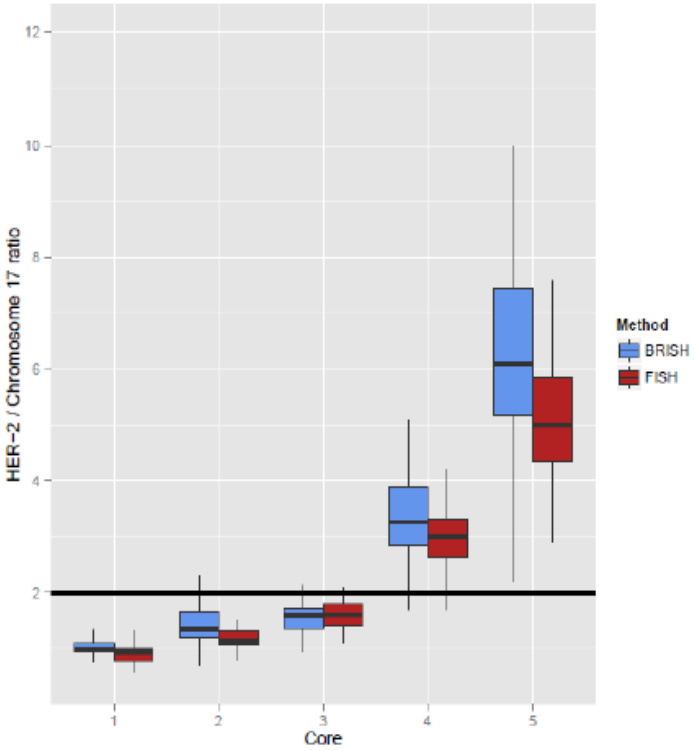




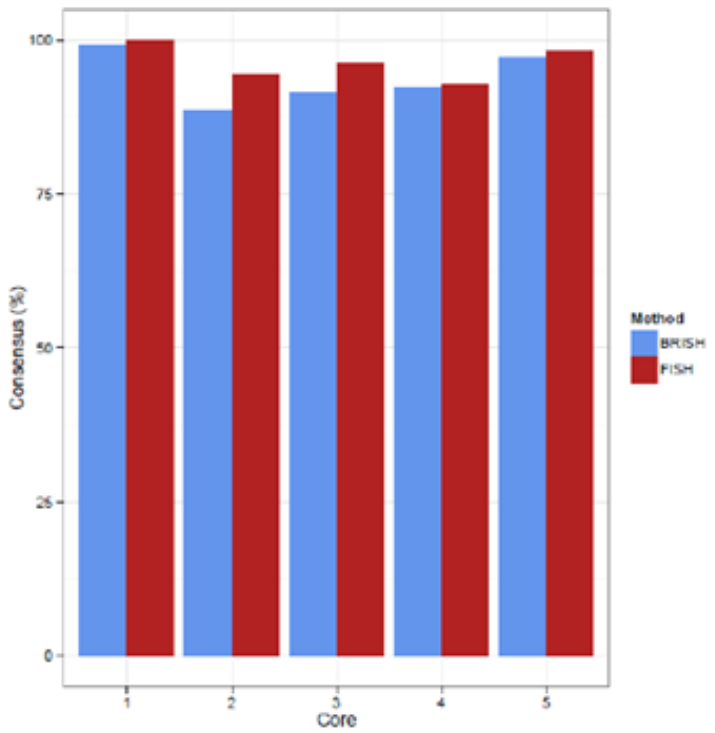


IHC - Protocols and controls for Breast tumours

Slightly higher concordance regarding interpretation for FISH



NordiQC HER-2 ISH run H9: participants interpretation of amplification status



NordiQC HER-2 ISH run H9: consensus between participants and NordiQC

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

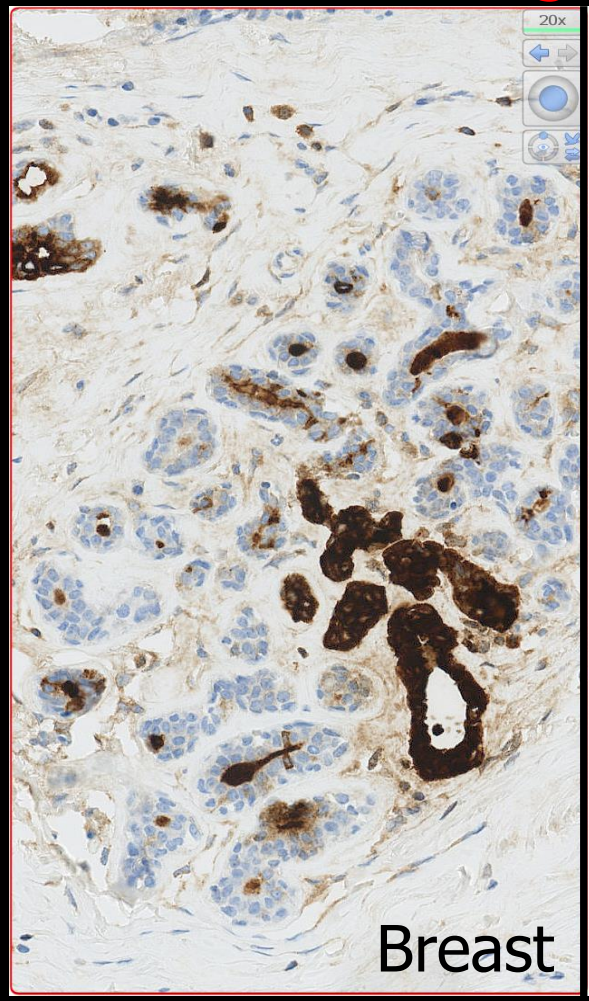
Breast panel:

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

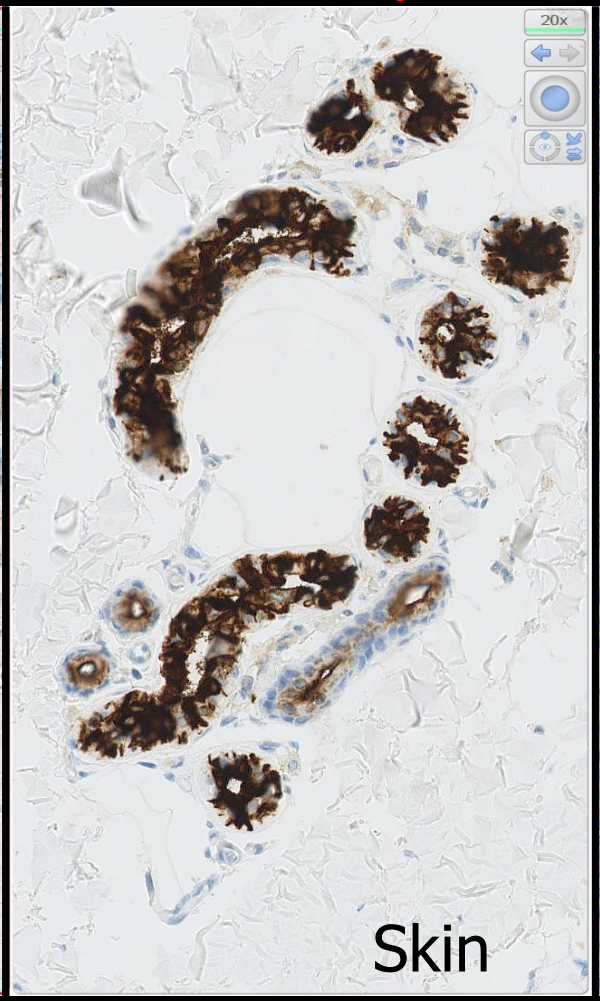
- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

IHC – Protocols and controls for Breast tumours

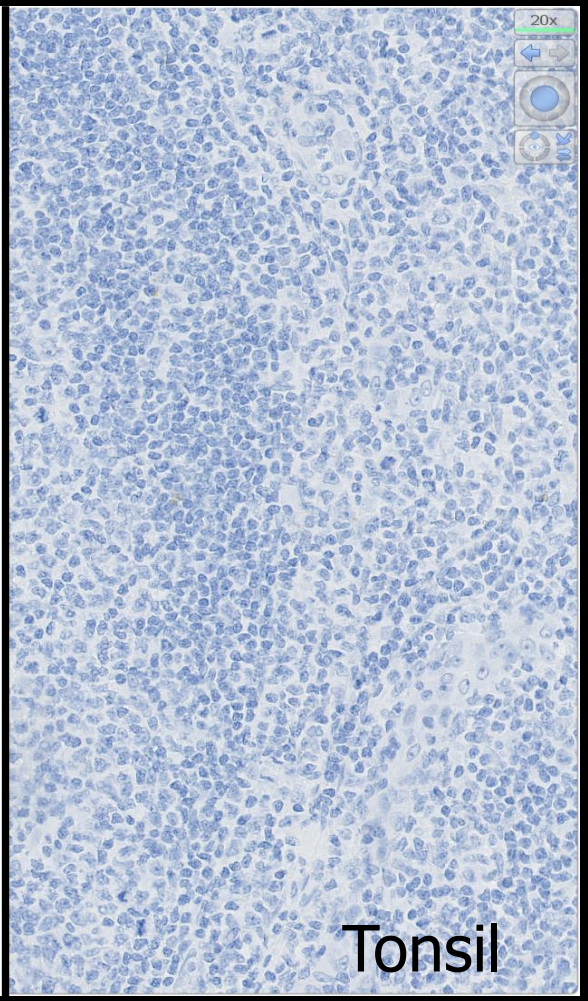
GCDFP15 / Mammaglobin reaction pattern



A moderate to strong, distinct cytoplasmic staining reaction in scattered ductal epithelial cells and in apocrine metaplastic cells.



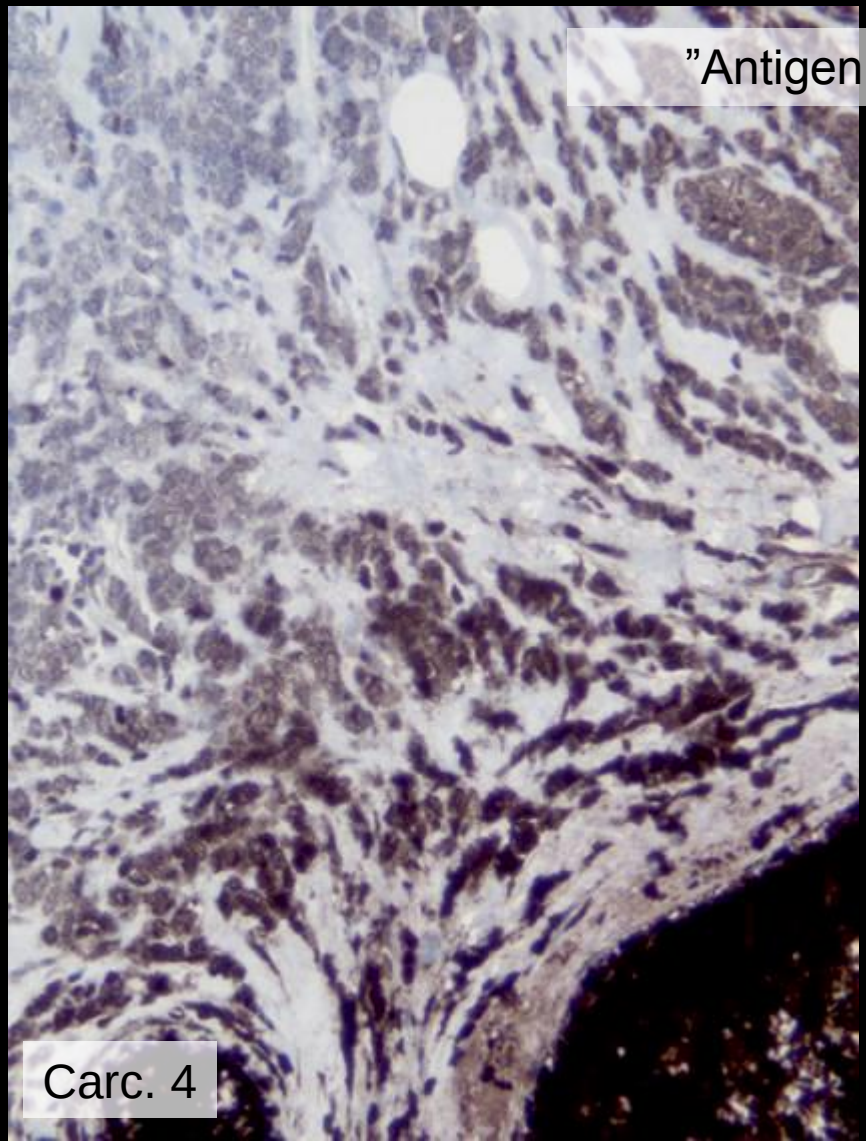
A moderate to strong, distinct cytoplasmic staining reaction of the majority of the epithelial cells of the eccrine sweat glands



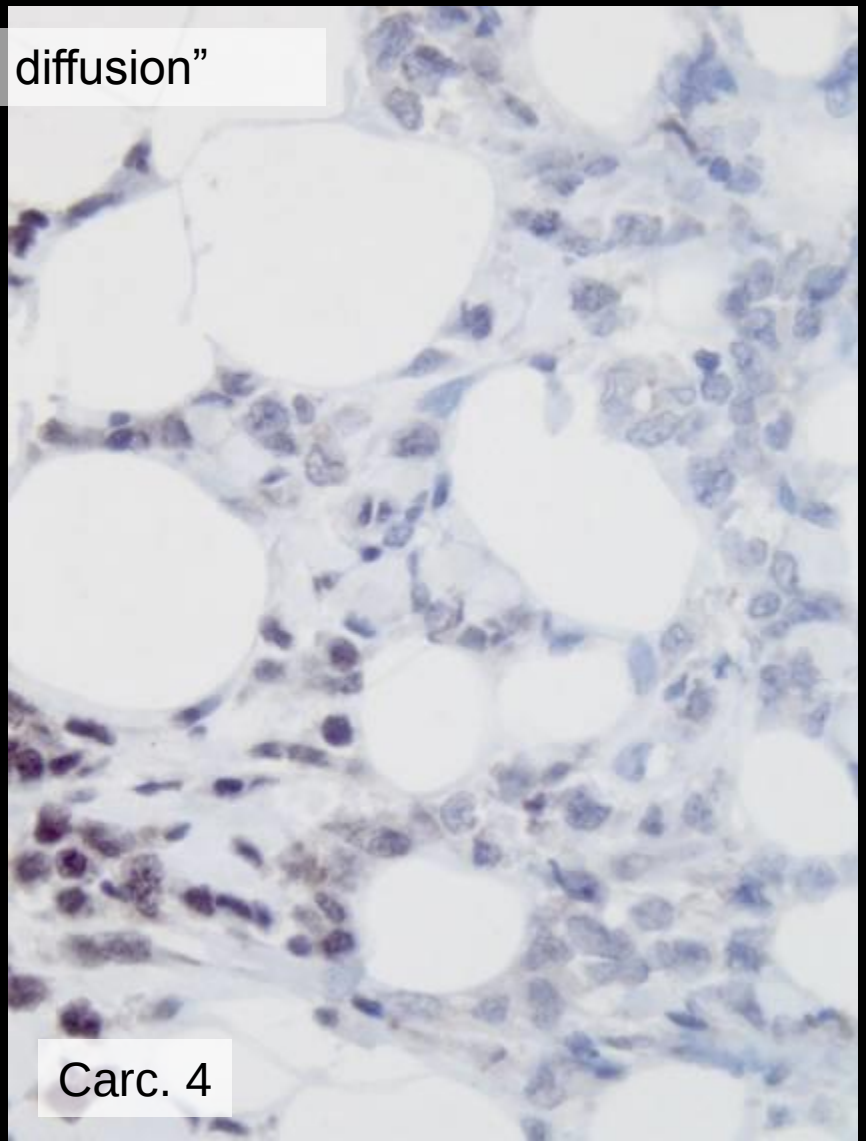
No staining reaction should be seen.

IHC – Protocols and controls for Breast tumours

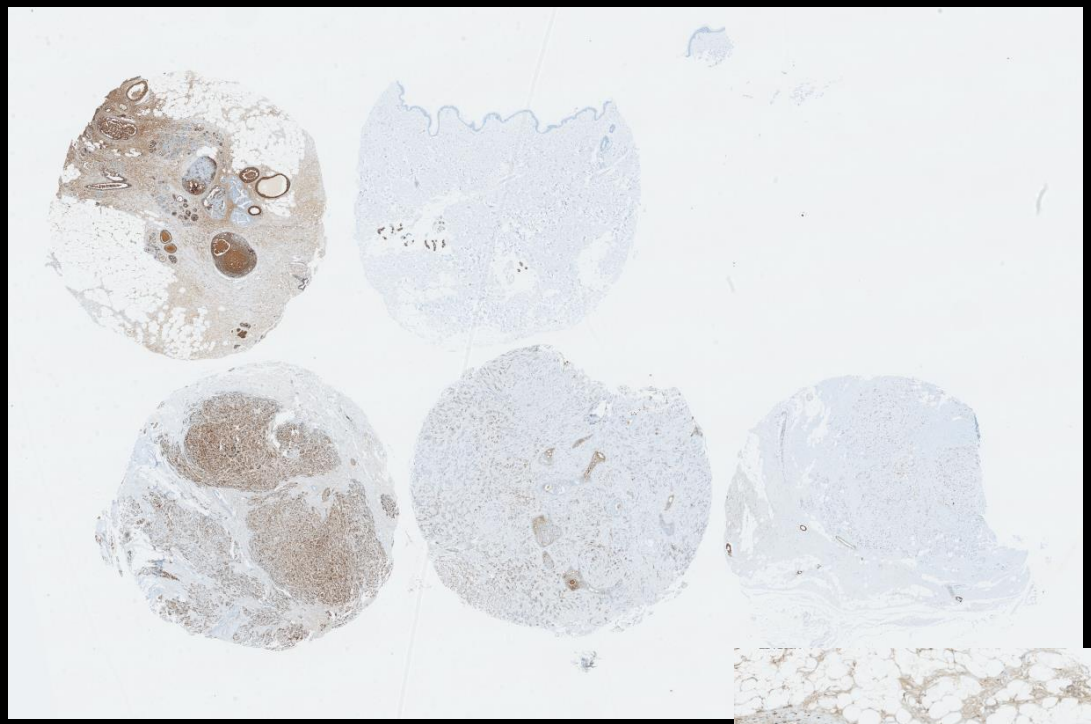
GCDFP15 / Mammaglobin reaction pattern



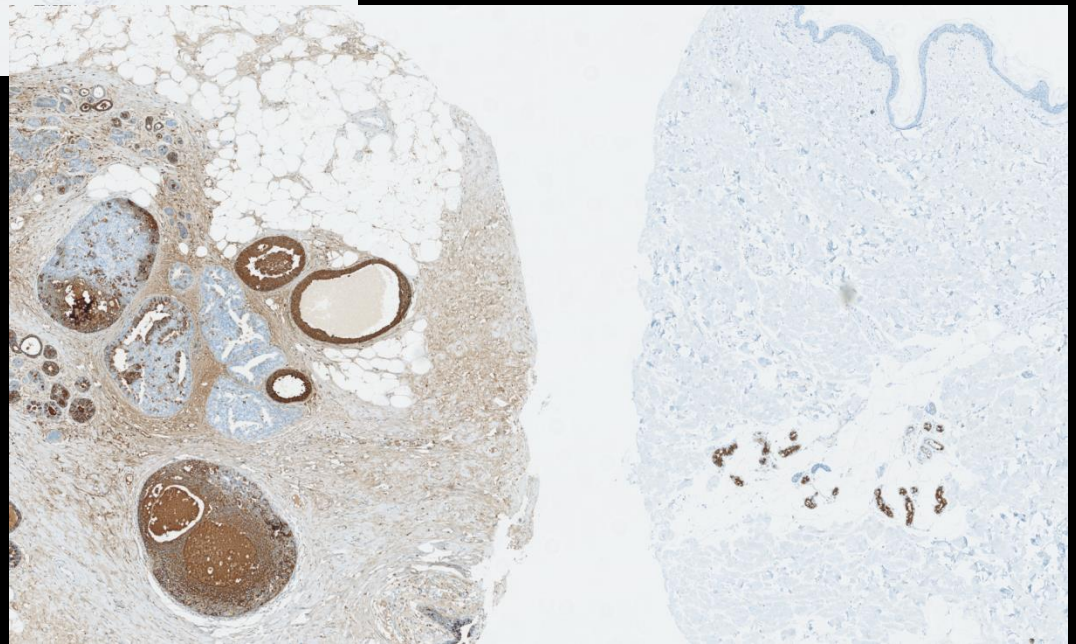
”Antigen diffusion”



IHC – Protocols and controls for Breast tumours

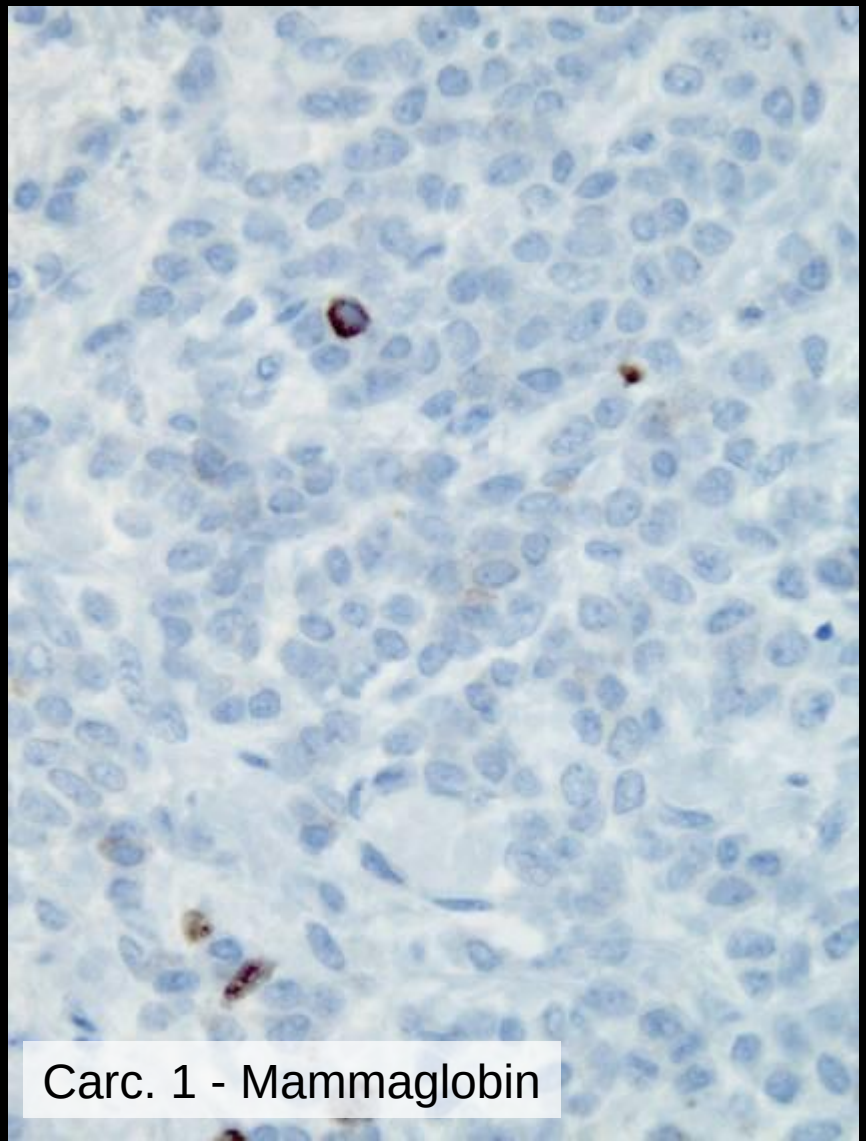


Local diffusion

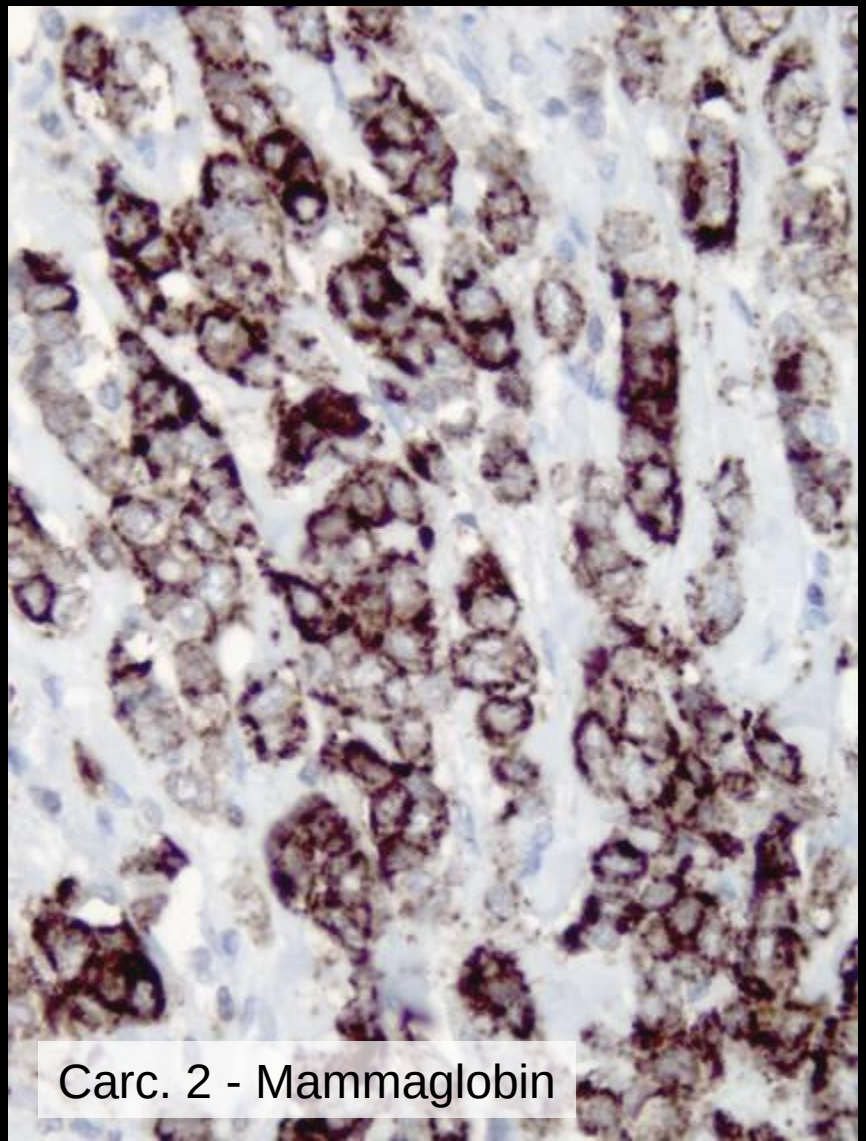


IHC – Protocols and controls for Breast tumours

GCDFP15 / Mammaglobin reaction pattern



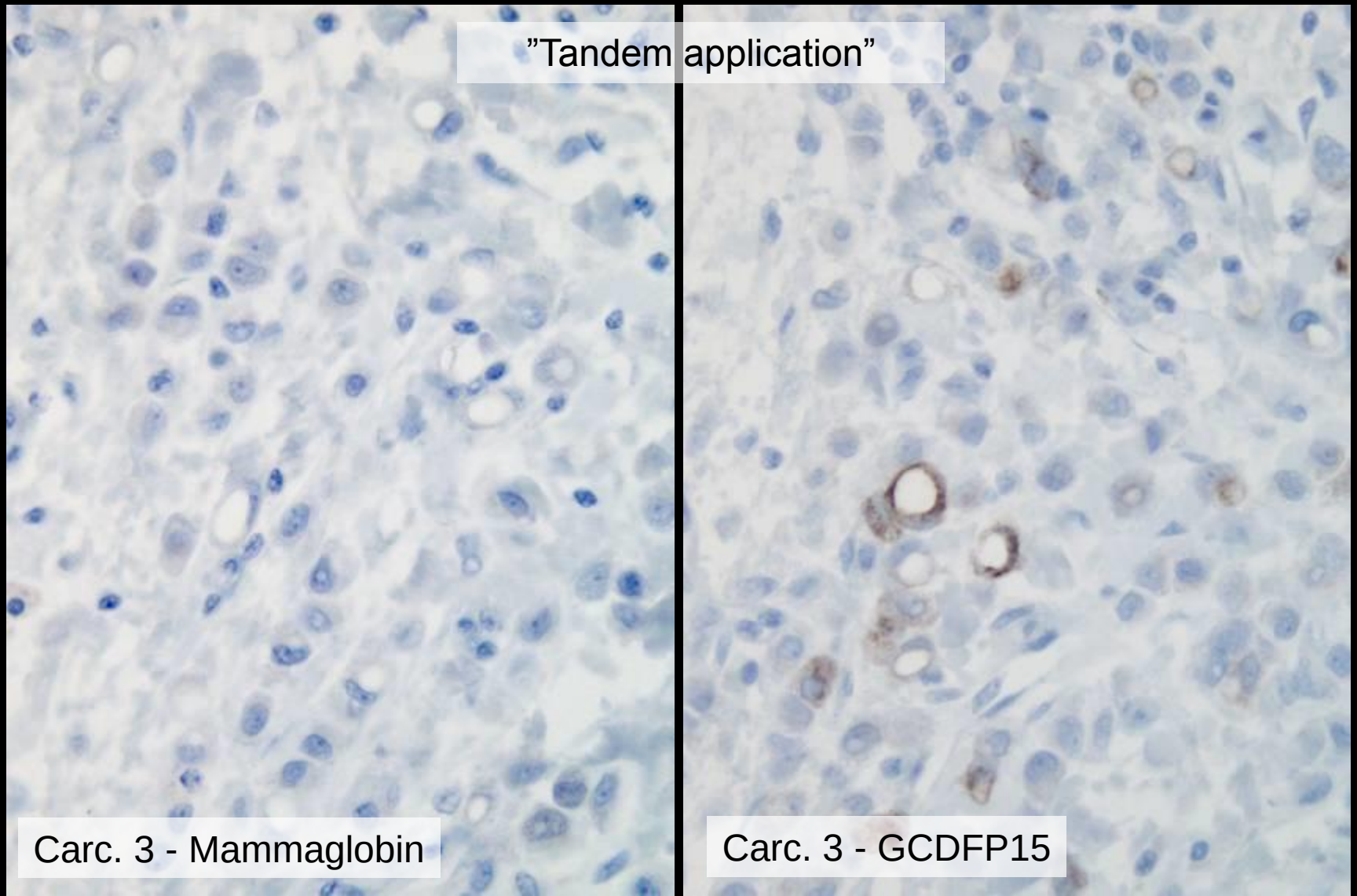
Carc. 1 - Mammaglobin



Carc. 2 - Mammaglobin

IHC – Protocols and controls for Breast tumours

GCDFP15 / Mammaglobin reaction pattern



IHC – Protocols and controls for Breast tumours

Table 1. Abs and assessment marks for GCD, run 36

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **23A3**	43 12 10 2 2 1 1 1	Leica/Novocastra Thermo/Neomarkers Dako Cell Marque Diagnostic Biosystems Labs Inc. Vector Lab. Abcam	25	33	8	6	81 %	88 %
mAb clone **D6**	6 2 1 1 1	Covance/Signet ID Labs Biocare Invitrogen Sanbio	4	5	1	1	82 %	86 %
mAb **SPM135**	1	Spring Bioscience	0	1	0	0	-	-
rmAb **EP1582Y**	2 1	Cell Marque Zytomed systems	1	2	0	0	-	-
rmAb **EP95**	1	Epitomics	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **23A3 IS/IR077**	20	Dako	10	10	0	0	100 %	100 %
mAb clone **23A3 PA0350**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **23A3 257M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **23A3 MS-1170**	1	Thermo/Neomarkers	0	0	1	0	-	-
mAb clone **23A3 MAD-001638QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone **EP1582Y 760-4386**	18	Ventana	10	7	1	0	94 %	94 %
rmAb clone **EP1582Y AN481-5M**	1	Biogenex	0	1	0	0	-	-
Total	131		50	62	12	7		
Proportion			38 %	48 %	9 %	5 %	86 %	

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

Ins.:

Omission of HIER

and/or

too low conc.

RTU > Conc.

(difficult to calibrate… what is best control…)

IHC – Protocols and controls for Breast tumours

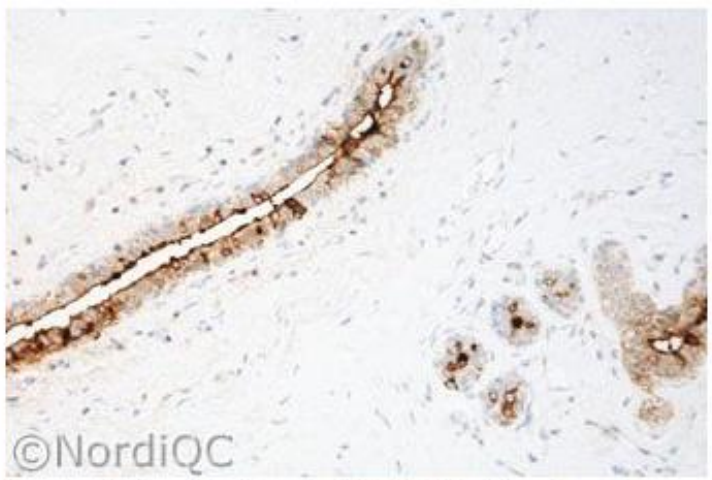


Fig. 1a. Optimal staining for GCDFP-15 of the breast hyperplasia using the mAb clone 23A3 optimally calibrated as a concentrate, HIER in an alkaline buffer and a polymer based detection system. The majority of the ductal epithelia cells show a distinct moderate to strong cytoplasmic staining reaction. Also compare with Fig. 2a – same protocol.

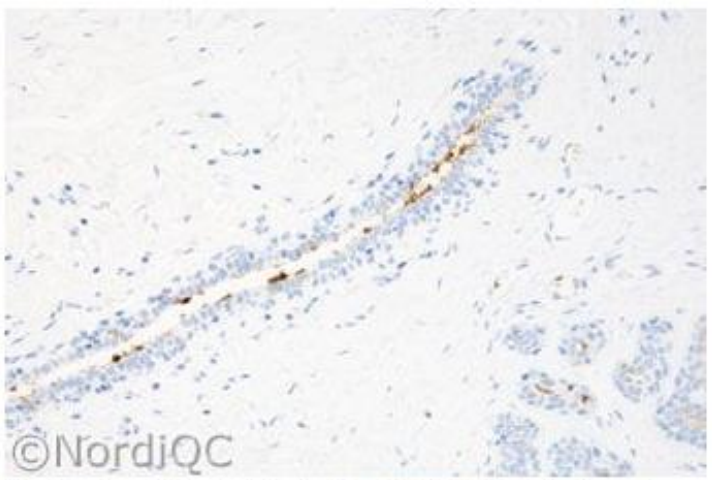


Fig. 1b. Insufficient staining for GCDFP-15 of the breast hyperplasia applying the mAb clone 23A3 as a concentrate using exactly the same protocol settings as used in Fig 1a, except for a 20 fold dilution of the primary antibody. The proportion and the intensity of the cells demonstrated are significantly reduced compared to the result in Fig. 1a. Also compare with Fig. 2b – same protocol.

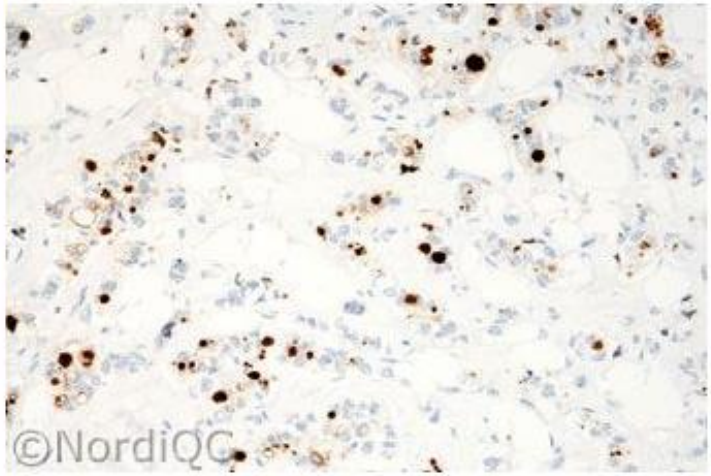


Fig. 2a. Optimal staining for GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong dot-like cytoplasmic staining reaction.

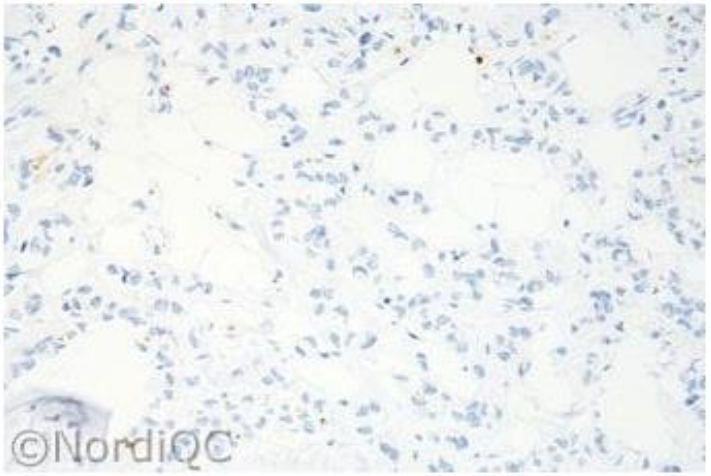


Fig. 2b. Insufficient staining GCDFP-15 of the breast carcinoma no. 5 using same protocol as in Fig. 1b. - same field as in Fig. 2a. Only scattered neoplastic cells show a faint dot-like reaction.

IHC – Protocols and controls for Breast tumours

Table 1. Abs and scores for mammaglobin, run 25

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **304-1A5**	14 4	Dako BioLogo	7	8	2	1	83 %	100 %
pAb **53625**	1	AnaSpec. Inc.	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **304-1A5**	2	Dako, IR074	2	0	0	0	-	-
rmAb clone **31A5**	2	Ventana, 760-4623	2	0	0	0	-	-
Total	23		11	8	2	2	-	-
Proportion			48 %	35 %	9 %	9 %	83 %	100 %

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

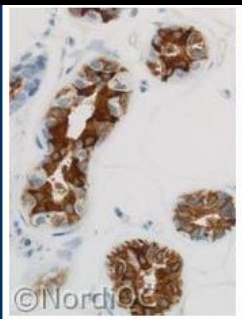


Fig. 1a. Optimal staining for mammaglobin using the mAb clone 304-1A5 optimally calibrated and with HIER.
Left: Skin: The majority of the epithelial cells of the eccrine sweat glands show a distinct cytoplasmic reaction.
Right: Breast: The apocrine metaplastic cells and few ductal epithelial cells show a distinct cytoplasmic reaction.
Also compare with Figs. 2a & 3a – same protocol.

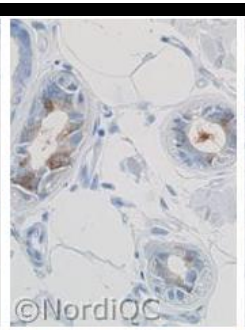


Fig. 1b. Insufficient staining for mammaglobin using the mAb clone 304-1A5 too diluted.
Left: Skin: Only scattered epithelial cells of the eccrine sweat glands show a weak cytoplasmic reaction.
Right: Breast: The epithelial cells are virtually negative and only extracellular mucus is demonstrated. Also compare with Figs. 2b & 3b – same protocol.

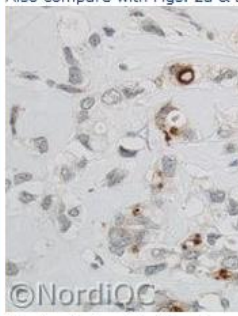


Fig. 3a. Optimal staining for mammaglobin of the breast carcinoma no. 5 using same protocol as in Fig. 1a & 2a. The majority of the neoplastic cells show at least a weak cytoplasmic reaction and focally a dot-like staining.

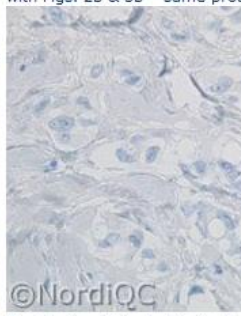


Fig. 3b. Insufficient staining for mammaglobin of the breast carcinoma no. 5 using same protocol as in Fig. 1b & 2b. None or only a dubious reaction is seen in the neoplastic cells.

Ins.:

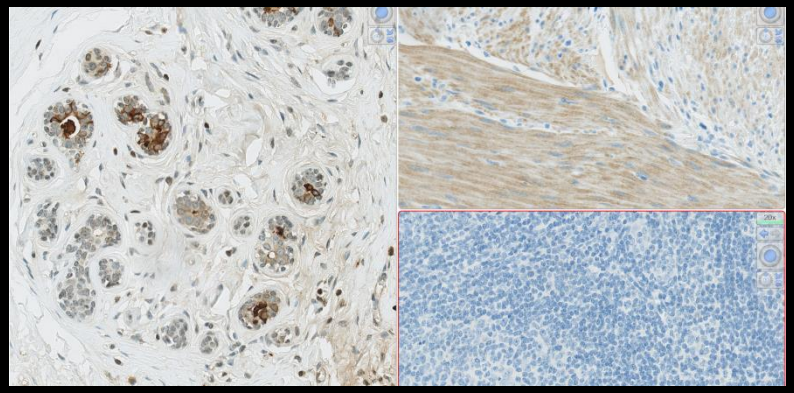
Too low/high conc.

HIER and calibration mandatory for optimal results

IHC – Protocols and controls for Breast tumours

Breast panel: GCDFP15 & Mammaglobin
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb 23A3	HIER High	1:10 - 75	3-step	Dako	2- & 3-step
mAb D6	HIER High	1:4 - 100	3-step	-	-
rmAb EP1582Y	HIER High	1:500-1.000	3-step	Ventana	2- & 3-step
mAb 304-105	HIER High	1:50 - 400	2- & 3-step	Dako	2-step
mAb 31A5	HIER High	-	-	Ventana	2-step

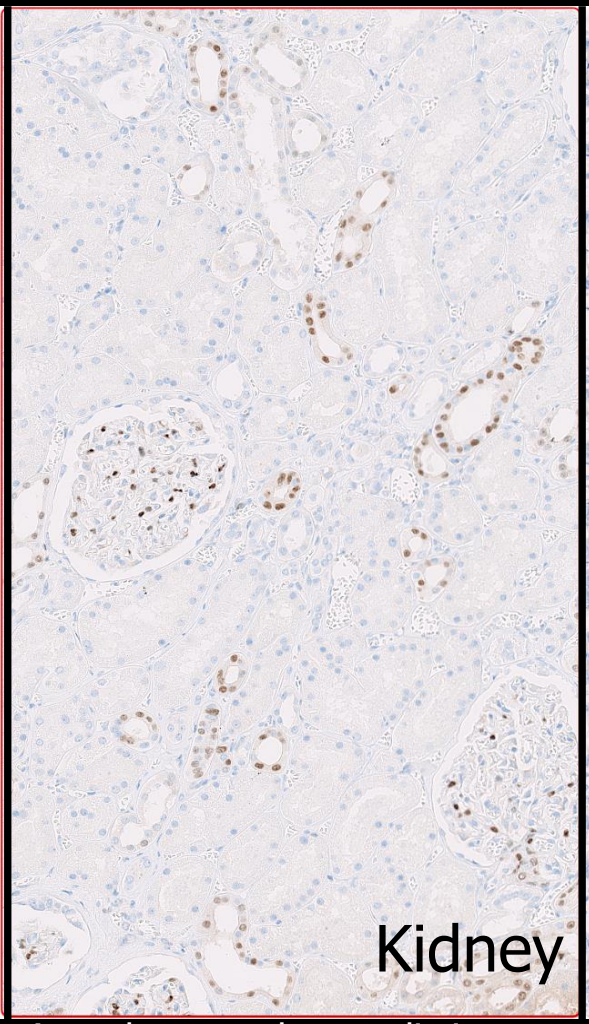


rmAb clone EP1582Y can show positive staining reaction in smooth muscle cells

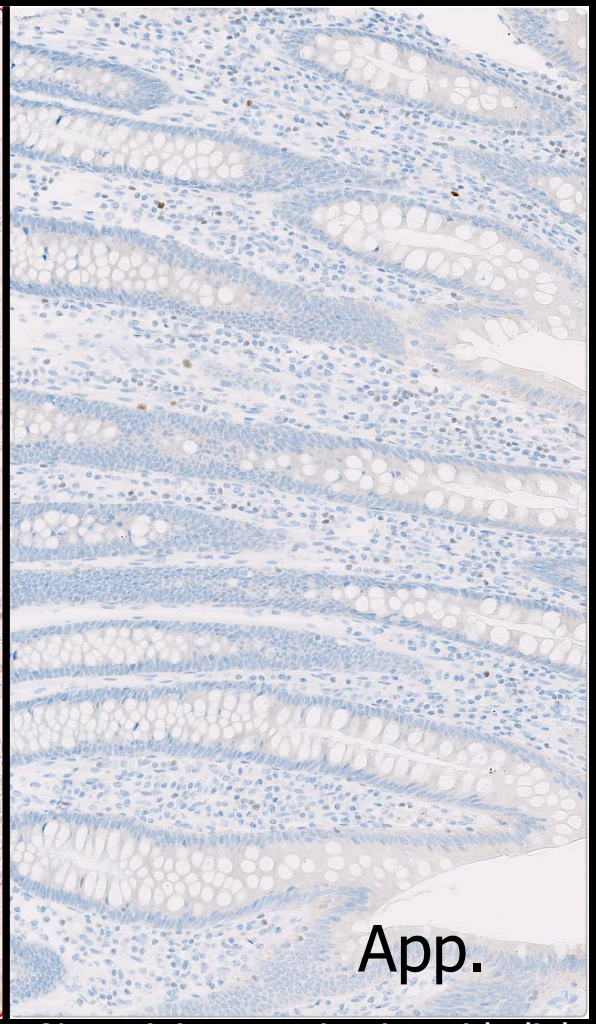
IHC – Protocols and controls for Breast tumours

GATA3 reaction pattern

An at least weak nuclear staining reaction of the majority of T-cells in the T-zones in the tonsil.



An at least moderate, distinct nuclear staining reaction of virtually all epithelial cells in collecting ducts and podocytes in glomeruli in the kidney.

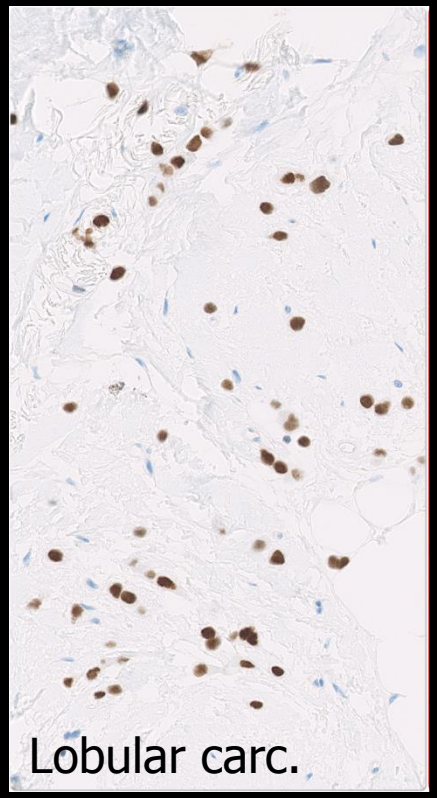


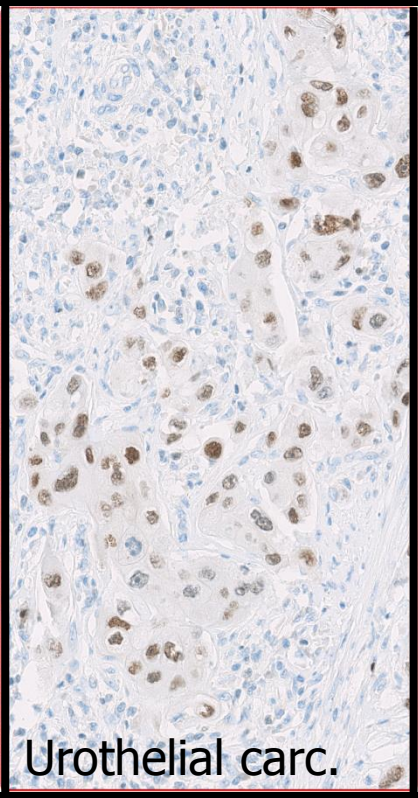
No staining reaction in epithelial cells should be seen.

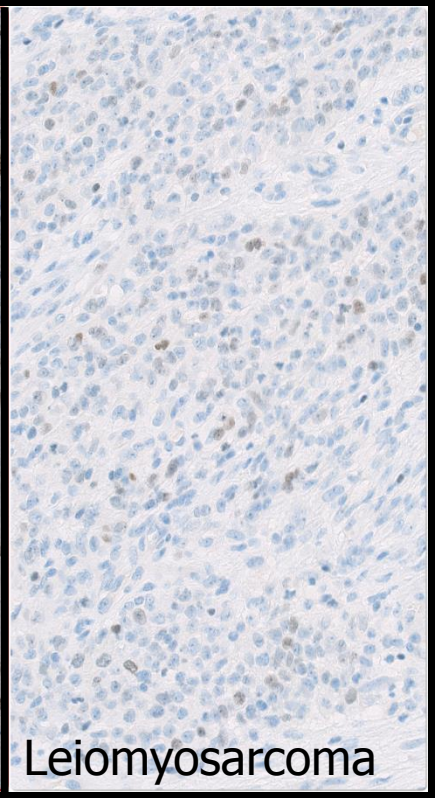
IHC – Protocols and controls for Breast tumours

Breast panel: GATA3
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
mAb L50-8023	HIER TE	1:70-500	2- & 3-step	Ventana	2- & 3-step


Lobular carc.


Urothelial carc.


Leiomyosarcoma

GATA3:

Highly sensitive for Breast carcinomas (& Urothelial carcinoma). But also seen in other neoplasias

IHC - Protocols and controls for Breast tumours

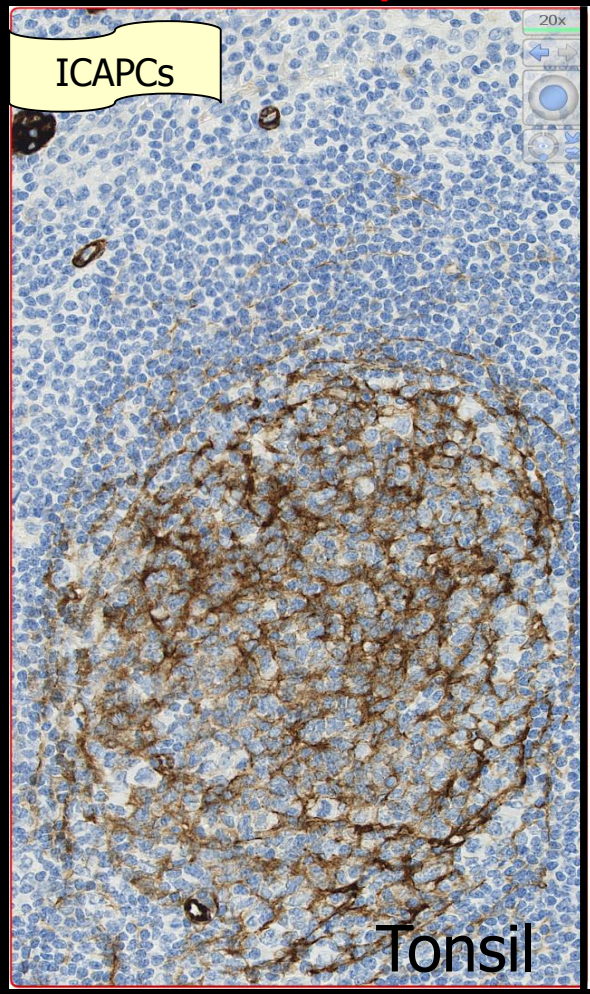
Breast panel:

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

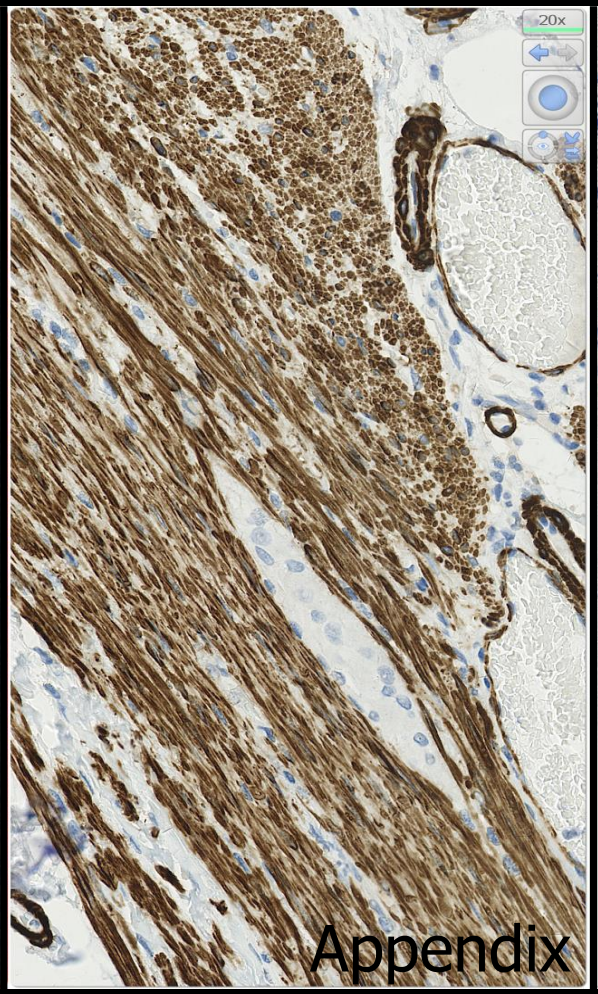
- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

IHC – Protocols and controls for Breast tumours

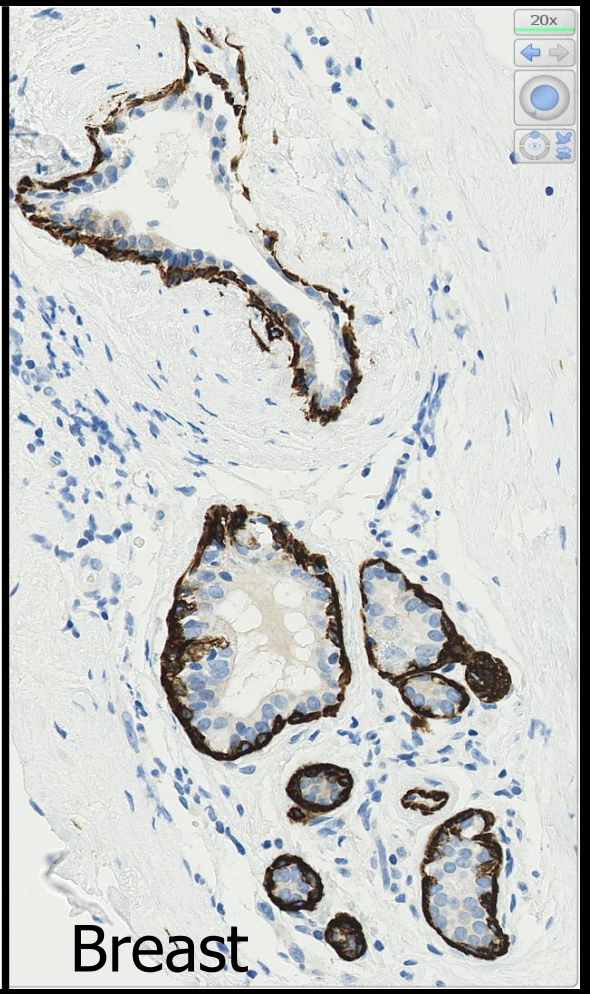
SMH reaction pattern



A weak to moderate, distinct cytoplasmic staining reaction in the follicular dendritic network of germinal centres. No staining should be seen in epithelial cells.



A moderate to strong, distinct cytoplasmic staining reaction of all smooth muscle cells in muscularis propria and vessels. No staining in epithelium.



A moderate to strong cytoplasmic staining reaction must be seen in myoepithelium. No staining reaction should be seen in luminal epithelial cells.

IHC – Protocols and controls for Breast tumours

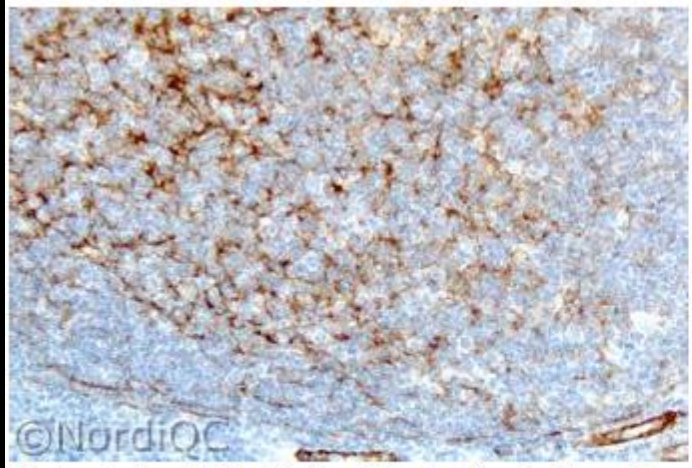


Fig. 1a. Optimal SMH staining of the tonsil using the mAb clone SMMS-1 correctly calibrated and with HIER in an alkaline buffer (Tris-EDTA pH 9). Both the smooth muscle cells of the vessels and dendritic follicular cells of the germinal centre show a moderate and distinct staining. No staining is seen in the lymphocytes.

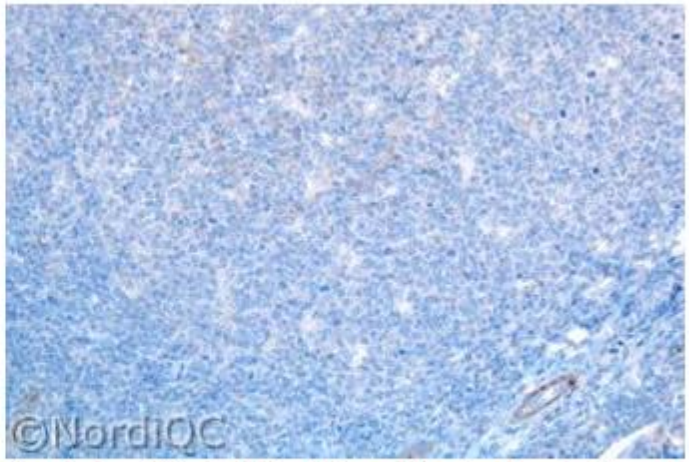


Fig. 1b. Insufficient SMH staining of the tonsil using the mAb clone SMMS-1 too diluted. Only the smooth muscle cells of the vessels are demonstrated, while the dendritic follicular cels of the germinal centre are virtually negative. Also compare with Fig. 2b - same protocol.

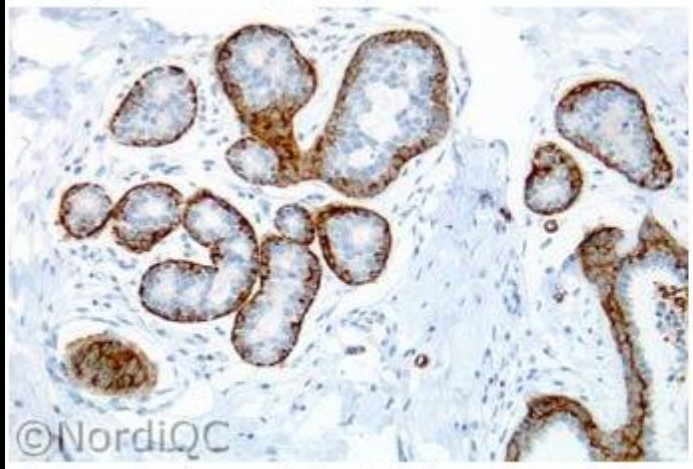


Fig. 2a. Optimal SMH staining of the breast hyperplasia using same protocol as in Fig. 1a. Virtually all the glandular myoepithelial cells show a strong and distinct reaction with no background reaction.

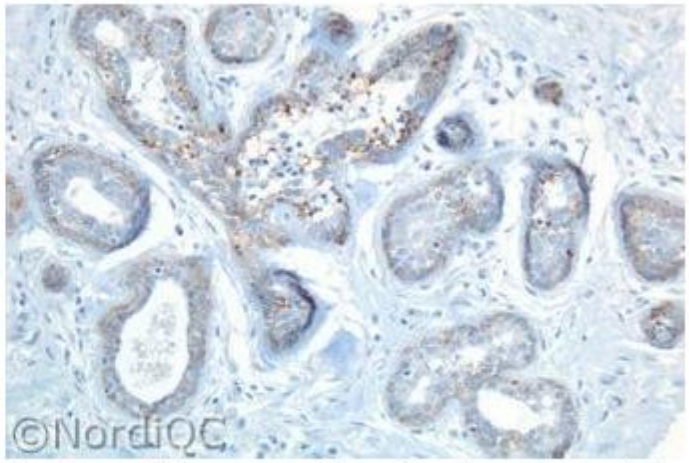


Fig. 2b. Insufficient SMH staining of the breast hyperplasia using same protocol as in Fig. 1b.
The myoepithelial cells only show a weak, ambiguous staining.

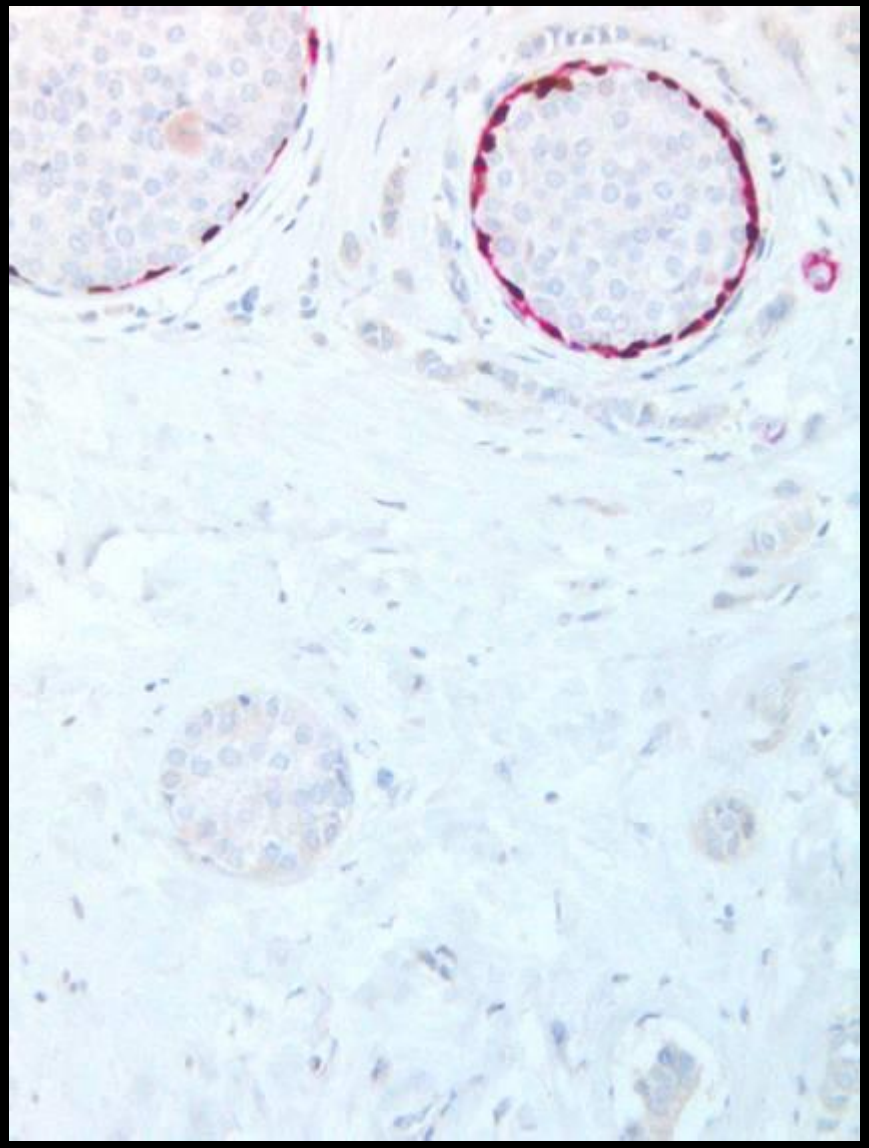
mAb clone SMMS-1

HIER in alk. pH

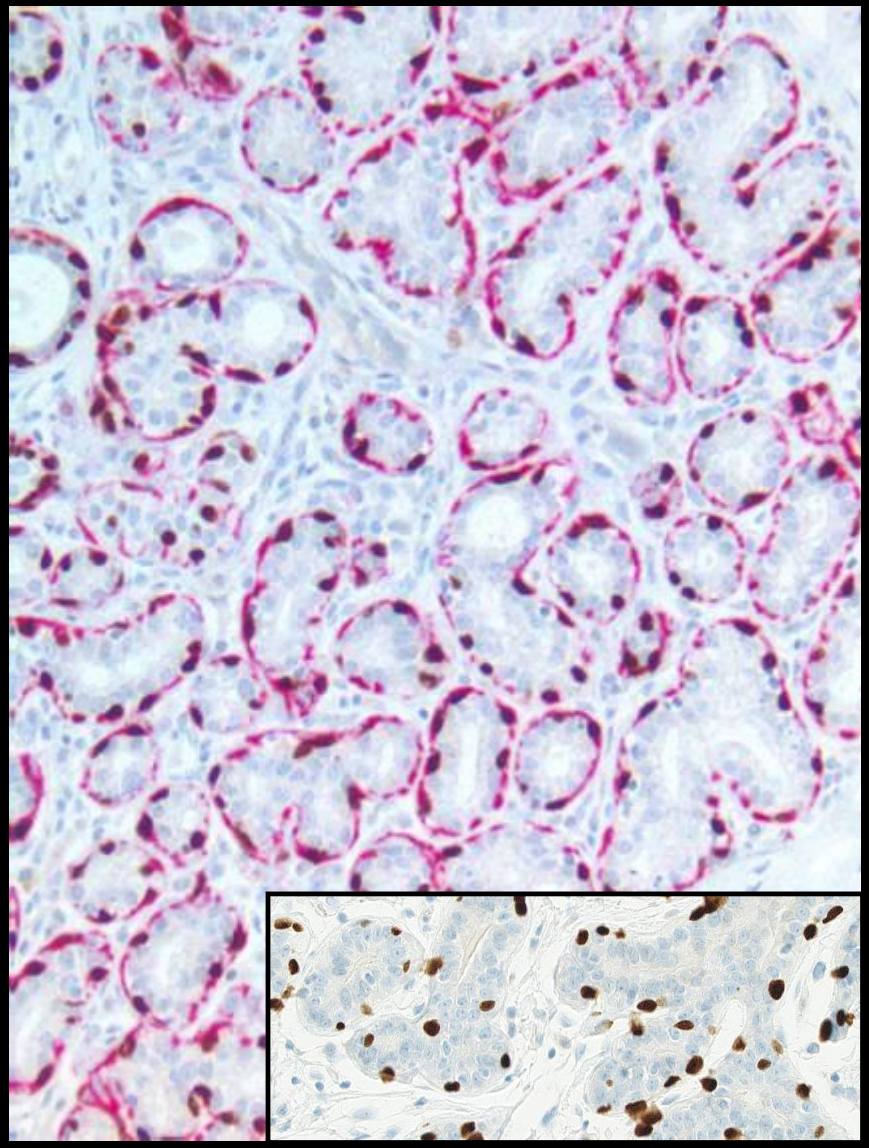
HIER in alk. pH + proteolysis

Insufficient HIER provided a too low sensitivity

IHC – Protocols and controls for Breast tumours



p63 / SMH



p63 / SMH

IHC – Protocols and controls for Breast tumours

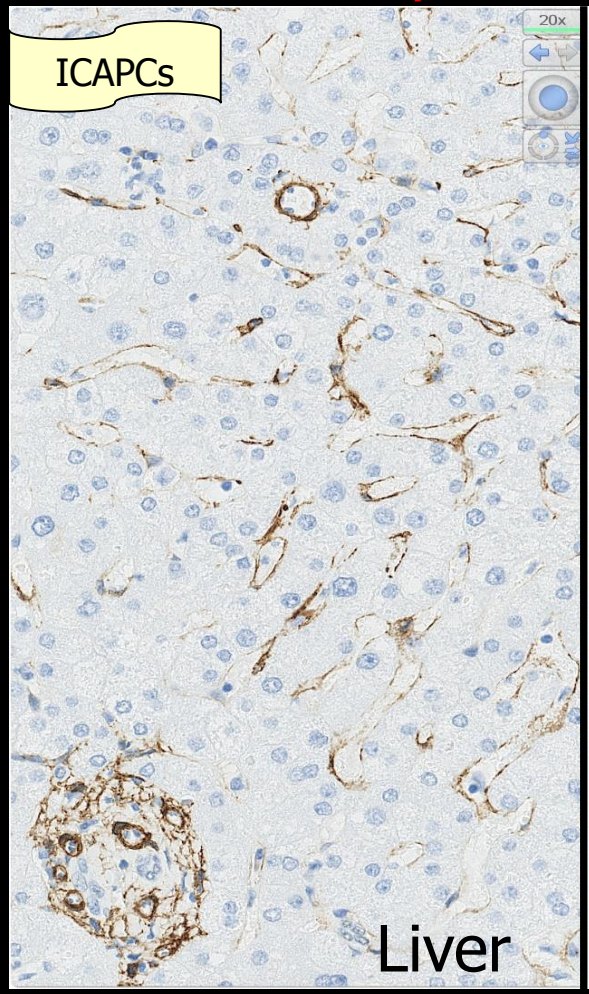
Breast panel: SMH

Basic protocol settings for an optimal staining result (NQC)

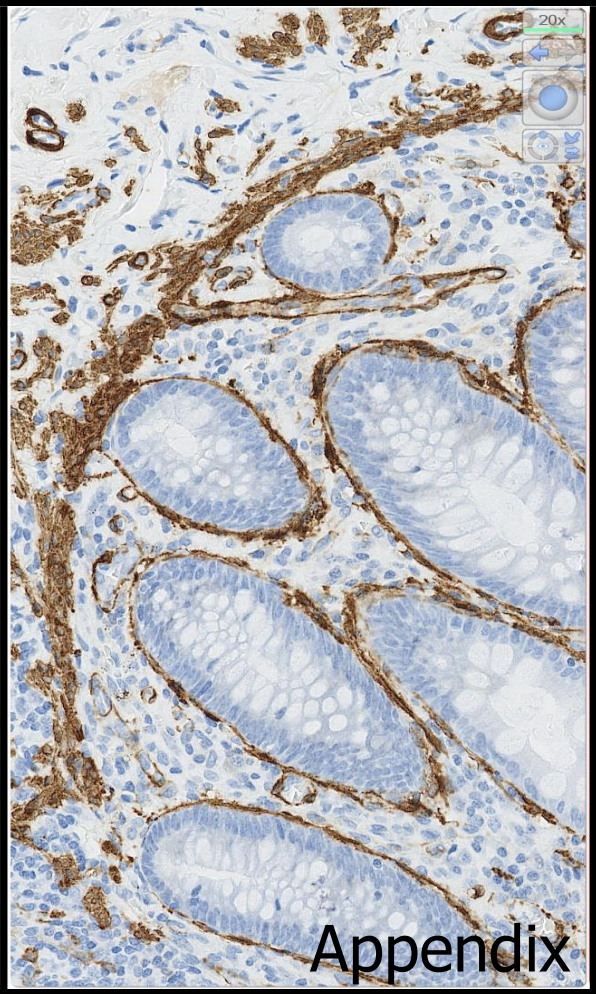
	Retrieval	Titre	Detection	RTU	Detection
mAb SMMS1	HIER High	1:200-1.500	2 & 3-step	Ventana	3-step
rmAb EPR5336	*HIER High*	*1:50 - 100*	*3-step*	-	-

IHC – Protocols and controls for Breast tumours

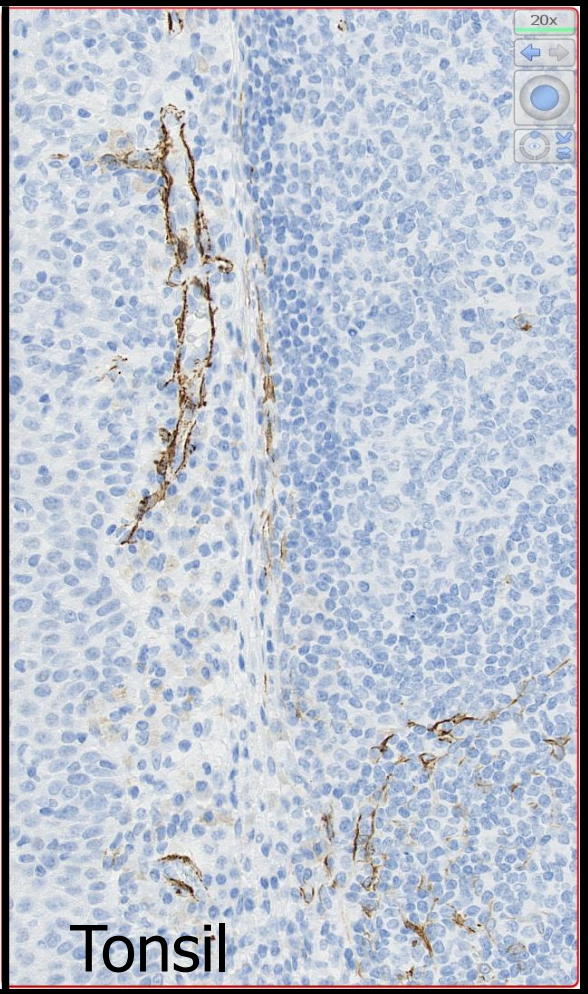
ASMA reaction pattern



A moderate to strong, distinct cytoplasmic staining of the majority of the perisinusoidal cells in the liver. No staining should be seen in hepatocytes.



A strong, distinct cytoplasmic staining of all the smooth muscle cells in the muscularis propria, lamina muscularis mucosae and myofibroblasts lining the crypts.



A moderate to strong cytoplasmic staining must be seen in smooth muscle cells – e.g. vessels
No staining should be seen in lymphocytes and epithelial cells.

IHC – Protocols and controls for Breast tumours

Table 1. **Antibodies and assessment marks for ASMA, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1A4**	98 6 5 1 1 1 1 1 1	Dako Thermo/NeoMarkers Sigma Aldrich AbD Serotec Biocare BioGenex Genemed Immunologic Spring Bioscience Zytomed	34	49	23	10	72%	85%
mAb clone **asm-1**	10	Leica/Novocastra	2	4	4	0	60%	100%
mAb clone **BS66**	1	Nordic Biosite	0	1	0	0	-	-
rmAb clone **E184**	1	Biocare	0	0	0	1	-	-
rmAb clone **EP188**	1	Epitomics	1	0	0	0	-	-
rmAb clone **SP171**	1	Spring Bioscience	0	0	1	0	-	-
Unknown	2	Unknown	1	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **1A4 IR/IS611**	44	Dako	23	13	7	1	82%	91%
mAb clone **1A4 760-2833**	44	Ventana/Cell Marque	0	6	29	9	14%	-
mAb clone **1A4 202M-9x**	3	Cell Marque	0	0	2	1	-	-
mAb clone **1A4 MAD-001195QD**	3	Master Diagnostica	0	0	3	0	-	-
mAb **1A4 PM001**	1	Biocare	0	0	1	0	-	-
mAb clone **1A4 AM128-5M**	1	BioGenex	0	1	0	0	-	-
mAb clone **1A4 Kit-0006**	1	Maixin	0	0	1	0	-	-
mAb clone **asm-1 PA0943**	5	Leica/Novocastra	1	3	1	0	80%	100%
Total	234		62	77	73	22	-	
Proportion			27%	33%	31%	9%	60%	

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

IHC – Protocols and controls for Breast tumours

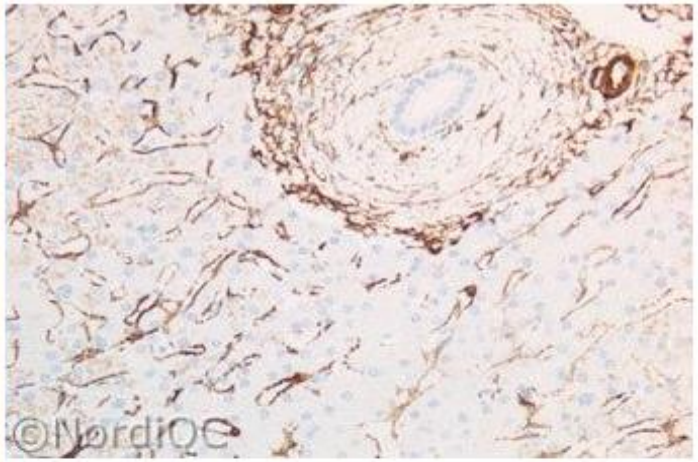


Fig. 1a. Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. The smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (a weak granular staining is due to lipofuscin).

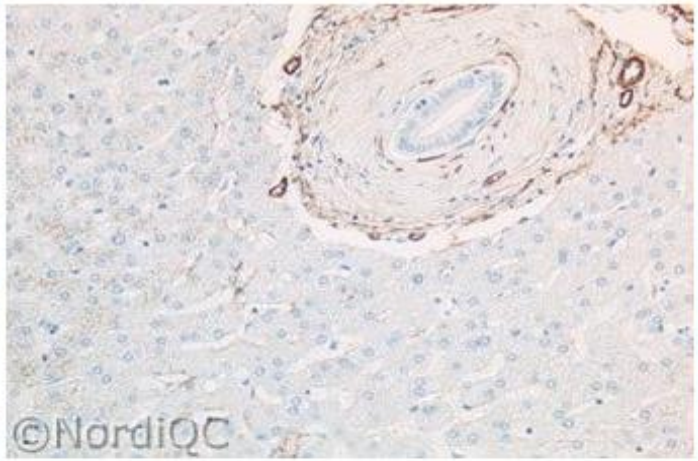


Fig. 1b. Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER – same field as in Fig. 1a. The smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative. Also compare with Figs. 2b & 3 b same protocol.

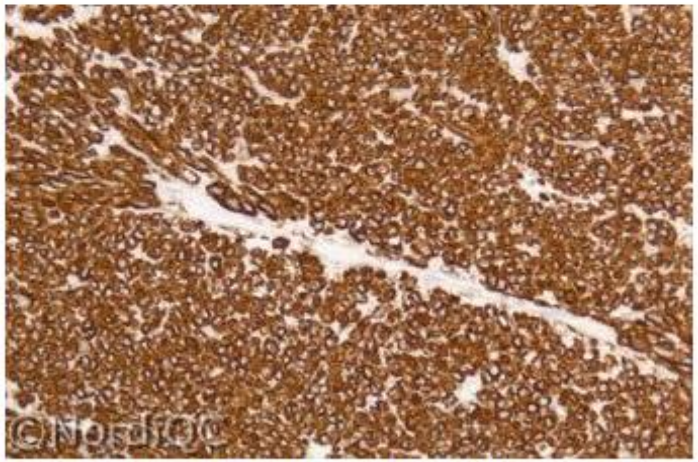


Fig. 2a. Optimal ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction.

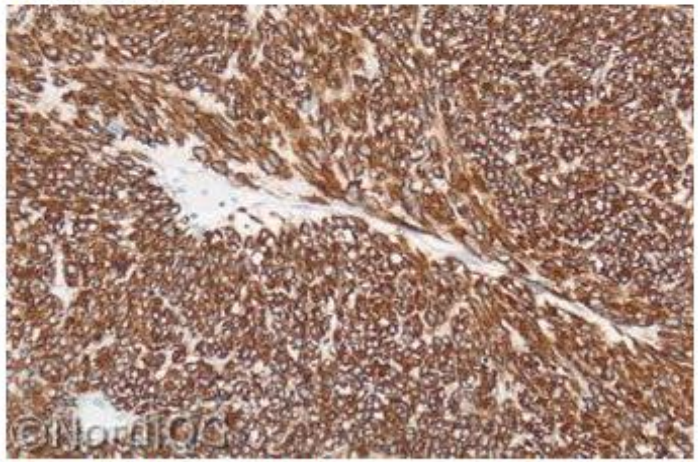


Fig. 2b. ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same insufficient protocol as in Fig. 1b. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction – same field as in Fig. 2a. However, also compare with Fig. 3b – same protocol.

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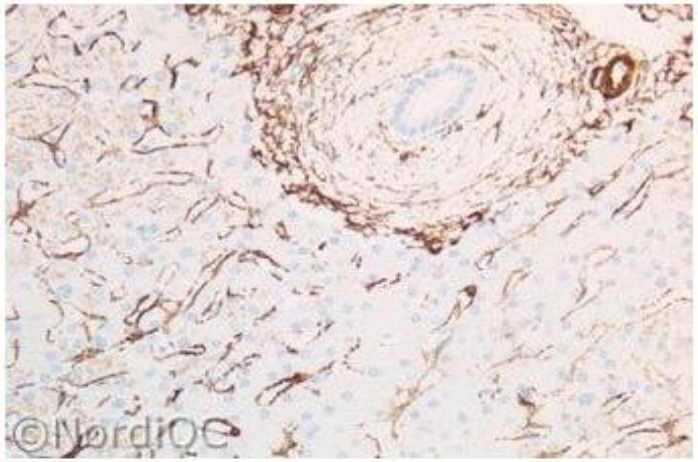

Fig. 1a. Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. The smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (a weak granular staining is due to lipofuscin).

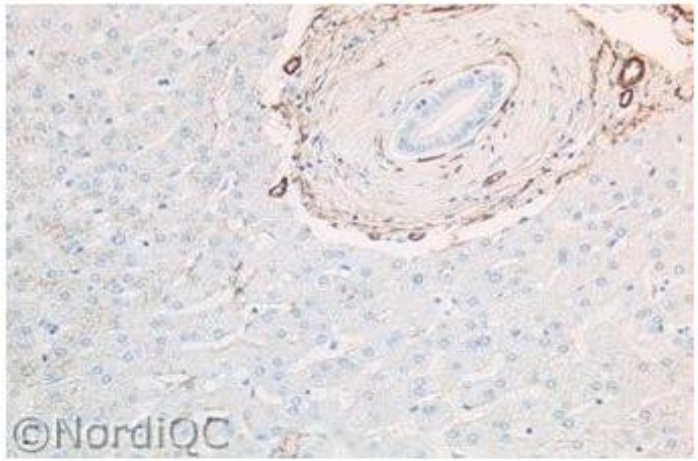

Fig. 1b. Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER – same field as in Fig. 1a. The smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative. Also compare with Figs. 2b & 3 b same protocol.

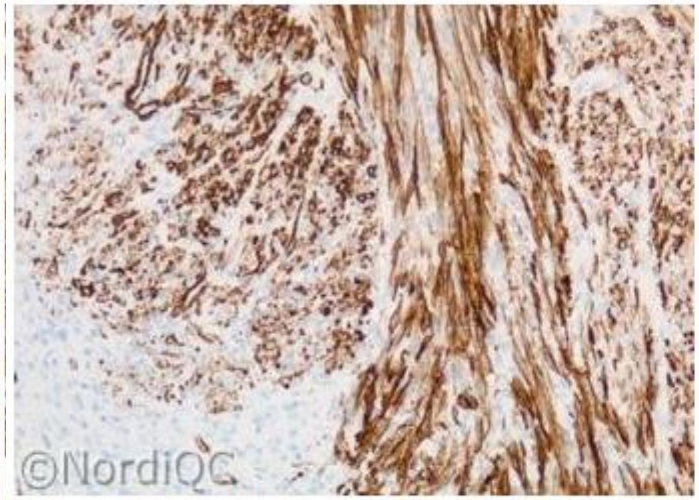

Fig. 3a. Optimal ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong and distinct reaction with no background reaction.

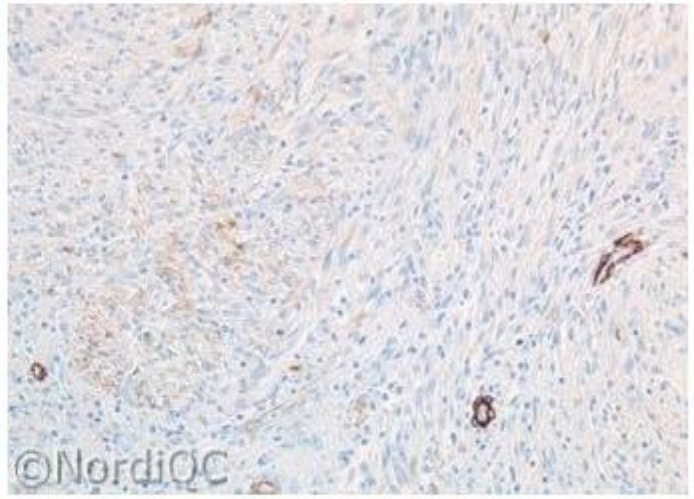

Fig. 3b. Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 3a.

IHC – Protocols and controls for Breast tumours

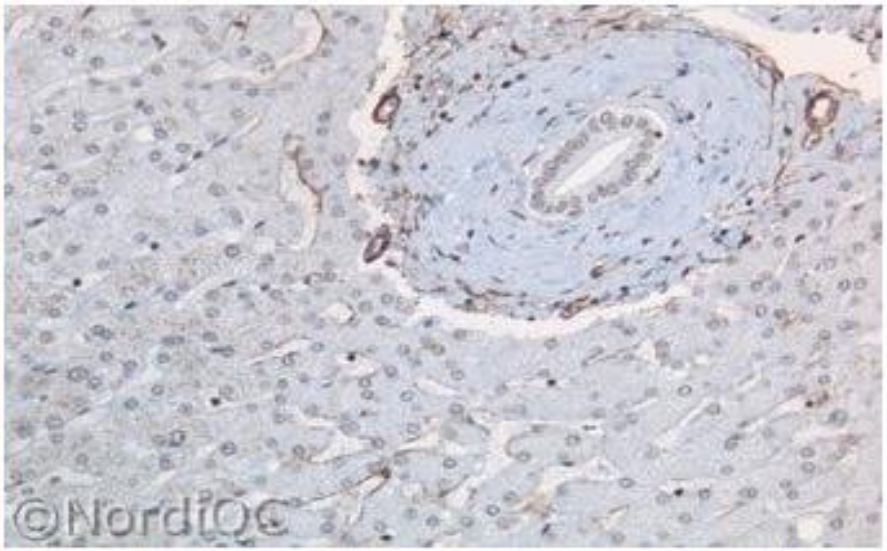

Fig. 4a. Insufficient ASMA staining of the liver using the mAb clone 1A4 with HIER in Cell Conditioning 1 (CC1) on the BenchMark XT, Ventana.
Scattered perisinusoidal smooth muscle cells are demonstrated, but the liver cells and the epithelial cells of the bile duct show a false positive nuclear reaction. This pattern was frequently seen when the mAb clone 1A4 was applied with HIER in CC1 and stained on the BenchMark XT, Ventana. Compare with Fig. 1a.

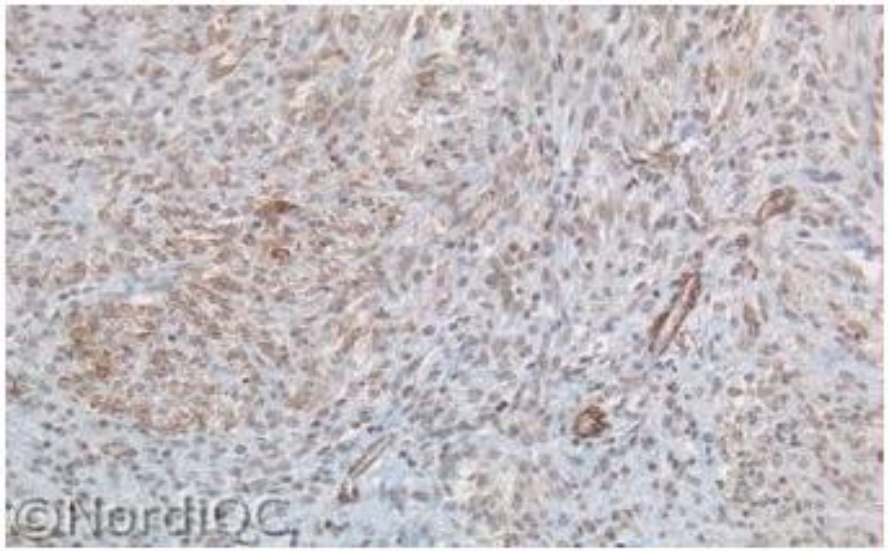

Fig. 4b. Left: Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multi block using same protocol as in Fig. 4a. The neoplastic cells show a false positive positive nuclear reaction, while the specific cytoplasmic reaction is virtually absent. Compare with Fig. 3a - same field.

Table 3. **Proportion of optimal results for ASMA for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **1A4**	14/25** (56%)	0/2	1/29 (3%)	0/2	5/7 (71%)	1/3

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

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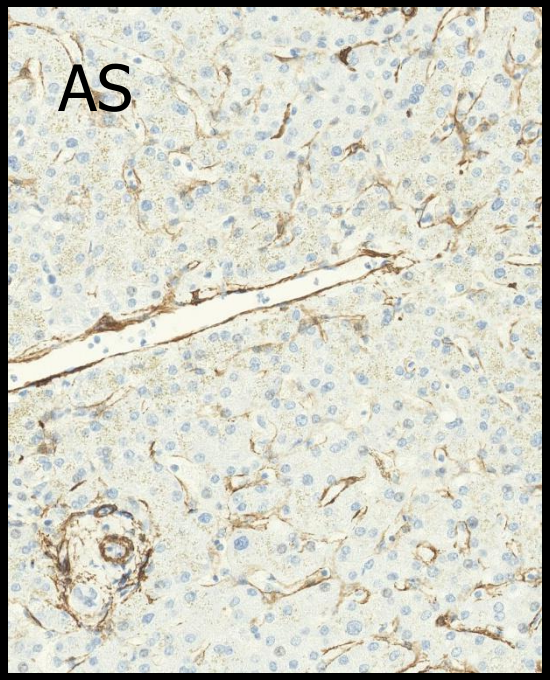
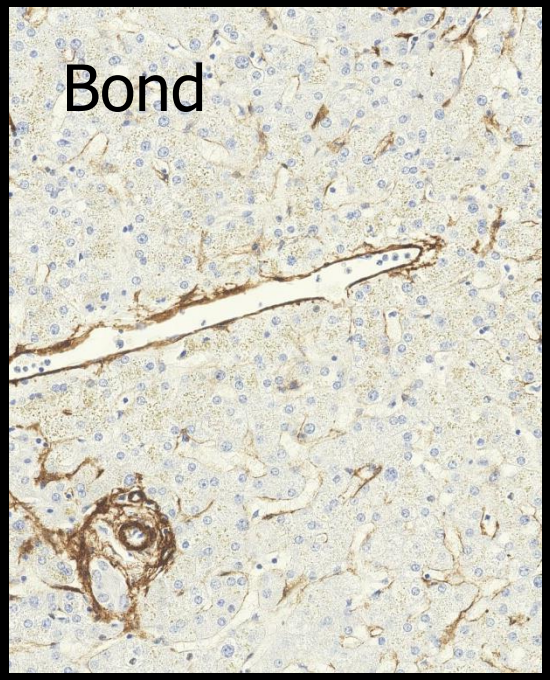
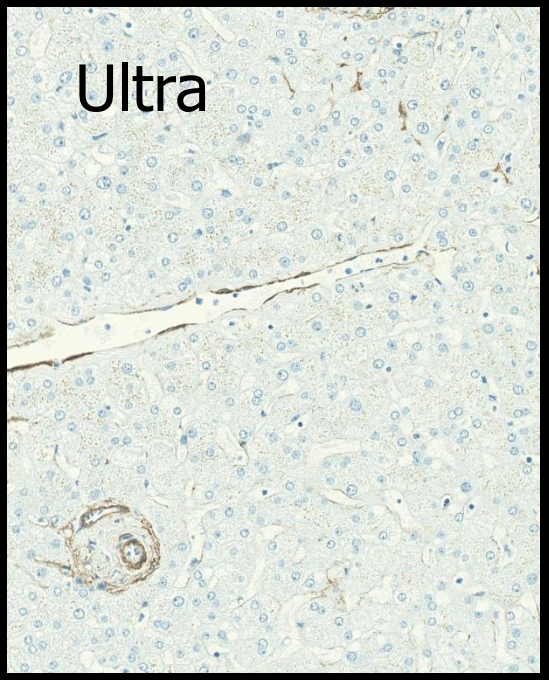






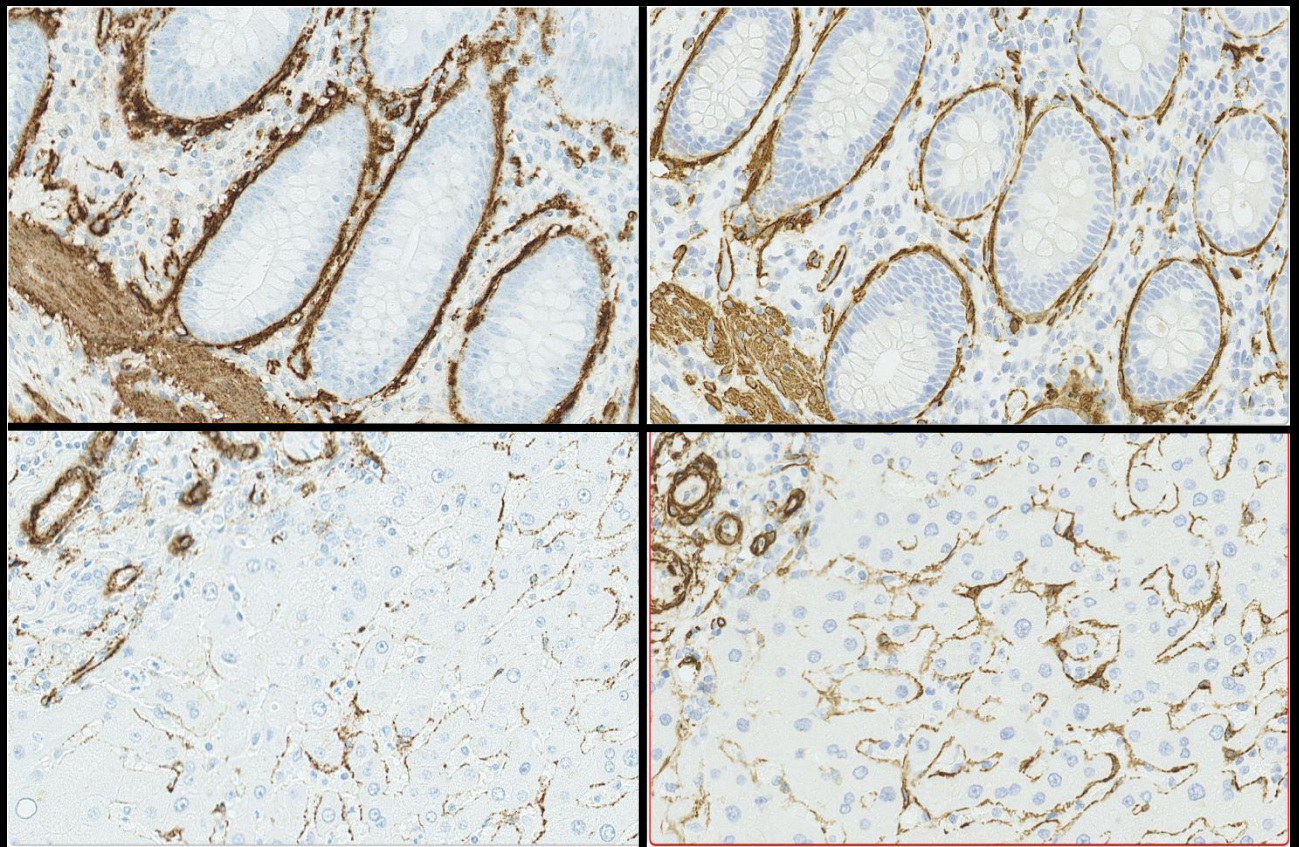
Protocol for clone depending on IHC stainer

mAb clone 1A4 (Dako*)	**Dako AS48**	**Leica Bond III**	**VMS Ultra**
Titre	1:100-500* / RTU	1:200-500*	*1:100-300**
Retrieval	HIER TRS High	HIER ER 2	*None*
Detection	2- or 3-step	3-step	*3-step*

IHC – Protocols and controls for Breast tumours

Breast panel: ASMA – Ventana BenchMark
Basic protocol settings for an optimal staining result (NQC)

	Retrieval	Titre	Detection	RTU	Detection
rmAb EP188	P2 4M + CC1M	1:200	3-step OP + AMP	-	-



ASMA:

Left: EP188,
VMS platform,
combined retrieval
OptiView + AMP

Right: 1A4
Dako platform
HIER
EnVision FLEX

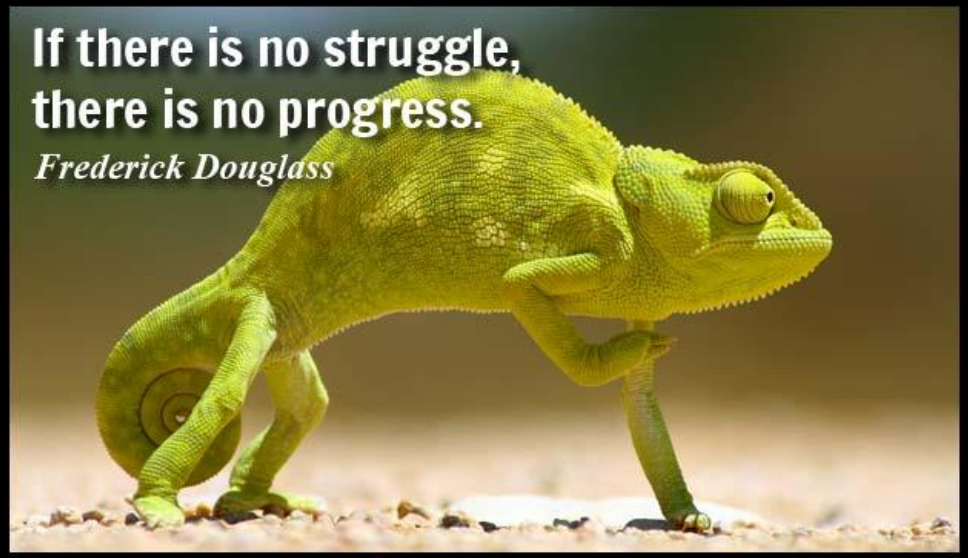
IHC – Protocols and controls for Breast tumours

Performance history
This was the fourth NordiQC assessment of ASMA and as shown in table 2 the pass rates have been constant at a relatively low level throughout all runs.

Table 2. **Proportion of sufficient results for ASMA in the four NordiQC runs performed**

	Run 10 2004	Run 21 2007	Run 27 2009	Run 44 2015
Participants, n=	71	106	124	234
Sufficient results	62%	63%	64%	60%

Conclusion
The mAb clones **1A4**, **asm-1** and rmAb clone **EP188** could all be used to obtain an optimal staining result. Using the two most widely used ASMA antibodies (clone 1A4 and asm-1) HIER and careful calibration of the titre of the primary antibody were the main prerequisites for optimal results.
The performance of clone 1A4 seems to be influenced by the stainer platform as a significantly reduced proportion of sufficient results was observed when used on the Ventana BenchMark platform compared to Dako Autostainer and Leica BOND platforms.



IHC - Protocols and controls for Breast tumours

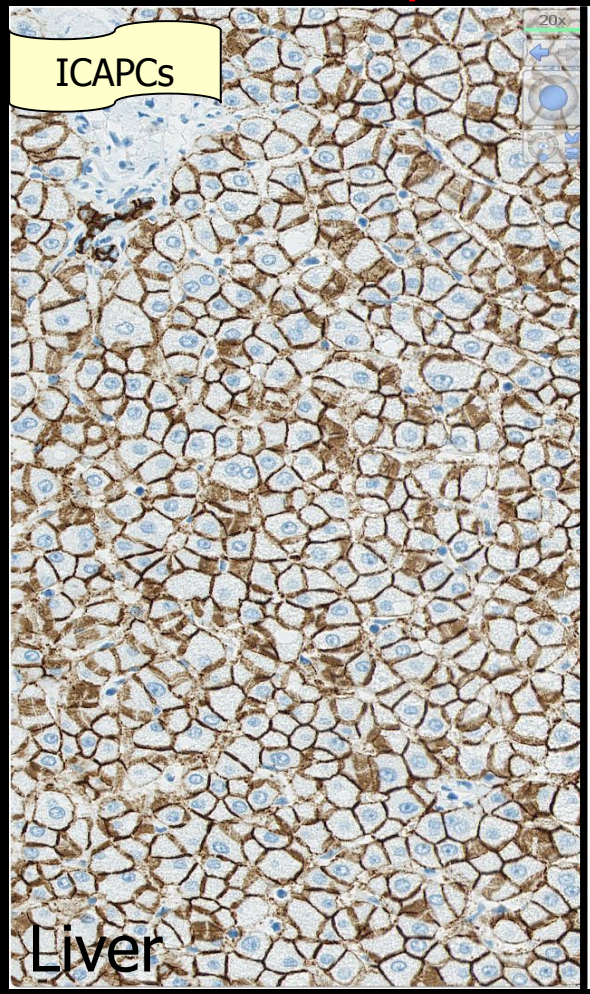
Breast panel:

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

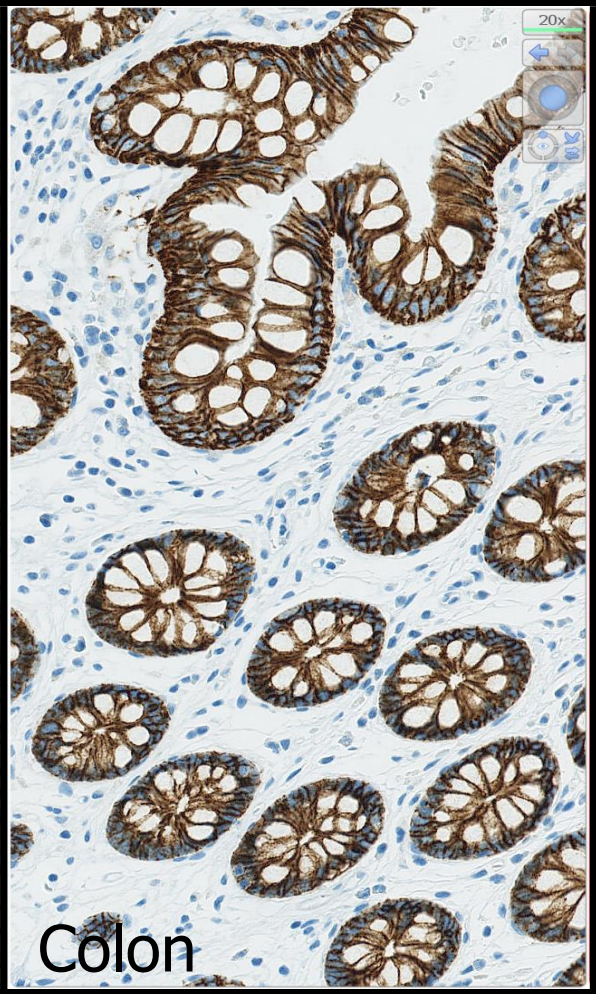
- Is it primary breast ?
- Is it invasive ?
- Is it lobular or ductal ?
- Which teraphy ?

IHC – Protocols and controls for Breast tumours

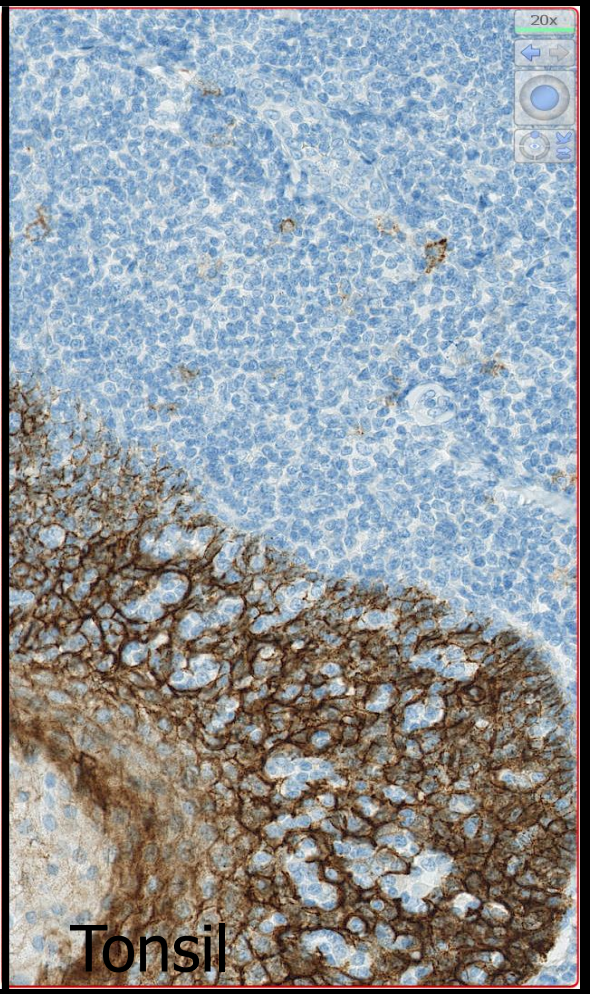
ECAD reaction pattern



An at least weak to moderate membranous staining reaction of virtually all the hepatocytes.



A moderate to strong, distinct membranous staining reaction of virtually all the columnar epithelial cells in the colon / appendix.



A moderate to strong, distinct membranous staining reaction of virtually all squamous epithelial cells. No staining reaction of the vast majority of lymphocytes.

IHC – Protocols and controls for Breast tumours

Table 1. **Antobodies and assessment marks for ECAD run 39**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **36**	1	BD Biosciences	0	0	1	0	-	-
mAb clone **36B5**	16 1	Leica/Novocastra Vector	2	13	2	0	88 %	100 %
mAb clone **4A2C7**	5	Invitrogen/Zymed	0	3	2	0	60 %	-
mAb clone **BSH38**	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **ECH-6**	1	Zytomed	1	0	0	0	-	-
mAb clone **HECD-1**	9 2 1 1	Invitrogen/Zymed Immunologic Abcam Biocare	7	6	0	0	100 %	100 %
mAb clone **NCH-38**	90 5	Dako Thermo/NeoMarkers	59	30	6	0	94 %	94 %
mAb clone **SPM471**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb clone **EP6**	2	Epitomics	0	2	0	0	-	-
rmAb clone **EP700Y**	6 1 1 1 1	Cell Marque Biocare Bio SB Thermo/NeoMarkers Zytomed	0	7	1	2	70 %	-
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use Antibodies								
mAb clone **36** **790-4497**	51	Ventana	6	12	32	1	35 %	38 %
mAb clone **36B5** **PA0387**	10	Leica	0	10	0	0	100 %	-
mAb clone **NCH-38** **IR/IS059**	44	Dako	40	4	0	0	100 %	100 %
mAb clone **NCH-38** **GA059**	1	Dako	1	0	0	0	-	-
rmAb clone **EP700Y** **760-4440**	16	Ventana/Cell Marque	0	15	1	0	94 %	-
rmAb clone **EP700Y** **246R-1x**	1	Cell Marque	0	1	0	0	-	-
rmAb clone **EP700Y** **MAD-000051QD**	1	Master Diagnostica	0	1	0	0	-	-
Total	271		117	106	45	3	-	
Proportion			43 %	39 %	17 %	1 %	82 %	

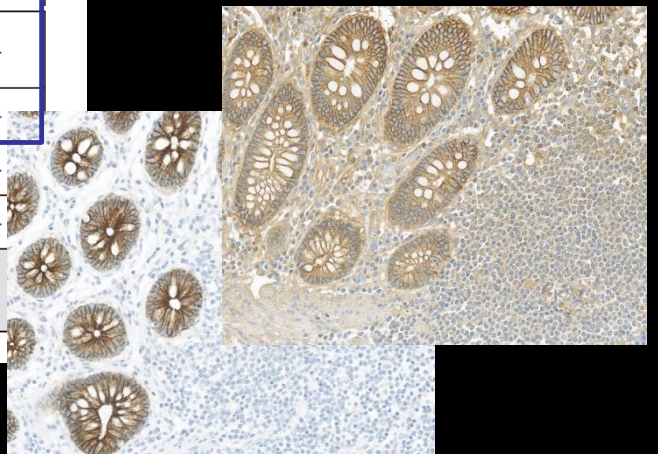
1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below.

mAb clones
HECD-1 & NCH-38 most successful

HIER
2 or 3-step mul/pol.

mAb clone 36 aberrant nuclear staining reaction

rmAb clone EP700y inferior signal-to-noise



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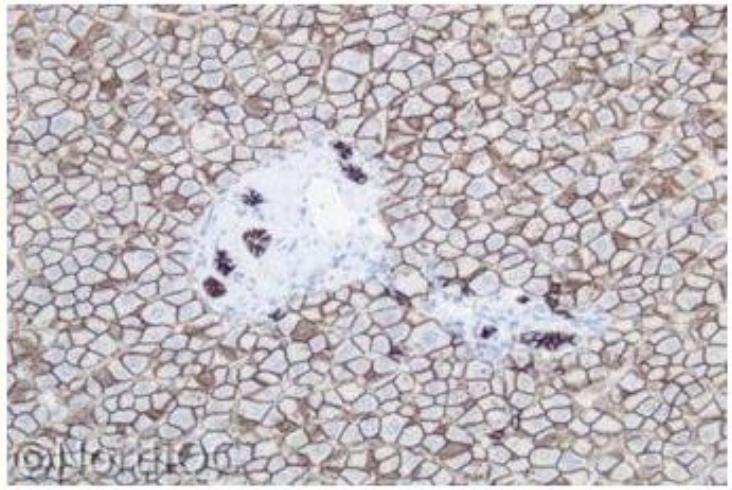


Fig. 1a. Optimal staining for ECAD of the liver using the mAb clone NCH-38 with HIER. Virtually all the hepatocytes show a moderate distinct membranous reaction, while the epithelial cells of the bile ducts show a strong staining.

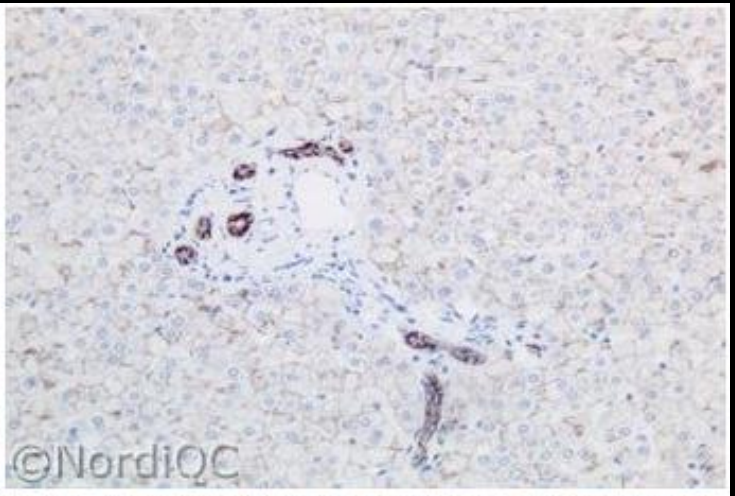


Fig. 1b. Staining for ECAD of the liver using an insufficient protocol based on the same mAb clone NCH-38 as in Fig. 1a, but in a too low concentration. The hepatocytes only show a weak disrupted membranous reaction – same field as in Fig. 1a.

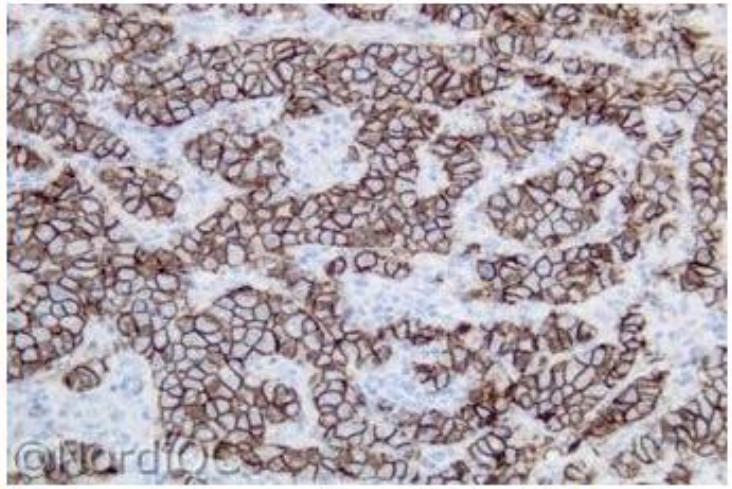


Fig. 2a. Optimal ECAD staining of the ductal breast carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a strong distinct membranous reaction with no background reaction.

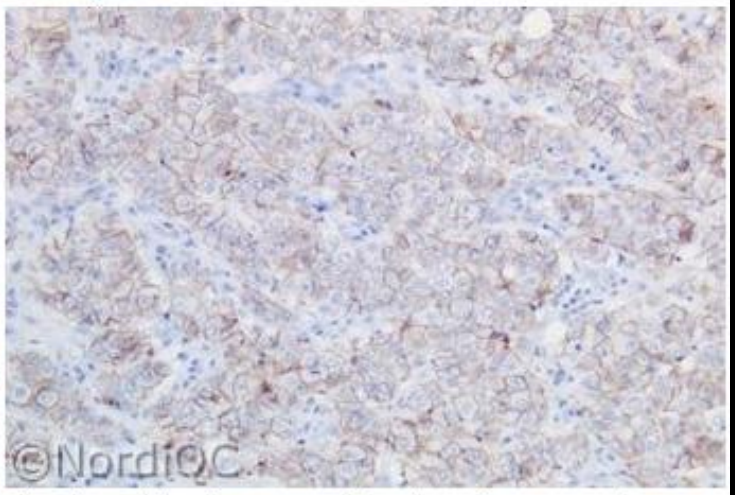


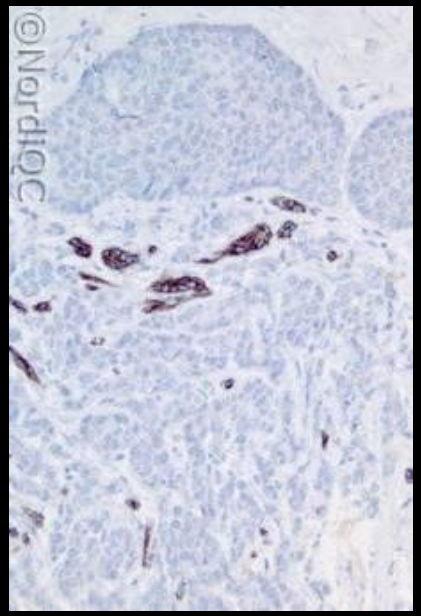
Fig. 2b. Staining for ECAD of the ductal breast carcinoma using same insufficient protocol as in Fig. 1b. The neoplastic cells only show a weak diffuse membranous reaction – also compare with Fig. 3b – same protocol.

E-Cadherin

Liver

CSQI:

Hepatocytes



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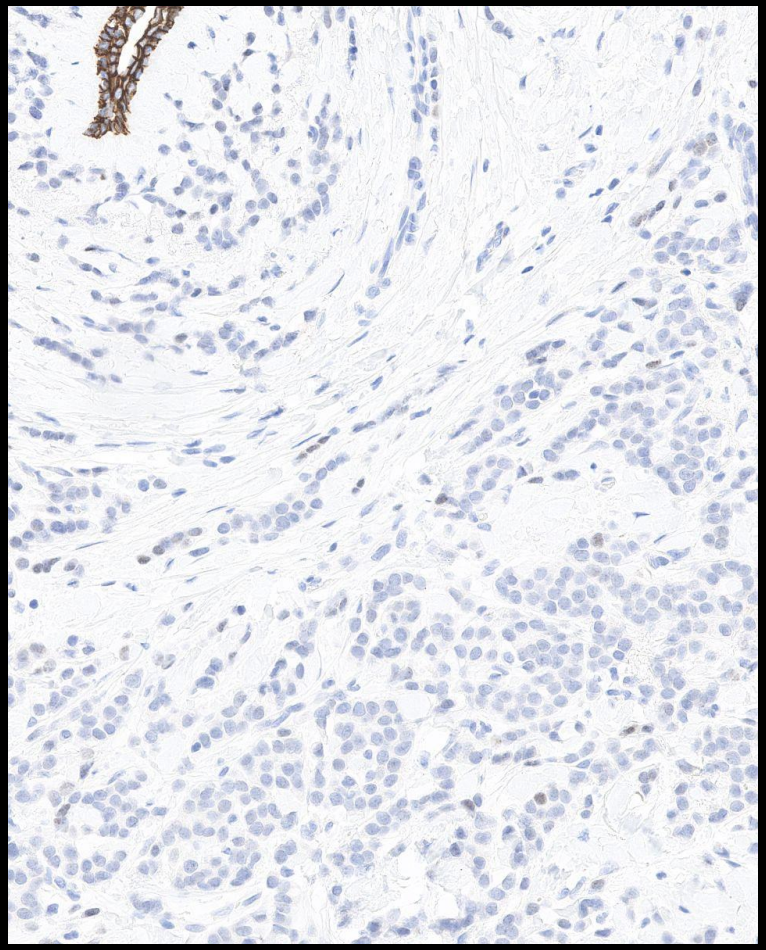
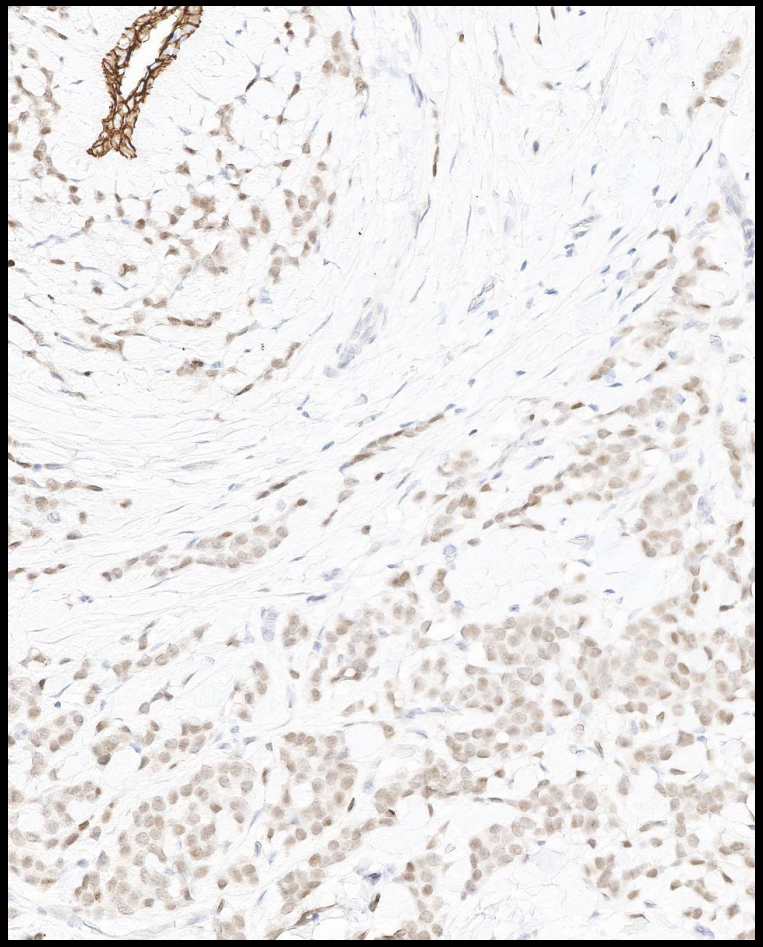




Lobular breast carcinoma

mAb clone HECD-1 or NCH-38

mAb clone 36

Technical ? Biology ?

IHC – Protocols and controls for Breast tumours

Histopathology 2008, **52**, 325–330. DOI: 10.1111/j.1365-2559.2007.02949.x

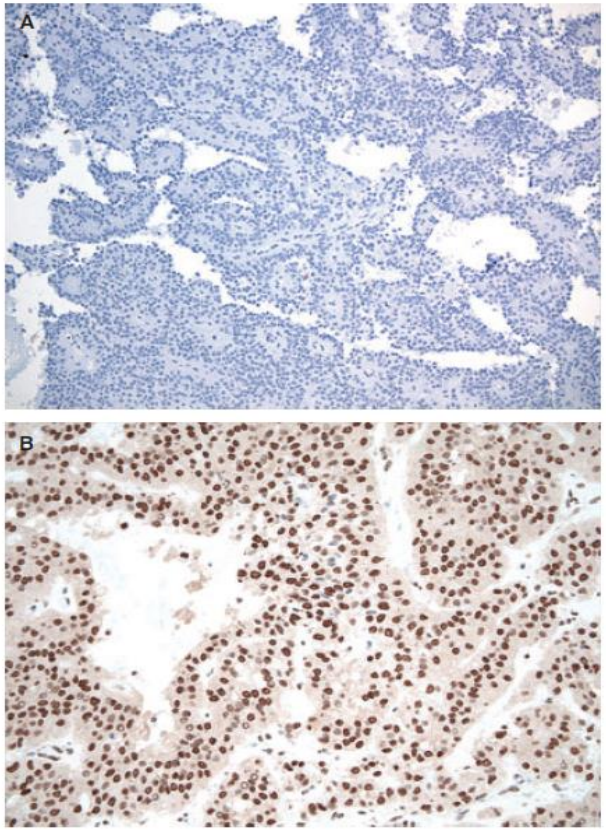
Membrane loss and aberrant nuclear localization of E-cadherin are consistent features of solid pseudopapillary tumour of the pancreas. An immunohistochemical study using two antibodies recognizing different domains of the E-cadherin molecule

R Chetty & S Serra

Department of Pathology, University Health Network/Toronto Medical Laboratories, University of Toronto, Toronto, ON, Canada

Table 1. Clinicopathological features and immunohistochemistry

Case	Gender	Age (years)	Size (mm)	Site	β-Catenin	E-cadherin clone 36	E-cadherin clone 36B
1	F	32	52	Head	Nuc/cyto	Nuclear	Negative
2	F	40	68	Tail	Nuc/cyto	Nuclear	Negative
3	F	45	55	Body/tail	Nuc/cyto	Nuclear	Negative
4	F	39	35	Head	Nuc/cyto	Nuclear	Negative
5	F	24	45	Head	Nuc/cyto	Nuclear	Negative
6	F	31	88	Tail	Nuc/cyto	Nuclear	Negative
7	F	43	40	Tail	Nuc/cyto	Nuclear	Negative
8	M	45	35	Tail	Nuc/cyto	Nuclear	Negative
9	F	39	32	Tail	Nuc/cyto	Nuclear	Negative
10	F	20	31	Body/tail	Nuc/cyto	Nuclear	Negative
11	F	43	130	Tail	Nuc/cyto	Nuclear	Negative
12	F	39	63	Tail	Nuc/cyto	Nuclear	Negative
13	F	11	80	Body/tail	Nuc/cyto	Nuclear	Negative
14	F	61	90	Tail	Nuc/cyto	Nuclear	Negative
15	M	62	170	Body/tail	Nuc/cyto	Nuclear	Negative
16	F	40	45	Tail	Nuc/cyto	Nuclear	Negative
17	F	52	20	Body/tail	Nuc/cyto	Nuclear	Negative
18	F	19	53	Body/tail	Nuc/cyto	Nuclear	Negative
19	F	36	70	Tail	Nuc/cyto	Nuclear	Negative
20	F	13	50	Head	Nuc/cyto	Nuclear	Negative



mAb 36B5

mAb 36

Clone 36 reacts with cytoplasmic component
Nuclear localization might occur due to B-Cat mutation (has to be confirmed and no data on breast tumours).

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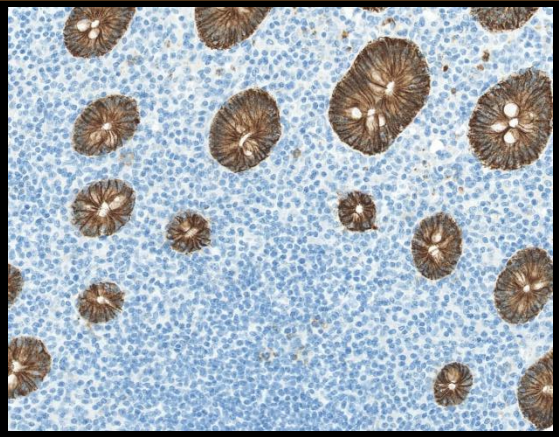
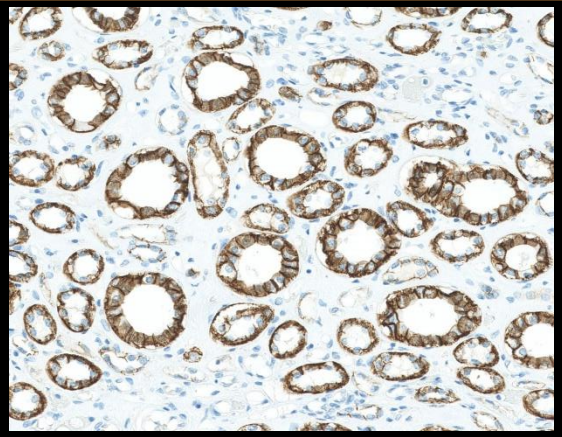
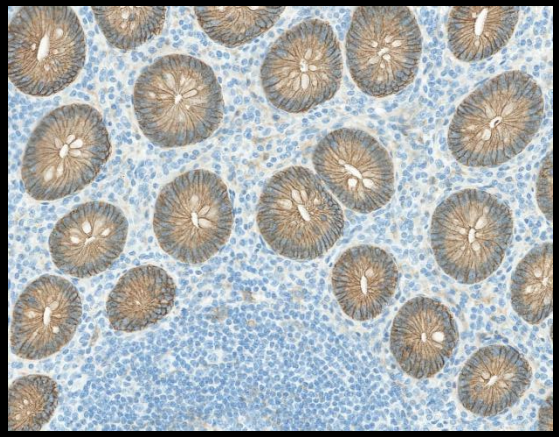
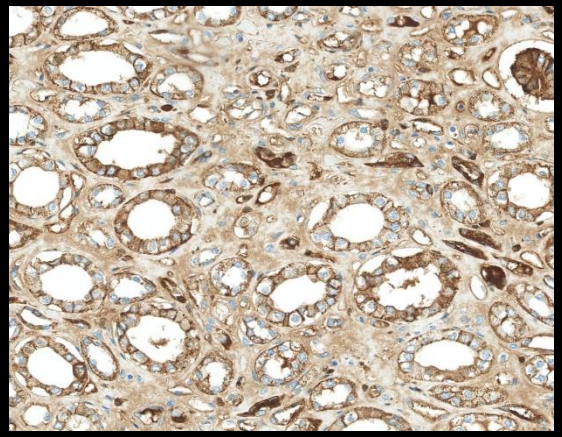




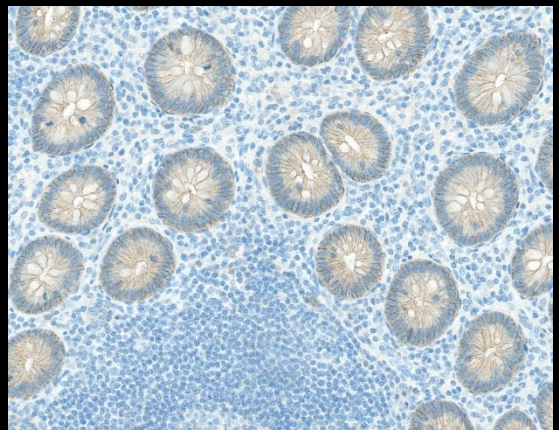
NCH-38 vs EP700Y

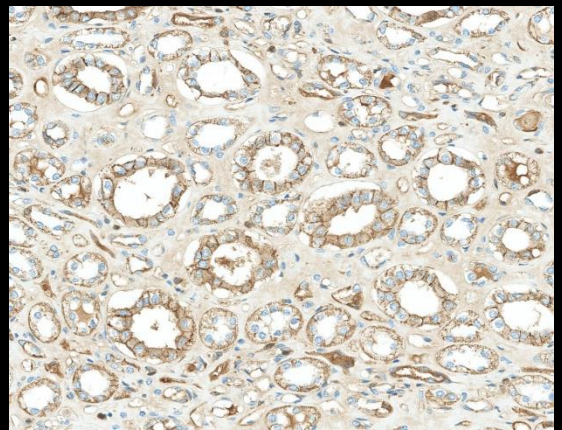
Colon - Kidney
NCH-38





Colon - Kidney
EP700Y Titre A





Colon – Kidney
EP700Y Titre B

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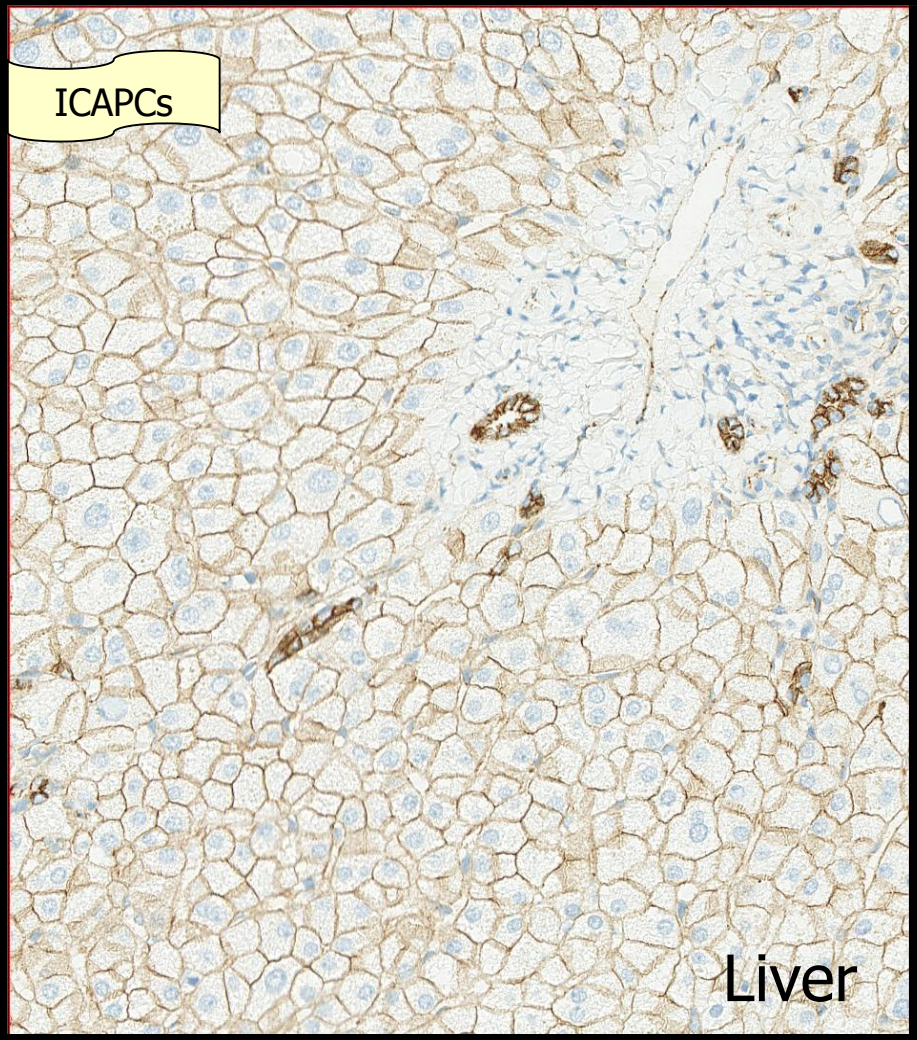
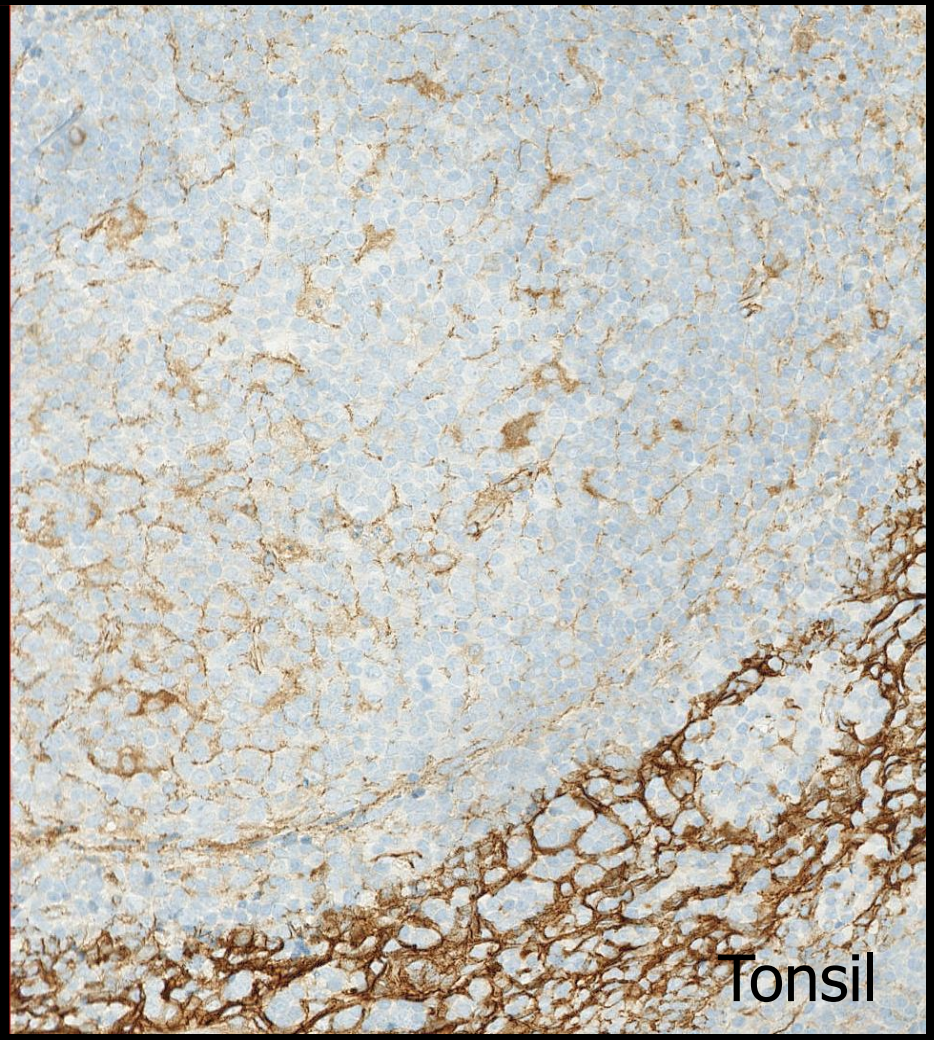


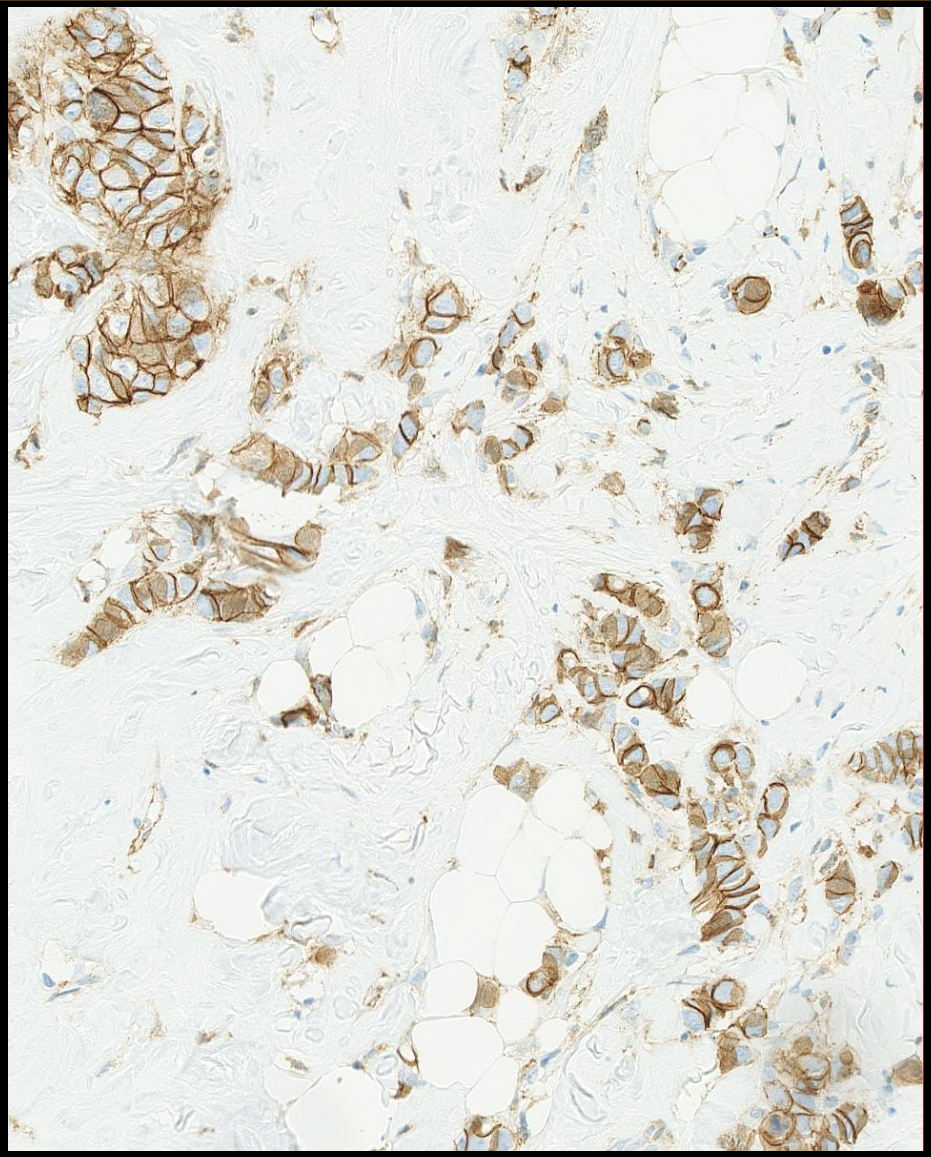


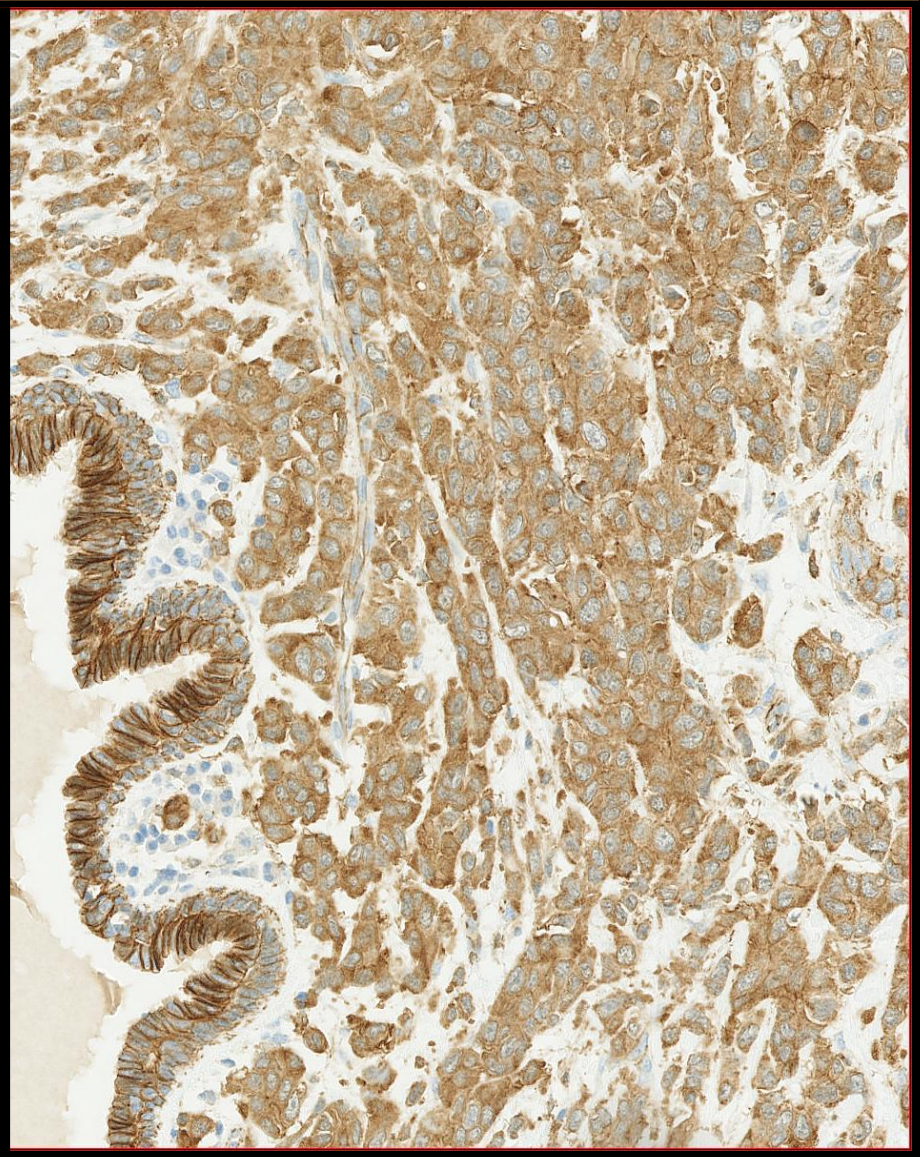
p120 Catenin

An at least weak to moderate membranous staining reaction of virtually all the hepatocytes.
A moderate to strong pre-dominantly membranous staining reaction must be seen all epithelial cells of bile ducts.

An at least weak to moderate membranous staining reaction of germinal centre macrophages and the follicular dendritic network.

IHC – Protocols and controls for Breast tumours





p120 Catenin

Ductal carc.

Lobular carc.

IHC – Protocols and controls for Breast tumours

Breast panel: E-Cadherin (& *p120*)
Basic protocol settings for an optimal staining result (NQC)

E-CAD	Retrieval	Titre	Detection	RTU	Detection
mAb NCH-38	HIER High	1:25-100	2- & 3-step	Dako	2- & 3-step
mAb HECD-1	HIER High	1:200–1.000	2- & 3-step	-	-
mAb 36B5	HIER High	1:50	2- & 3-step	-	-
mAb ECH-6	HIER High	1:100	2-step	-	-
mAb 36	*HIER High*	*-*	*-*	*Ventana*	*2-step**

* *Short incubation time 8-16 min. and 2-step multimer*

p120	Retrieval	Titre	Detection	RTU	Detection
mAb MRQ-5	*HIER high*	*1:25-100*	*2- & 3-step*	-	-