NORDIQC DATA FOR BREAST MARKERS

Antibody selection, protocols and controls

NordiQC Workshop, October 5-7th 2022

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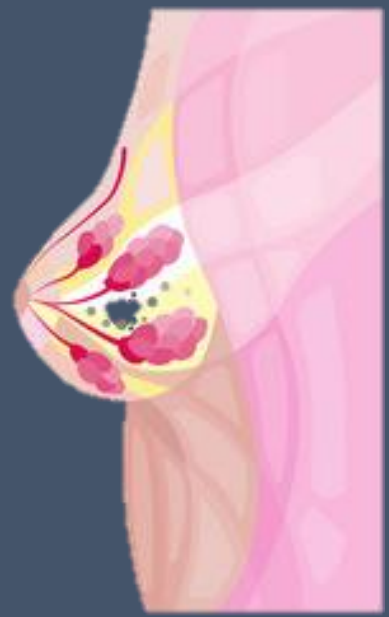
AGENDA

- NordiQC results for selected markers
- Clones - successful vs. less successful
- Tricky markers – pitfalls
- iCAPS

NORDIQC EQA DATA FOR IHC BREAST MARKERS

		Purpose	Last run	Pass rate	No of labs
Type I IHC tests	GATA3	Breast vs non-breast	Run 63, 2021	68%	320
	Mammaglobin	Breast vs non-breast	Run 25, 2009	83%	23
	GCDFP15	Breast vs non-breast	Run 36, 2012	86%	131
	CK5	CIS vs invasive	Run 65, 2022	71%	311
	SMH	CIS vs invasive	Run 50, 2017	78%	114
	p63	CIS vs invasive	Run 61, 2021	79%	324
	E-Cadherin	Ductal vs lobular	Run 53, 2018	89%	298
Type II IHC tests	KI67	PI index	Run B22, 2016	93%	409
	ER	Predictive for Tamoxifen	Run B33, 2022	89%	407
	PR	Predictive for Tamoxifen	Run B33, 2022	91%	405
	HER2 IHC	Predictive for Herceptin	Run B33, 2022	93%	386
	HER2 BRISH	Predictive for Herceptin	Run H21, 2022	56%	154
	PD-L1 IC	Predictive for Tecentriq	Run C11, 2022	59%	141
	PD-L1 TPS/CPS	Predictive for Keytruda	Run C11, 2022	81%	225



KEY-POINTS FOR BEST PROTOCOLS

- Clone selection
- RTUs – “Plug and Play” or “Play and Plug”?
- Efficient HIER, preferable in an alkaline buffer
- Use of right detection system

- Use of iCAPS

CLONE PERFORMANCE FOR SELECTED BREAST MARKERS

Marker	Successful clones (pass rate)	Less successful clones (pass rate)
GATA3	mAb L50-823, rmAb SP368	mAb HG3-31
CK5*	mAb XM26, rmAb SP27	mAb D5/16 B4
SMH	mAb SMMS1	-
p63	mAbs 4A4 & DAK-p63	mAb 7JUL
E-Cadherin	mAbs NCH-38, 36 & 36B5	rmAb EP700Y
KI67	mAb MIB-1, rmAb 30.9	-
ER	rmAbs SP1 & EP1, mAb 6F11	-
PR	mAbs 16 & PgR1294, rmAbs 1E2 & Y85	-
HER2 IHC	rmAbs 4B5 & DG44, Dako pAb	mAb CB11
PD-L1 IC	rmAb SP142	*Non-SP142*
PD-L1 TPS/CPS*	mAb 22C3, rmAb SP263	*rmAb SP142*

*for pitfalls: see ppt for lung-markers

ICAPS FOR SELECTED BREAST MARKERS

Marker	IHC critical assay performance controls Low expression	Negative tissue controls No expression	
GATA3	Tonsil: T-helper-cells in the T-zones and germinal centers.	Tonsil: B-cells, squamous epithelial cells, endothelial cells.	Link
Mammaglobin	Skin: Epithelial cells of eccrine sweat glands.	Tonsil: All cell types.	Link
GCDFP15	Skin: Epithelial cells of eccrine sweat glands.	Tonsil: All cell types.	Link
CK5	Pancreas: Scattered epithelial cells of intercalated ducts.	Liver. All cell types.	Link
Smooth MHCM	Tonsil: Follicular dendritic cells in germinal centers.	Tonsil: Epithelial cells.	Link
p63	Placenta: Cytotrophoblastic cells.	Appendix: Epithelial- and smooth muscle cells.	Link
E-Cadherin	Liver: Hepatocytes.	Appendix: Stromal cells, smooth muscle cells, endothelial cells.	Link
KI67	Tonsil: B-cells in the light zones of the germinal centers.	Liver: Hepatocytes	Link
ER	Tonsil: Squamous epithelial cells, T-cells in germinal centres.	Tonsil: B-cells in mantle zone and germinal centres.	Link
PR	Cervix: Basal squamous epithelial cells.	Tonsil: All cells types (especially focus on lymphocytes in germinal centres).	Link
PD-L1 IC	Tonsil: T-cells and macrophages in germinal centres.	Tonsil: Normal squamous epithelial cells, lymphocytes.	Link
PD-L1 TPS/CPS	Tonsil: Germinal center macrophages and T-cells.	Tonsil: Stratified normal squamous epithelial cells and vast majority of lymphocytes.	Link

GATA3 – PITFALLS/POINTS OF ATTENTION

Table 1. **Antibodies and assessment marks for GATA3, Run 63**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823**	88 24 4 3 3 2 2 1 1	Cell Marque Biocare BD Pharmingen Zytomed Systems Gennova Bio-SB Immunologic Anacrom DBS	31	40	33	24	56%	25%
rmAb clone **EP368**	5 1	Cell Marque Quartett	4	-	1	1	67%	67%
mAb clone **HG3-31**	2	Santa Cruz	-	-	-	2	-	-
rmAb clone **ZR65**	1	Zeta Corporation	-	-	1	-	-	-
Conc total	137		35	40	35	27	55%	26%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **L50-823 760-4897**[3]	56	Ventana/Roche	36	12	8	-	86%	64%
mAb clone **L50-823 760-4897**[4]	67	Ventana/Roche	41	16	7	3	85%	61%
mAb clone **L50-823 390M-17,18,10**	42	Cell Marque	14	12	13	3	62%	33%
mAb clone **L50-823 PM 405AA**	12	BioCare Medical	5	3	2	2	67%	42%
mAb clone **L50-823 MAD-000632QD**	3 1	Master Diagnostica Vitro SA	1	2	1	-	-	-
mAb clone **L50-823 CGM-0130**	1	Celnovte	-	1	-	-	-	-
mAb clone **GATA3/6664 AMB89**	1	BioGenex	-	-	-	1	-	-
RTU total	183		97	46	31	9	78%	53%
Total	320		132	86	66	36		
Proportion			41%	27%	21%	11%	68%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (indicated in percentage if ≥5 asessed protocols).

Table 2. **Proportion of optimal results for GATA3 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibody	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark XT / Ultra		Leica Biosystems Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **L50-823**	1/12** (8%)	0/1	11/36 (31%)	0/1	15/46 (33%)	0/1	4/19 (21%)	-
rmAb clone **EP368**	1/1	-	2/2	-	0/1	-	0/1	-

* 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).

No RTU products for Dako and Leica users.
Use of conc. format of mAb L50-823 - and rmAb clone EP368 on Dako platforms - can obtain optimal results.

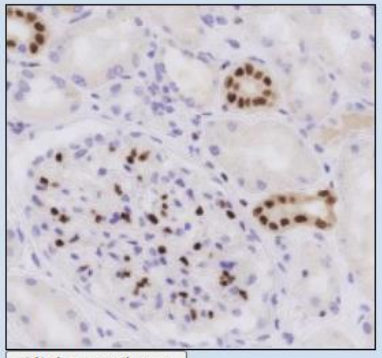
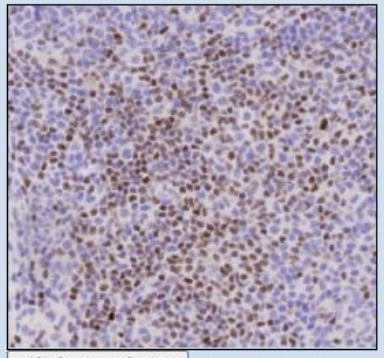
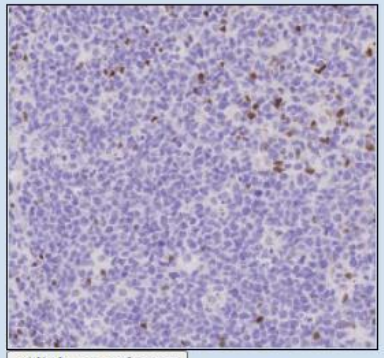
Recommended protocol settings:

- HIER in an alkaline buffer

- 40% pass rate for 2-step detection systems (8% optimal)
- 76% pass rate for 3-step detection systems (50% optimal)

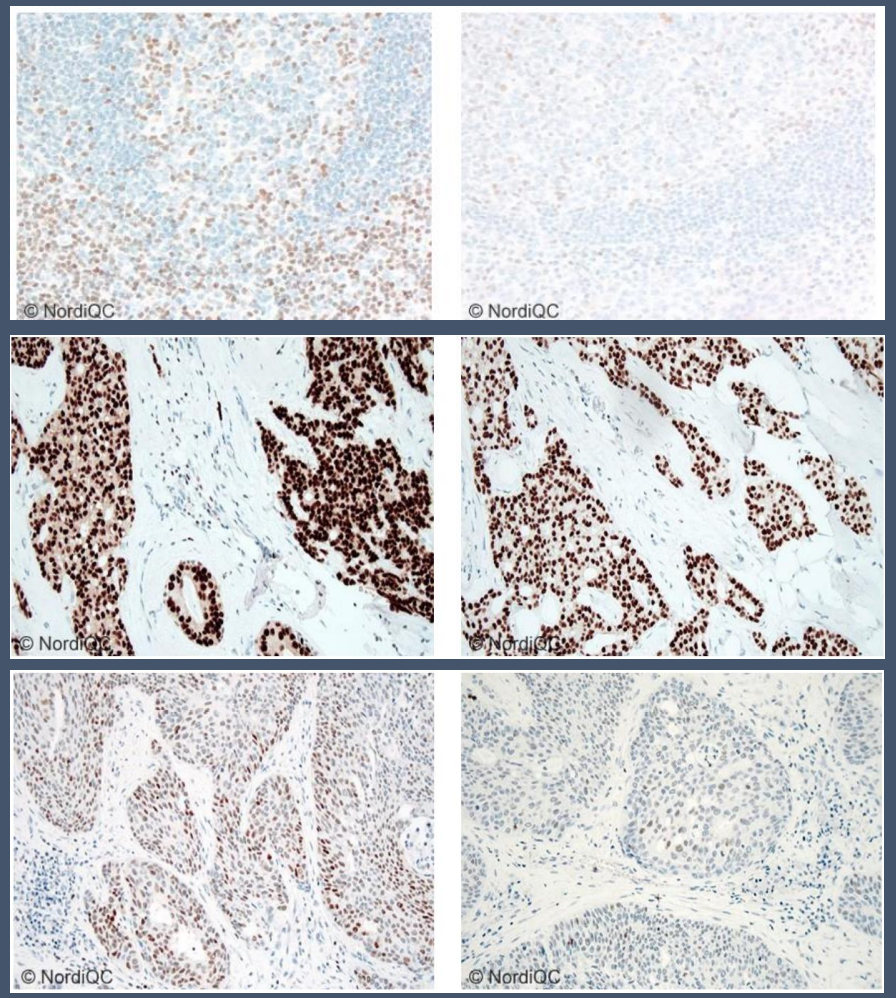
GATA3 – ICAPS

GATA3 - GATA3

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Kidney	Tonsil	Tonsil
Description	Virtually all epithelial cells in the collecting ducts and podocytes in renal glomeruli must show a moderate to strong distinct nuclear staining reaction.	The vast majority of T helper cells in the T zones and within germinal centers must show an at least weak to moderate distinct nuclear staining reaction.	No staining of B-cells, squamous epithelial cells and endothelial cells.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal protocol settings

Too diluted Ab



Tonsil

Breast carc.

Urothelial carc.

SMH - PITFALLS/POINTS OF ATTENTION

Table 4. **Proportion of sufficient and optimal results for SMH for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb SMMS-1 **IR/IS066**	100% (9/9)	67% (6/9)	2/3	0/3
Leica BOND mAb S131 **PA0493**	2/2	2/2	-	-
VMS Ultra/XT mAb SMMS-1 **760-2704**	1/3	0/3	91% (21/23)	57% (13/23)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Table 3. **Proportion of optimal results for SMH for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	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 **SMMS-1**	3/10** (30%)	-	1/4	0/1	13/23 (57%)	0/1	2/5 (40%)	-

* 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)

Recommended settings with UltraView detection system.
Most common and successful modification: adding UV amplification or use of OptiView.

No RTU for Omnis is available. Four laboratories used the Autostainer RTU on the Omnis unsuccessfully.

Limited data for concentrated formats on Omnis, but possible to achieve an optimal staining.

NordiQC

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Recommended protocol for SMH
Obtained in run 50
21 Apr 2017

Immunostainer
Type: Dako Omnis

Primary antibody
Clone: SMMS-1
Producer: Dako
Product no. / lot no.: M3558 / 00004941
Diluent: Renoir Red
Dilution factor: 1:800
Incubation time / temperature: 20 min. / 32°C

Epitope retrieval, HIER
Device: On Board / On Machine
Buffer: Dako Omnis Target Retrieval Solution, High pH
Heating time at max. temp.: 30 min.
Maximum heating temp.: 97°C

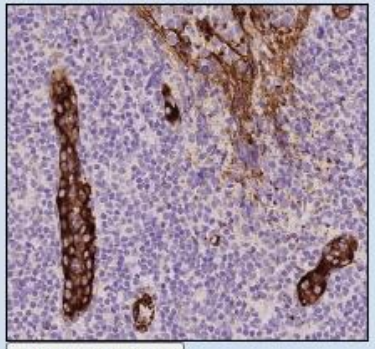
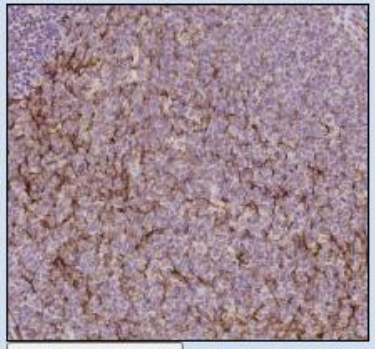
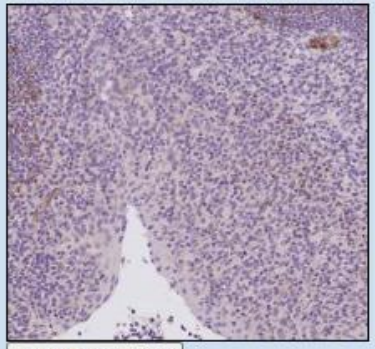
Visualization system
Producer: Dako Omnis
Product / no: EnVision Flex / GV800/GV823
Linker: Mouse LINKER
Incubation time linker: 10 min.
Incubation time polymer: 20 min.
Incubation temperature: 32°C

Chromogen
Producer: Dako Omnis
Product / no: DAB+ Substrate Chromogen System / GV825
Incubation time / temperature: 5 min. / 32°C
Enhancement: None

Disclaimer:
NordiQC makes every attempt to provide accurate and up-to-date information, yet NordiQC does not make any claim or warranty regarding the accuracy of the provided information nor does it represent that the contents of the web site and protocols reflect the most recent developments in immunohistochemistry at any point in time.

SMH - ICAPS

SMH - Myosin, smooth muscle heavy chain

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Tonsil	Tonsil	Tonsil
Description	Virtually all smooth muscle cells in vessels must show a moderate to strong cytoplasmic staining reaction.	The vast majority of follicular dendritic cells in the germinal centers must show a weak to moderate distinct cytoplasmic staining reaction.	No staining reaction in epithelial cells. *Note, smooth muscle cells in vessels and lamina muscularis will show a moderate to strong staining reaction.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

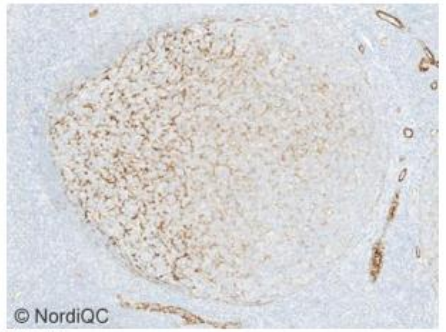


Fig. 2a
Optimal SMH staining of the tonsil using same protocol as in Fig. 1a.
A weak to moderate staining reaction is seen in the follicular dendritic network in the germinal center.
A high signal-to-noise ratio is observed.

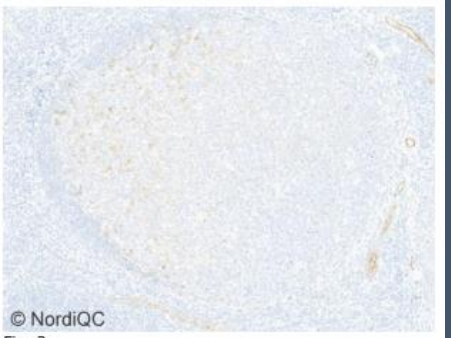


Fig. 2a
Insufficient SMH staining of the tonsil using same protocol as in Fig. 1b.
The follicular dendritic network in the germinal center is virtually negative and only vascular smooth muscle cells are demonstrated.

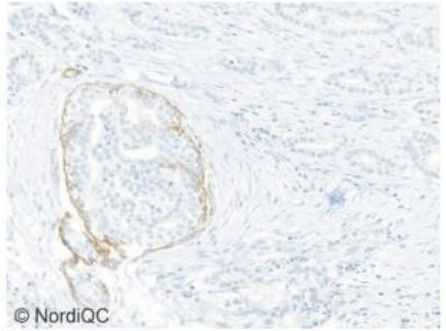


Fig. 5a
Optimal SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
A moderate, distinct and continuous staining reaction is seen in the myoepithelial cells lining the breast DCIS component, while the invasive components show no staining.

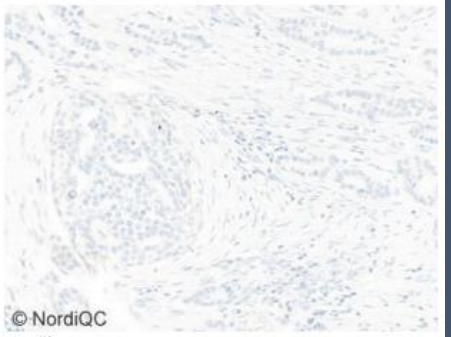


Fig. 5b
Insufficient SMH staining of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
No staining is seen in neither the DCIS nor the invasive components and thus not possible to differentiate these two entities.

P63 - PITFALLS/POINTS OF ATTENTION

Table 3. Proportion of sufficient and optimal results for p63 for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb 4A4 **790-4509**	57% (4/7)	0/7	88% (100/114)	52% (59/114)
Dako AS48 mAb DAK-p63 **IR662**	91% (11/12)	17% (2/12)	57% (4/7)	0/7
Dako Omnis mAb DAK-p63 **GA662**	85% (17/20)	25% (5/20)	100% (13/13)	62% (8/13)
Leica Bond mAb 7JUL **PA0103**	1/4	0/4	0/6	0/6

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Table 2. **Proportion of optimal results for p63 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	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 **4A4**	0/3**	0/1	1/2	-	9/20 (45%)	-	1/7 (14%)	0/1
mAb clone **DAK-p63**	0/3	-	4/9 (44%)	0/1	17/24 (71%)	-	0/9	-
mAb clone **7JUL**	-	-	-	-	0/4	-	0/6	0/1

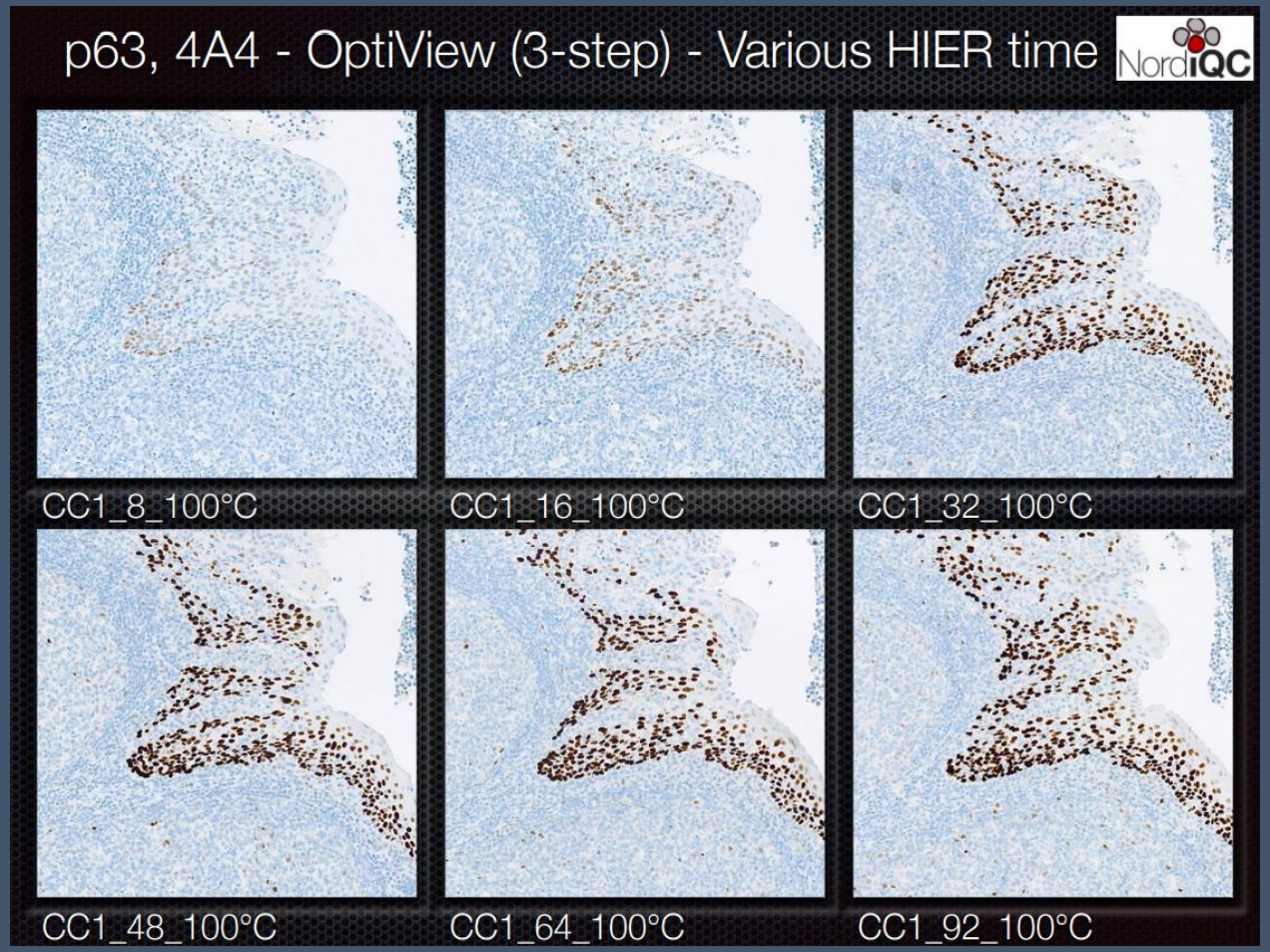
* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer).

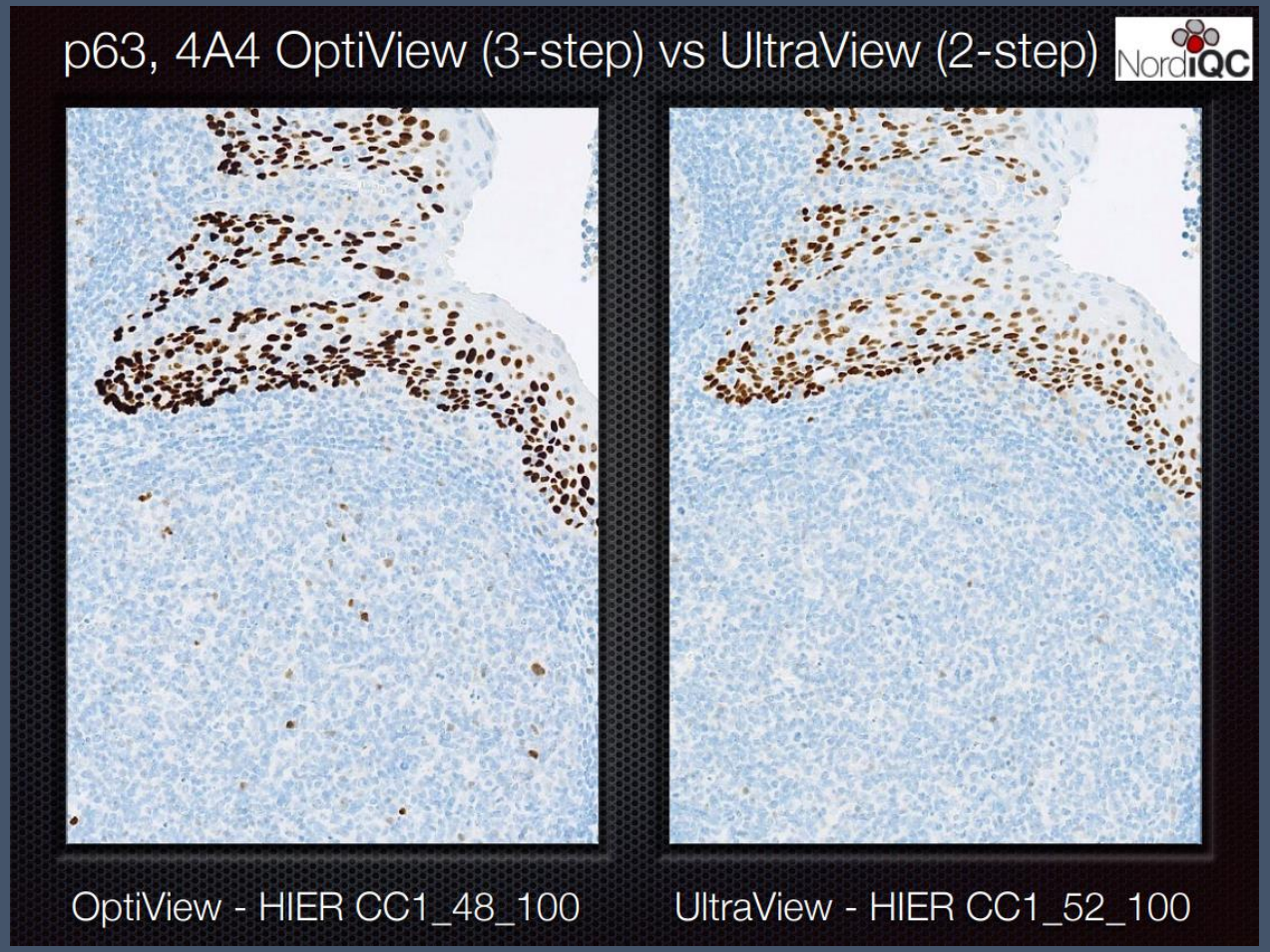
Vendor recommended protocol based on UltraView and 16-20 min. incubation of primary Ab.
Most common and successful modification was prolonging incubation time and use of OptiView or UltraView with amplification.

Vendor recommended protocol based on HIER in TRS Low pH.
Most successful modification was using HIER in TRS High pH.

Less successful performance for 7JUL on the Bond platform.
Limited data for Bond users, but conc. 4A4 might be the best solution.

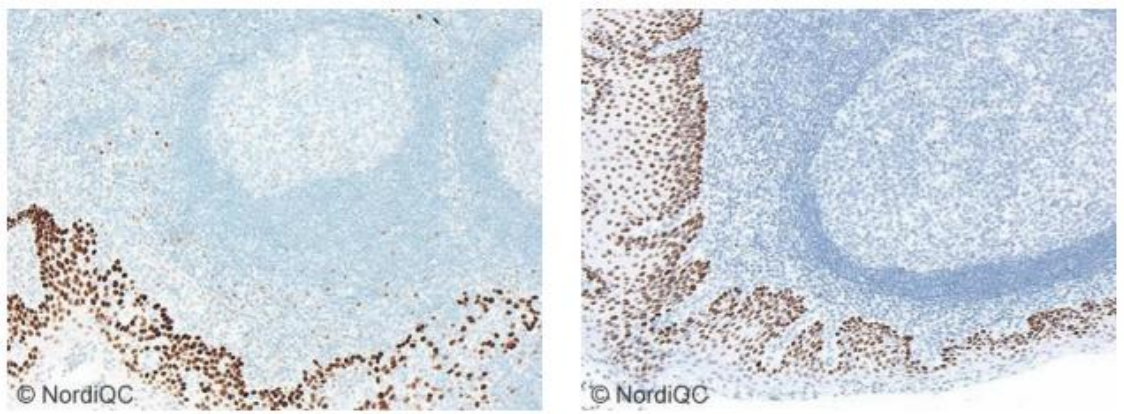
P63 - PITFALLS/POINTS OF ATTENTION



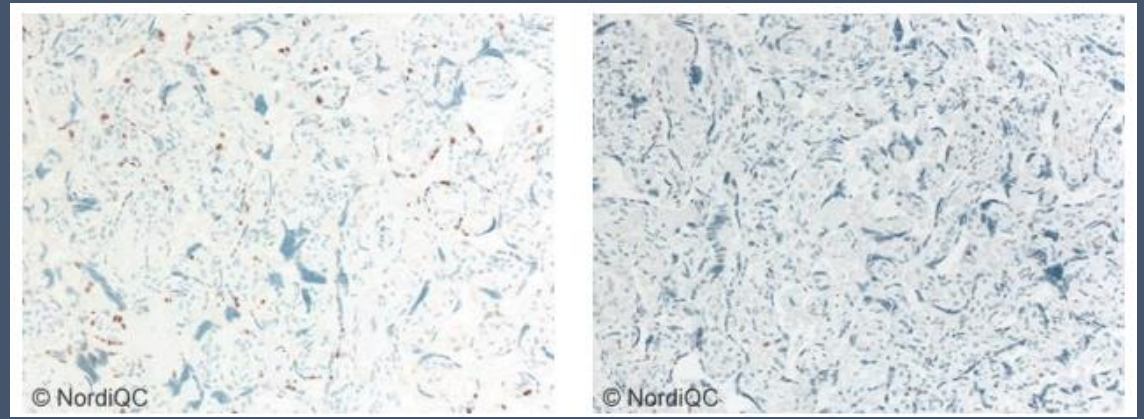


Courtesy Ole Nielsen, Dept. of Pathology, OUH, Denmark

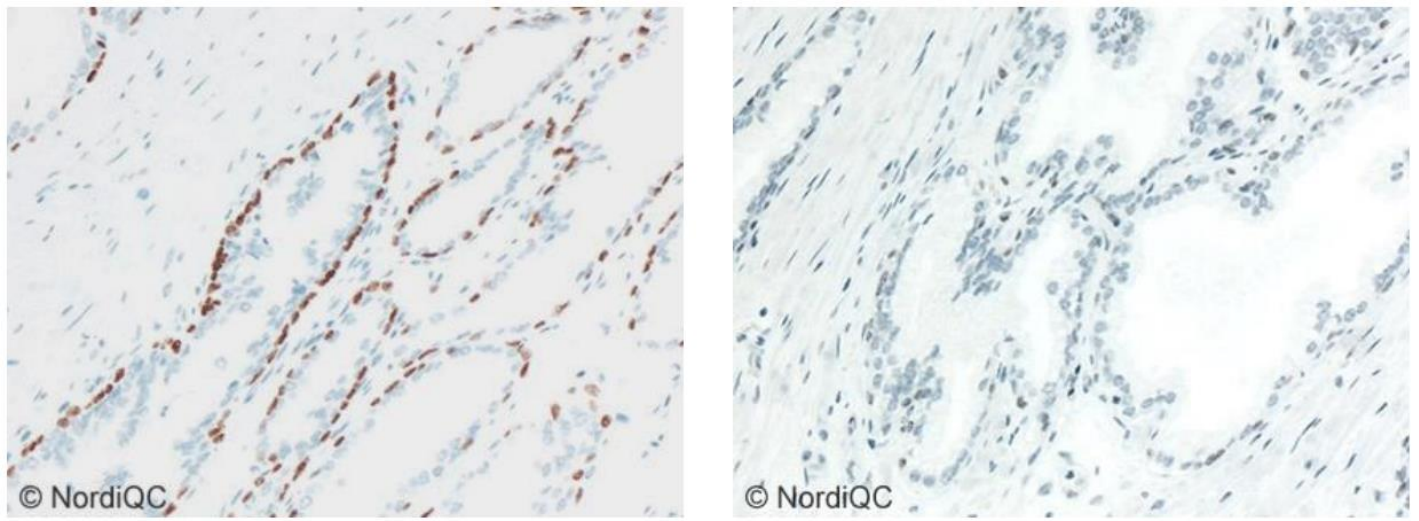
P63 - ICAPS



Left: Strong staining reaction is seen in in squamous epithelium cells in tonsil. Scattered lymphocytes shows a weak, but distinct nuclear staining reaction.



Left: Dispersed cytotrophoblastic cells shows an at least weak to moderate, distinct nuclear staining reaction.



Left: Basal cells in prostate hyperplasia show a moderate to strong nuclear staining reaction.

ECAD - PITFALLS/POINTS OF ATTENTION

Table 1. **Antibodies and assessment marks for ECAD, run 53**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **NCH-38**	82 1 1	Agilent/Dako Immunologics Thermo S./Neomarkers	57	22	4	1	94%	98%
mAb clone **36**	1 1	BD Biosciences Biogenex	0	1	0	1	-	-
mAb clone **36B5**	13	Leica/Novocastra	2	10	1	0	92%	100%
mAb clone **4A2C7**	4	Life Tech./Invitrogen	2	2	0	0	-	-
mAb clone **BS38**	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **DBM15.49**	1	Diagnostic BioSystems	1	0	0	0	-	-
mAb clone **ECH-6**	2	Zytomed Systems	1	0	1	0	-	-
mAb clone **HECD-1**	9 1	Life Tech./Invitrogen Takara Bio Inc.	4	5	0	1	90%	100%
mAb clone **GM016**	1	Genemed	1	0	0	0	-	-
mAb clone **SPM471**	1	Thermo S./Neomarkers	0	0	1	0	-	-
rmAb **EP700Y**	5	Cell Marque	0	4	1	0	-	-
rmAb **EP6**	1	Zeta Corporation	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **36 790-4497**	68	Roche/Ventana	54	11	3	0	96%	100%
mAb clone **GM016 8229-C010**	2	Sakura Finetek	2	0	0	0	100%	-
mAb clone **NCH-38 GA059**	31	Agilent/Dako	31	0	0	0	100%	100%
mAb clone **NCH-38 GA059**[3]	6	Agilent/Dako	5	1	0	0	-	-
mAb clone **NCH-38 IS/IR059**	27	Agilent/Dako	26	1	0	0	100%	100%
mAb clone **NCH-38 IS/IR059**[3]	6	Agilent/Dako	4	2	0	0	-	-
mAb clone **MX020 MAB-0738**	1	Maixin	0	1	0	0	100%	-
mAb clone **BS38 MAD-000643QD**	1	Master Diagnostica	1	0	0	0	100%	-
mAb clone **HECD-1 MAD-000761QD**	1	Master Diagnostica	1	0	0	0	100%	-
mAb clone **35B5 PA0387**	6	Leica/Novocastra	0	6	0	0	100%	-
rmAb clone **EP700Y 760-4440**	17	Roche/Ventana	0	2	15	0	13%	-
rmAb clone **EP700Y 246R-18**	6	Cell Marque	0	1	5	0	-	-
mAb clone **EP6 API3012**	1	Biocare Medical	0	1	0	0	100%	-
Total	298		192	72	31	3	-	
Proportion			65%	24%	10%	1%	89%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

Table 3. **Proportion of optimal results for ECAD for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	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 **NCH-38**	8/10** (80%)	-	1/1	-	32/42 (76%)	-	6/6 (100%)	0/2

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

Concentrated format of mAb NCH-38 works on the main IHC Systems

Table 4. **Proportion of sufficient and optimal results for ECAD for the most commonly used RTU IHC systems**

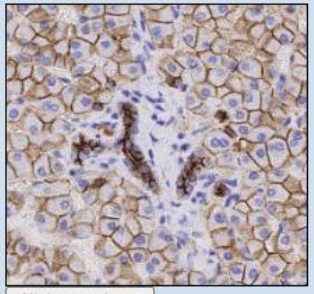
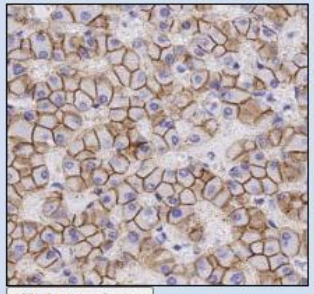
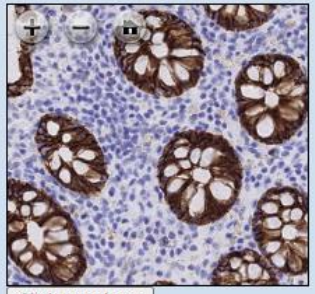
RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb NCH-38 **IS/IR059**	100% (10/10)	100% (10/10)	100% (13/13)	100% (13/13)
Dako Omnis mAb NCH-38 **GA059**	100% (21/21)	100% (21/21)	(3/3)	(3/3)
VMS Ultra/XT/GX mAb 36 **790-4497**	100% (11/11)	72% (8/11)	95% (54/57)	81% (46/57)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

RTU assays works as “plug-and-play” products.
The majority of RTU assays obtain high pass rates
– except assays based on rmAb EP700Y

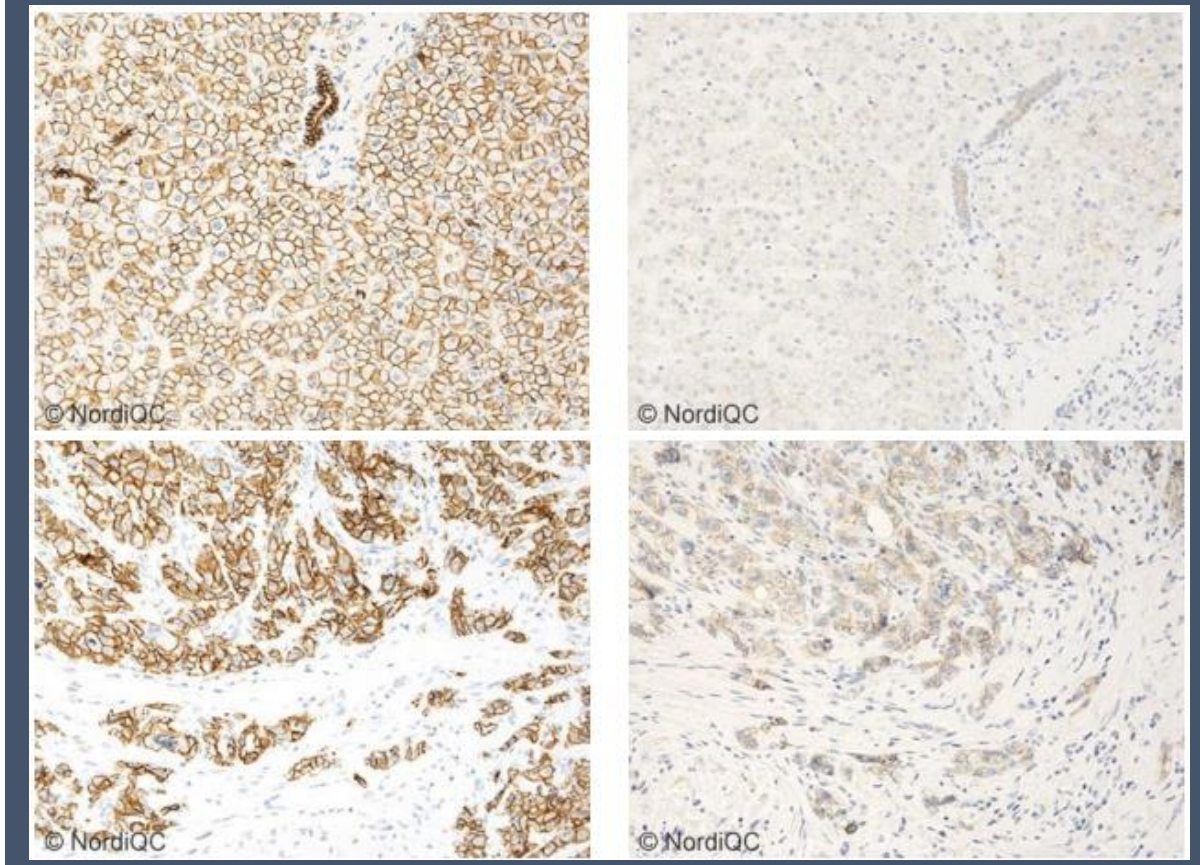
ECAD - ICAPS

ECAD - E-cadherin

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Liver	Liver	Appendix/colon
Description	Virtually all epithelial cells of bile ducts must show a strong membranous staining reaction.	Virtually all hepatocytes must show an at least weak to moderate, distinct membranous staining reaction.	No staining reaction in stromal cells such as lymphocytes, smooth muscle cells and endothelial cells should be seen. Dispersed plasma cells can show a weak membranous staining reaction. *Note, epithelial cells should show a strong membranous staining reaction.*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal staining result

Too diluted Ab



Liver

Ductal breast carc.

STILL AWAKE?

KI67 - PITFALLS/POINTS OF ATTENTION

Table 1. **Antibodies and assessment marks for Ki67, run B22**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BS4**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **GM001**	1	Genemed	1	0	0	0	-	-
mAb clone **K2**	2 1	Zytomed Leica/Novocastra	2	1	0	0	-	-
mAb clone **MIB-1**	122 1	Agilent/Dako VWR/Immunologic	72	36	13	2	88%	90%
mAb clone **UMAB107**	7	ZSBio	2	4	1	0	86%	80%
rmAb clone **SP6**	7 5 3 3 3 1 1	Thermo/Neomarkers Cell Marque Biocare Spring Bioscience Zytomed Master Diagnostica Diagnostic Biosystems	17	5	1	0	96%	95%
pAb **RB-1510**	1	Thermo/Neomarkers	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **GM001 60-0040-7**	1	Genemed	1	0	0	0	-	-
mAb clone **K2 PA0230**	4	Leica/Novocastra	2	2	0	0	-	-
mAb clone **Ki88 AM370**	1	Biogenex	0	1	0	0	-	-
mAb **MIB-1 IR626/IS626**	65	Agilent/Dako	34	25	5	1	91%	94%
mAb **MIB-1 GA626**	31	Agilent/Dako	25	5	1	0	97%	100%
mAb clone **MIB-1 AM297**	1	Biogenex	1	0	0	0	-	-
mAb clone **MM1 PA0118**	9	Leica/Novocastra	0	8	1	0	-	-
mAb clone **MX006 MAB-0672**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP6 275R**	4	Cell Marque	2	1	1	0	-	-
rmAb clone **SP6 PRM 325**	1	Biocare	0	1	0	0	-	-
rmAb clone **SP6 MAD-000310QD**	1	Master Diagnostica	0	1	0	0		
rmAb clone **30.9 790-4286**	131	Roche/Ventana	121	9	1	0	99%	100%
Total	409		282	100	24	3	-	
Proportion			69%	24%	6%	1%	93%	

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

Impact of Primary Antibody Clone, Format, and Stainer Platform on Ki67 Proliferation Indices in Breast Carcinomas

Rasmus Røge, MD,† Søren Nielsen,* Rikke Riber-Hansen, MD, PhD,‡ and Mogens Vyberg, MD*†*

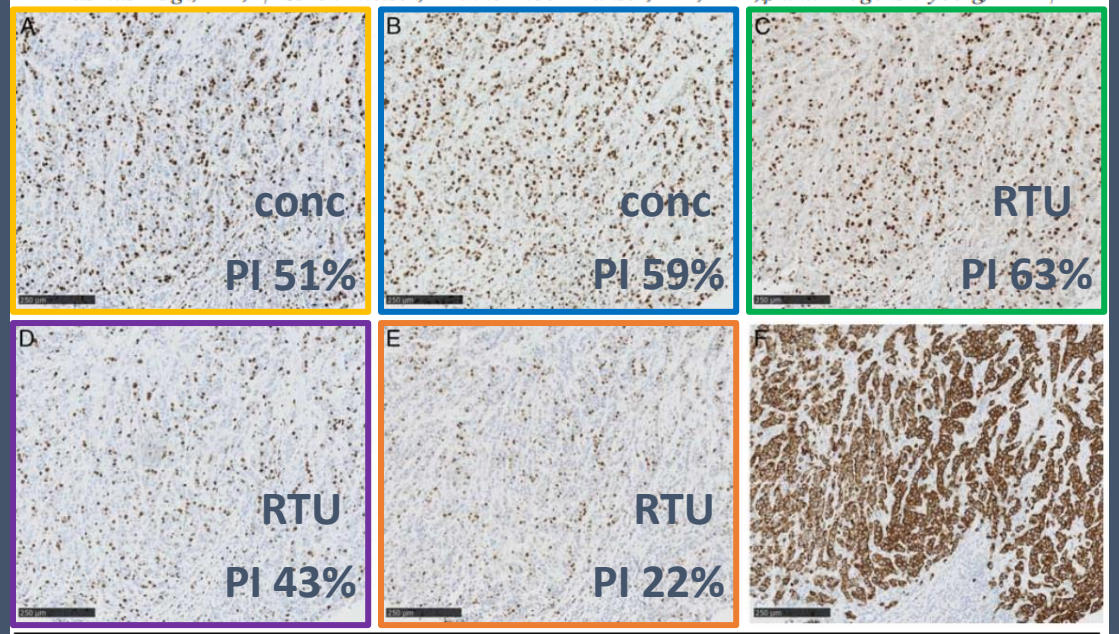


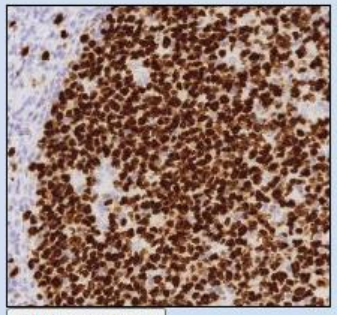
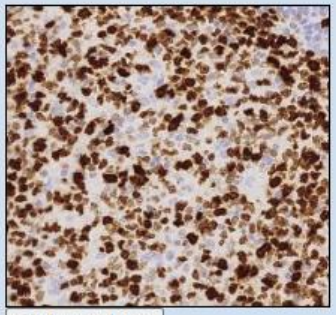
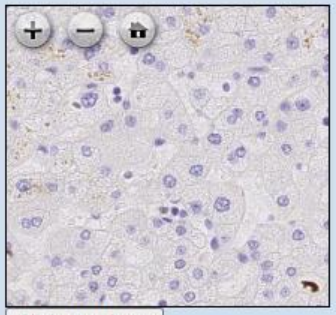
TABLE 2. Immunohistochemical Staining Protocols

Epitope	Clone	Vendor	Format	Antibody Dilution	Platform	Epitope Retrieval	HIER pH	Visualization System
Ki67	Mib1	Dako	Conc	1:200	Dako Autostainer	HIER	TRS high pH	FLEX+
Ki67	Mib1	Dako	Conc	1:100	Leica Bond	HIER	ER2 (high PH)	Refine
Ki67	Mib1	Dako	Conc	1:200	Ventana Ultra	HIER	CC1 (high pH)	Optiview
Ki67	Mib1	Dako	RTU	—	Dako Autostainer	HIER	TRS low pH	FLEX
Ki67	SP6	CellMarque	Conc	1:75	Dako Autostainer	HIER	TRS low pH	FLEX+, rabbit
Ki67	SP6	CellMarque	Conc	1:75	Leica Bond	HIER	ER2 (high pH)	Refine
Ki67	SP6	CellMarque	Conc	1:150	Ventana Ultra	HIER	CC1 (high pH)	Optiview
Ki67	MM1	Leica	RTU	—	Leica Bond	HIER	ER2 (high pH)	Refine
Ki67	30.9	Ventana	RTU	—	Ventana Ultra	HIER	CC1 (high pH)	Ultraview
PCK	AE1AE3	Dako	Conc	1:100	Dako Autostainer	HIER	TRS High	FLEX+ (mouse)
PCK	AE1AE3	Dako	Conc	1:75	Leica Bond	HIER	ER2 (high pH)	Refine
PCK	AE1AE3	Dako	Conc	1:150	Ventana Ultra	HIER	CC1 (high)	Optiview

Conc indicates concentrated; HIER, heat-induced epitope retrieval; PCK, pan-cytokeratin; RTU, ready-to-use.

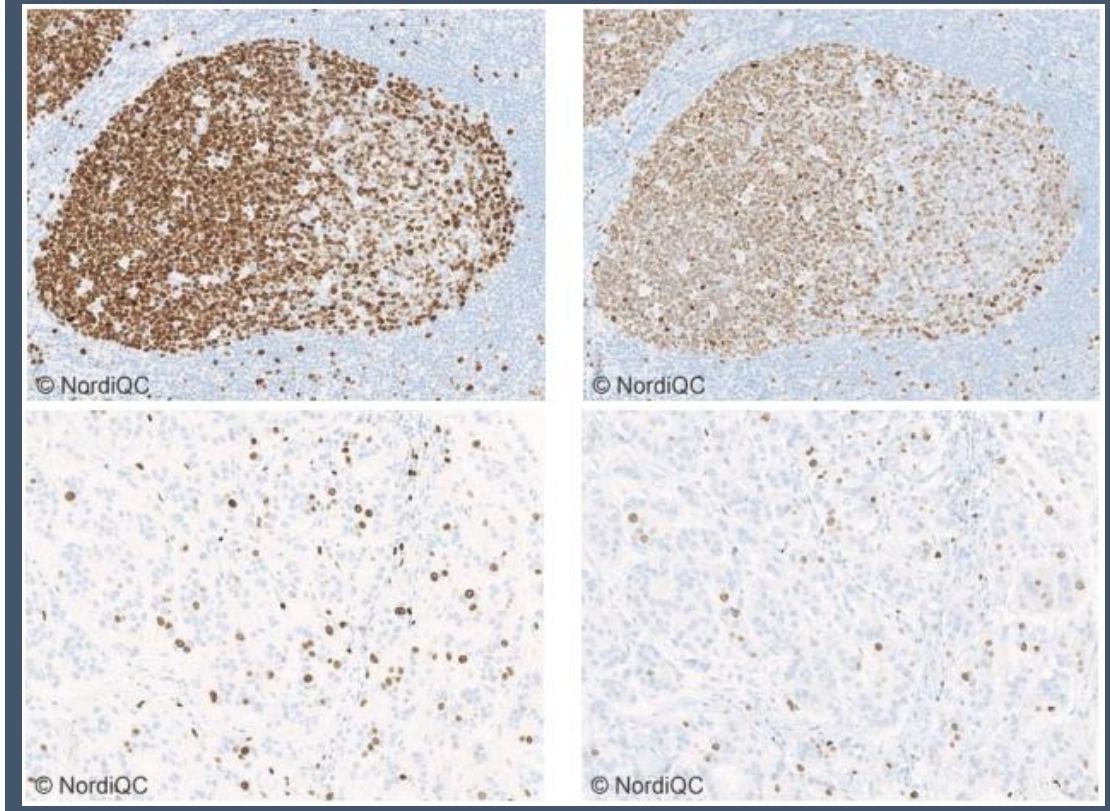
KI67 - ICAPS

Ki67 - Ki67

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Tonsil	Tonsil	Liver
Description	Virtually all B-cells in the dark zones of the germinal centres must show a moderate to strong nuclear staining reaction.	The vast majority of B-cells in the light zones of the germinal centres must show a weak to moderate nuclear staining reaction.	No nuclear staining reaction in the hepatocytes should be seen (<1% of hepatocytes should be positive) *Note, granulocytes in vessels can show a weak to moderate nuclear staining reaction (at present no explanation for this observation).*
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal protocol settings

Too diluted Ab



Tonsil

Breast carc.

ER – PITFALLS / POINTS OF ATTENTION

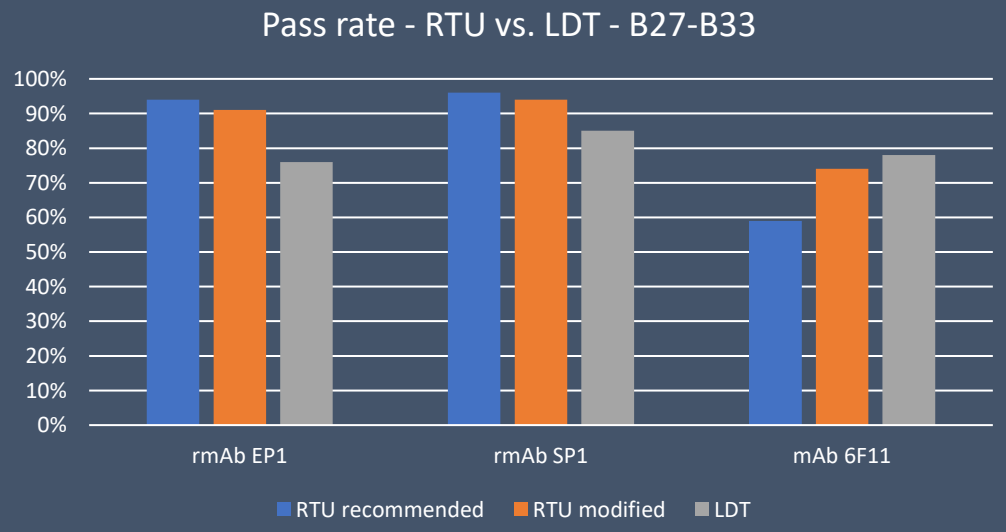
Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 rmAb EP1 **IR084/IS084**	2/3	1/3	21/22 (95%)	13/22 (59%)
Dako Omnis rmAb EP1 **GA084**	32/33 (97%)	29/33 (88%)	23/26 (88%)	14/26 (54%)
Leica Bond mAb 6F11 **PA009/PA0151**	1/3	0/3	7/10 (70%)	4/10 (40%)
VMS Ultra/XT/GX rmAb SP1 **790-4324/4325**	48/50 (96%)	24/50 (48%)	157/172 (91%)	90/172 (52%)

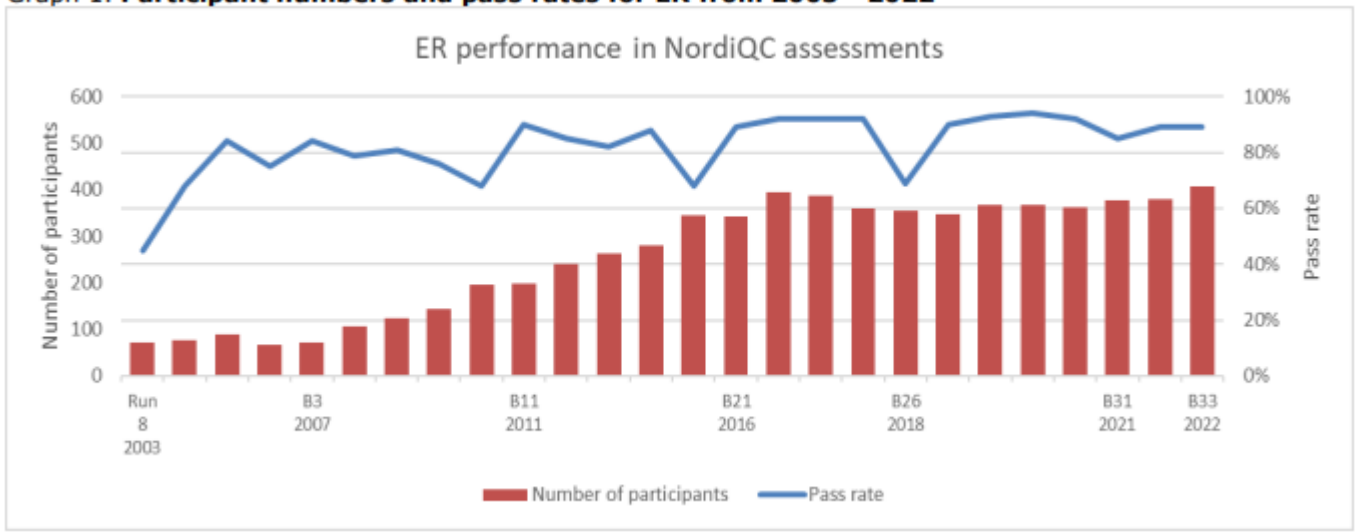
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer are included.

Even with these successful results, changing RTU assays requires internal validation.

For Dako and Ventana products, the most common modification was using a 3-step detection system.
For Leica, modification in HIER – changing from low till high pH buffer was made the majority of participants.

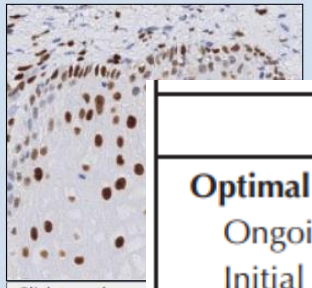
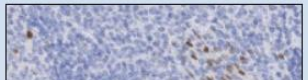


Graph 1. **Participant numbers and pass rates for ER from 2003 - 2022**



ER – ICAPS

ER - Estrogen Receptor

Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Cervix	Tonsil	Tonsil
Description	Virtually all squamous epithelial cells, columnar epithelial cells and stromal cells (except lymphocytes and endothelial cells) must show a moderate to strong nuclear staining reaction.	The vast majority of squamous epithelial cells and dispersed follicular dendritic cells / T-cells within the germinal centers must show an at least weak, distinct nuclear staining reaction.	No staining reaction in mant zone and germinal center cells should be seen.
Example	 Click to enlarge		

Estrogen and Progesterone Receptor Testing in Breast Cancer

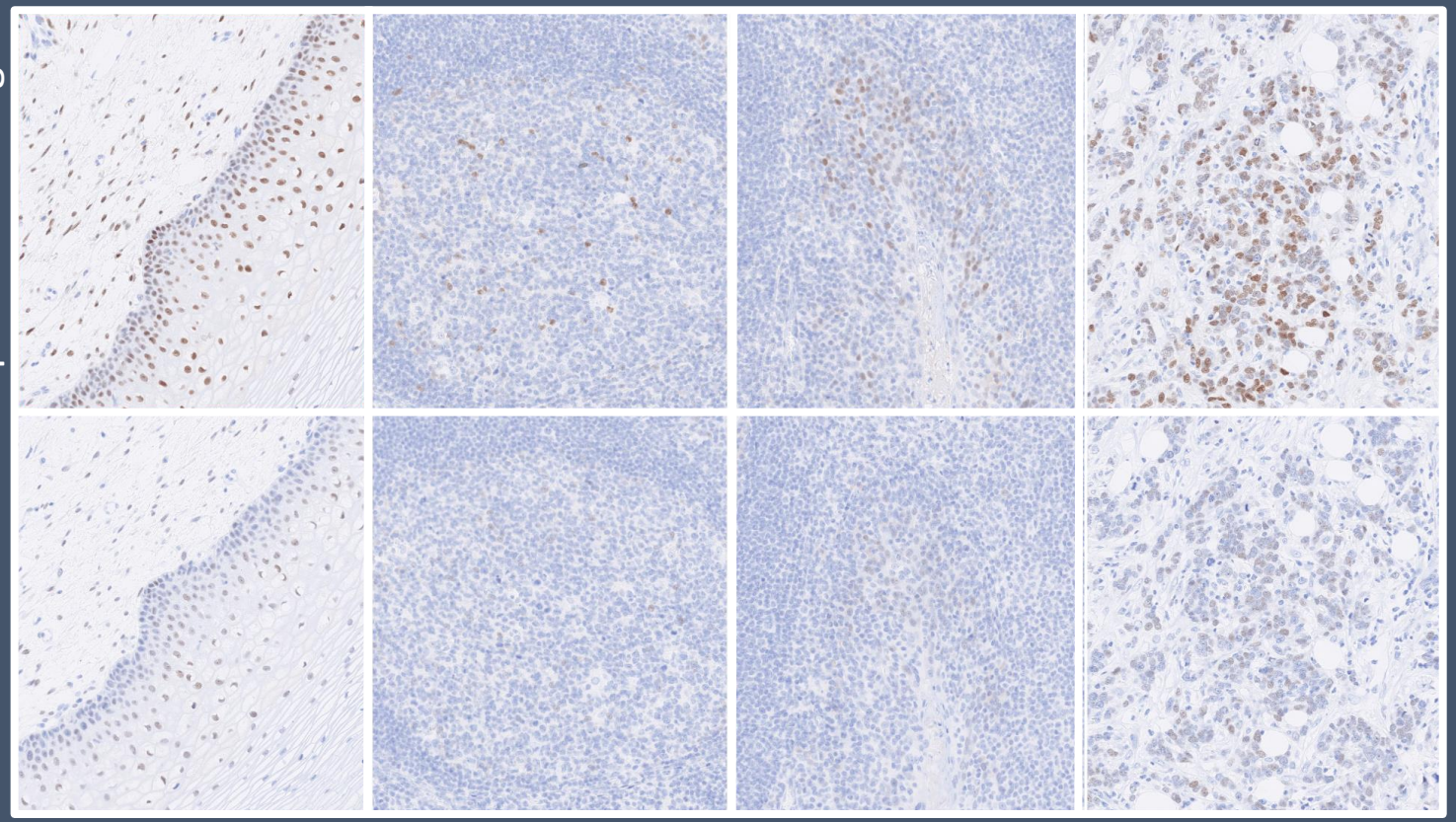
American Society of Clinical Oncology/College of American Pathologists Guideline Update

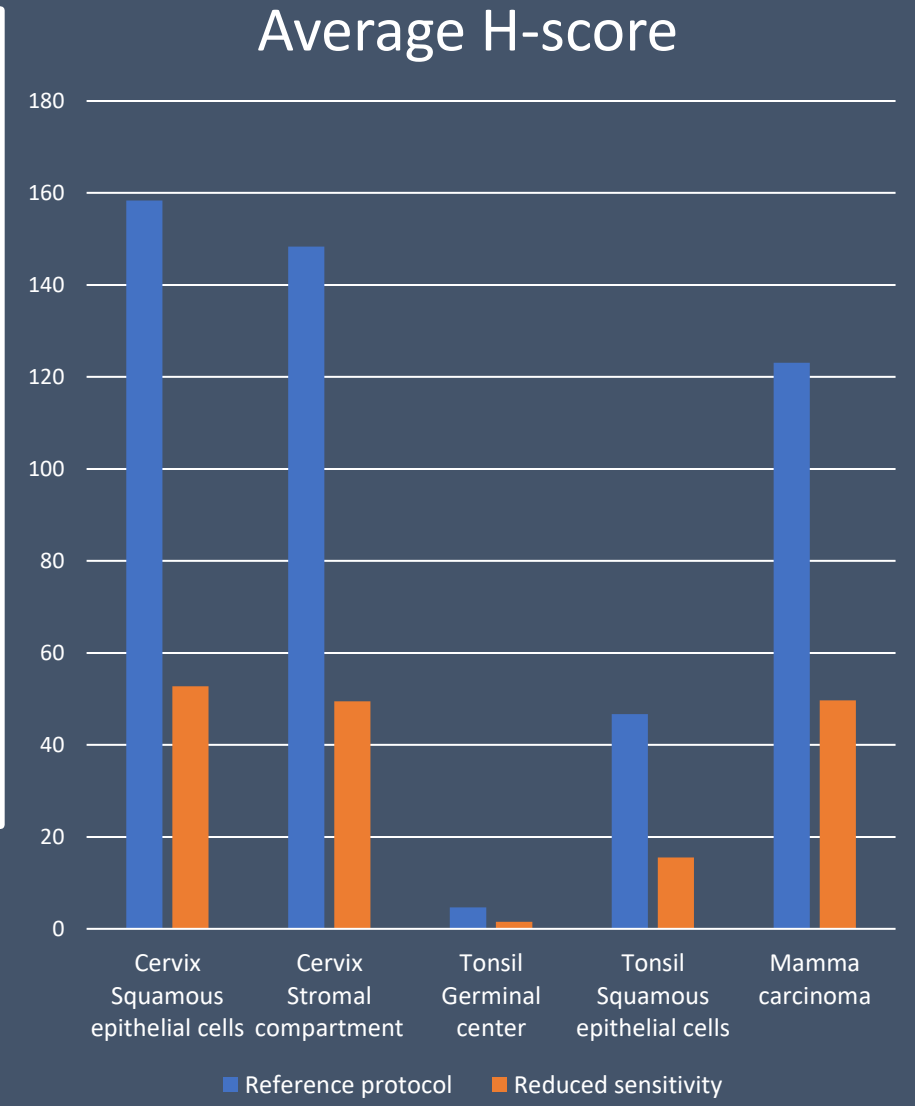
Kimberly H. Allison, MD[1]; M. Elizabeth H. Hammond, MD[2]; Mitchell Dowsett, PhD[3]; Shannon E. McKernin[4]; Lisa A. Carey, MD[5]; Patrick L. Fitzgibbons, MD[6]; Daniel F. Hayes, MD[7]; Sunil R. Lakhani, MD[8,9]; Mariana Chavez-MacGregor, MSc[10]; Jane Perlmutter, PhD[11]; Charles M. Perou, PhD[5]; Meredith M. Regan, ScD[12]; David L. Rimm, MD, PhD[13]; W. Fraser Symmans, MD[10]; Emina E. Torlakovic, MD, PhD[14,15]; Leticia Varella, MD[16]; Giuseppe Viale, MD[17,18]; Tracey F. Weisberg, MD[19]; Lisa M. McShane, PhD[20]; Antonio C. Wolff, MD[21]

Arch Pathol Lab Med—Vol 144, May 2020

2010 Recommendation	Updated Recommendation
Optimal internal QA procedures	**Optimal internal QA procedures**
Ongoing quality control and equipment maintenance.	Ongoing quality control and equipment maintenance are required.
Initial and ongoing laboratory personnel training and competency assessment.	Initial and ongoing laboratory personnel training and competency assessment should be performed.
Use of SOPs, including routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections on each tested slide, wherever possible.	SOPs should be used that include routine use of external control materials with each batch of testing and routine evaluation of internal normal epithelial elements or the inclusion of normal breast sections (or other appropriate control) on each tested slide, wherever possible. External controls should include negative and positive samples as well as samples with lower percentages of ER expression (such as tonsil). On-slide controls are recommended.

ER – ICAPS


VMS rmAb SP1 recommended protocol settings
VMS rmAb SP1 reduced sensitivity
Cervix
Tonsil Germinal centre
Tonsil Squamous epithelial cells
Breast carcinoma


Average H-score
180
160
140
120
100
80
60
40
20
0
Cervix Squamous epithelial cells
Cervix Stromal compartment
Tonsil Germinal center
Tonsil Squamous epithelial cells
Mamma carcinoma
Reference protocol
Reduced sensitivity

PR – PITFALLS / POINTS OF ATTENTION

Table 1. **Antibodies and assessment marks for PR, run B31**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16**	33 1	Leica Biosystems Monosan	20	12	-	2	94%	59%
mAb clone cocktail **16 + SAN27**	5	Leica Biosystems	2	2	1	-	80%	40%
rmAb clone **BP6081**	1	Biolynx	-	1	-	-	-	-
mAb clone **PgR 636**	13 1	Dako/Agilent Invitrogen	5	4	3	2	64%	36%
mAb clone **PgR 1294**	10	Dako/Agilent	8	1	1	-	90%	80%
mAb clone **PR88**	1	BioGenex	-	-	-	1	-	-
rmAb clone **SP2**	1 1	Diagnostic BioSystems Thermo Scientific	2	-	-	-	-	-
rmAb clone **SP42**	3	Zytomed	-	2	1	-	-	-
rmAb clone **YR85**	1	Fischer Scientific	-	1	-	-	-	-
rmAb clone **ZR4**	1	Zeta Corporation	1	-	-	-	-	-
Ready-To-Use antibodies							Suff.[1]	OR[2]
mAb clone **16** **PA0312 (VRPS[3])**	6	Leica Biosystems	6	-	-	-	100%	100%
mAb clone **16** **PA0312 (LMPS[4])**	12	Leica Biosystems	10	1	1	-	92%	83%
mAb clone **16** **MAD-000670QD**	2	Master Diagnostica	-	-	2	-	-	-
mAb **PgR 636** **IR/IS068 (VRPS[3])**	4	Dako/Agilent	3	1	-	-	-	-
mAb **PgR 636** **IR/IS068 (LMPS[4])**	26	Dako/Agilent	21	3	-	2	92%	81%
mAb **PgR 1294** **GA090 (VRPS[3])**	33	Dako/Agilent	10	22	1	-	97%	30%
mAb **PgR 1294** **GA090 (LMPS[4])**	20	Dako/Agilent	11	5	4	-	80%	55%
rmAb clone **1E2** **790-2223/4296 (VRPS[3])**	53	Ventana/Roche	44	9	-	-	100%	83%
rmAb clone **1E2** **790-2223/4296 (LMPS[4])**	141	Ventana/Roche	108	23	9	1	93%	77%
mAb clone **IHC751** **IHC751**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **SP2** **Kit-0013**	2	Maixin	2	-	-	-	-	-
rmAb clone **Y85** **8360-C010**	4	Sakura Finetek	4	-	-	-	-	-
mAb **PgR 636** **PM343**	1	Biocare Medical	-	1	-	-	-	-
Total	377		258	88	23	8		
Proportion			68%	23%	6%	2%	92%	

1) Proportion of sufficient results (optimal or good) (≥5 asessed protocols).
2) Proportion of optimal results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s) or other platforms.

Graph 1. **Pass rate in the NordiQC assessments for PR**

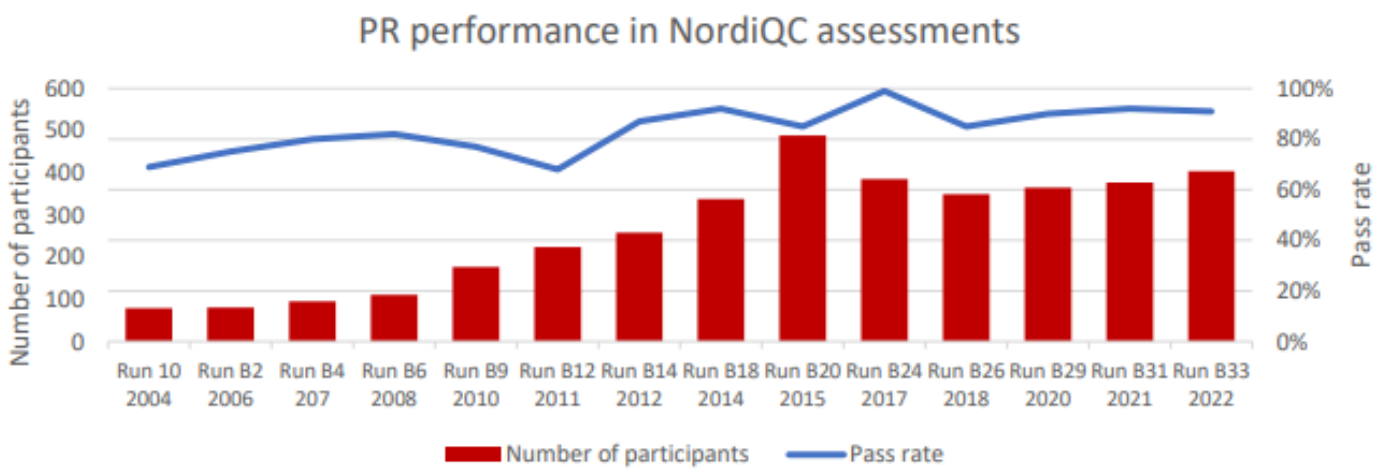


Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	Vendor recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/ BOND III mAb 16 **PA0312**	9/9 (100%)	9/9 (100%)	11/11 (100%)	7/11 (64%)
Dako Autotstainer+/ Autostainer Link mAb PgR 636 **IS068/IR068**	8/8 (100%)	6/8 (75%)	17/17 (100%)	15/17 (88%)
Dako Omnis mAb PgR 1294 **GA090**	33/41 (80%)	18/41 (44%)	22/23 (96%)	17/23 (74%)
Ventana BenchMark GX/XT/Ultra rmAb 1E2 **790-2223/790-4296**	62/63 (98%)	38/63 (60%)	128/142 (90%)	87/142 (61%)

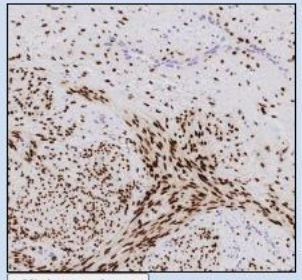
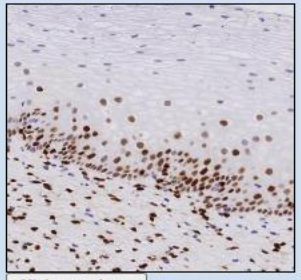
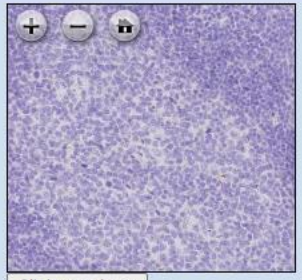
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer are included.

Autostainer RTU:
If using Flex+ a pass rate of 100%, 90% optimal.

Omnis RTU:
If using Flex+ a pass rate of 100%, 76% optimal.

PR – ICAPS

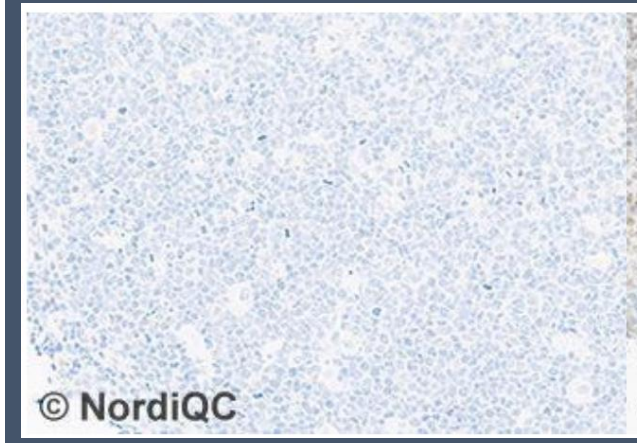
PR - Progesterone Receptor

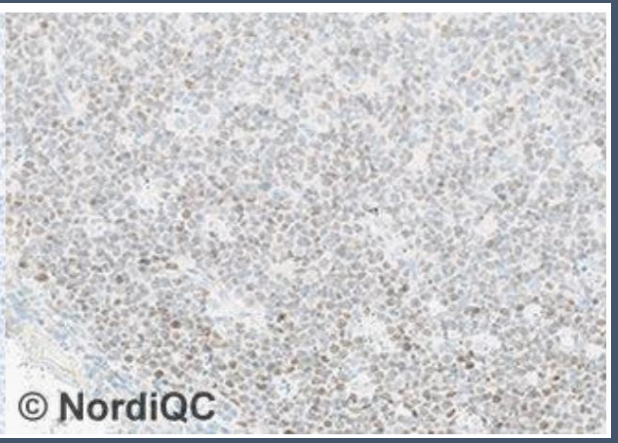
Control type	Positive tissue control High expression level	Positive tissue control Low expression levels	Negative tissue control
Tissue	Cervix	Cervix	Tonsil
Description	Virtually all columnar epithelial cells most of the stromal cells (except endothelial cells and lymphocytes) must show a moderate to strong nuclear staining reaction.	The vast majority of basal squamous epithelial cells must show an at least weak nuclear staining reaction. *Note, PR expression level can be reduced in some samples of uterine cervix e.g. due to post-menopausal status or phase of menstrual cycle.*	No and of central importance no nuclear staining reaction in germinal center lymphocytes should be seen.
Example	 Click to enlarge	 Click to enlarge	 Click to enlarge

Optimal protocol settings

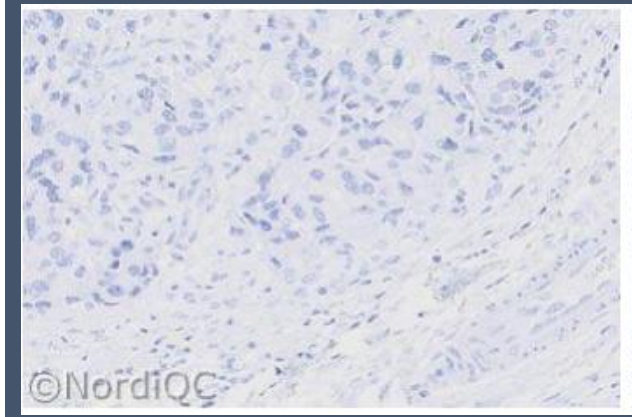
rmAb 1E2: Reduced HIER + prolonged inc. time of Ab

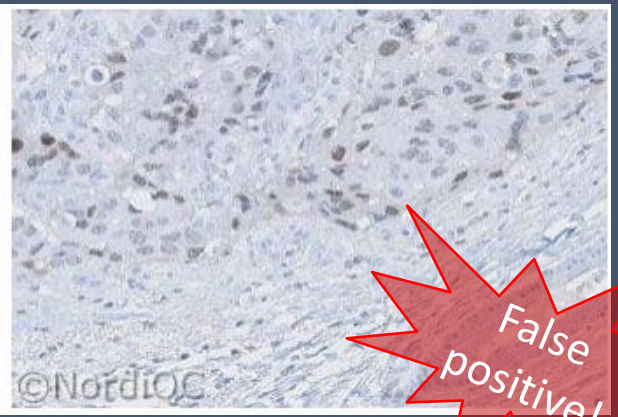
Tonsil





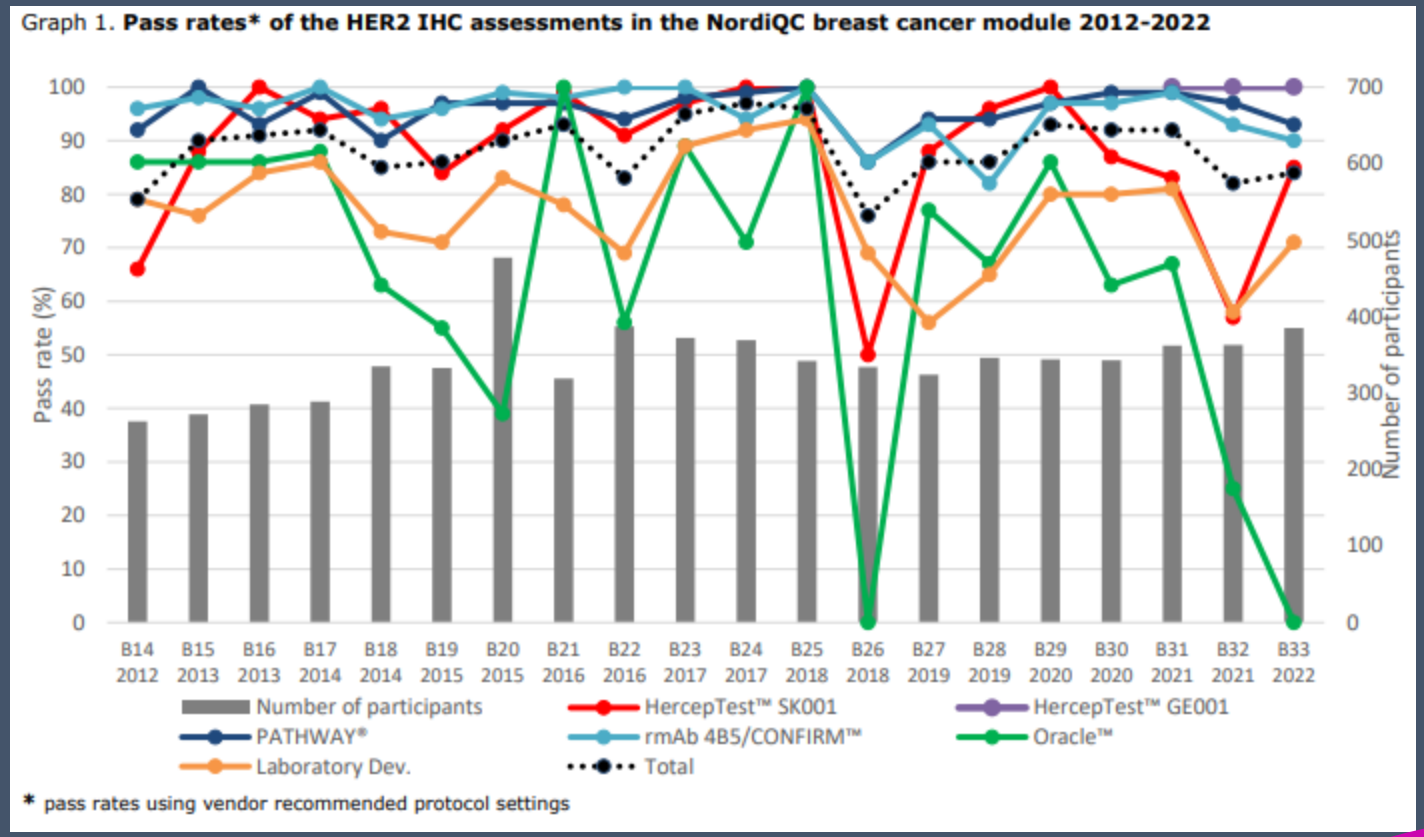
Breast carc.

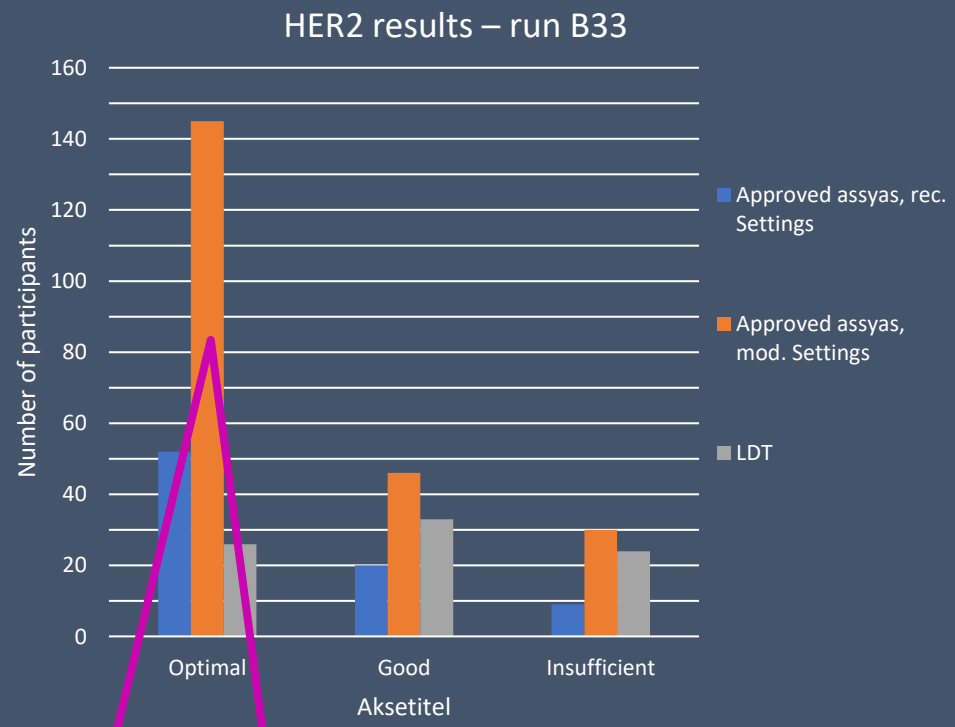




False positive!

HER2 – PITFALLS / POINTS OF ATTENTION





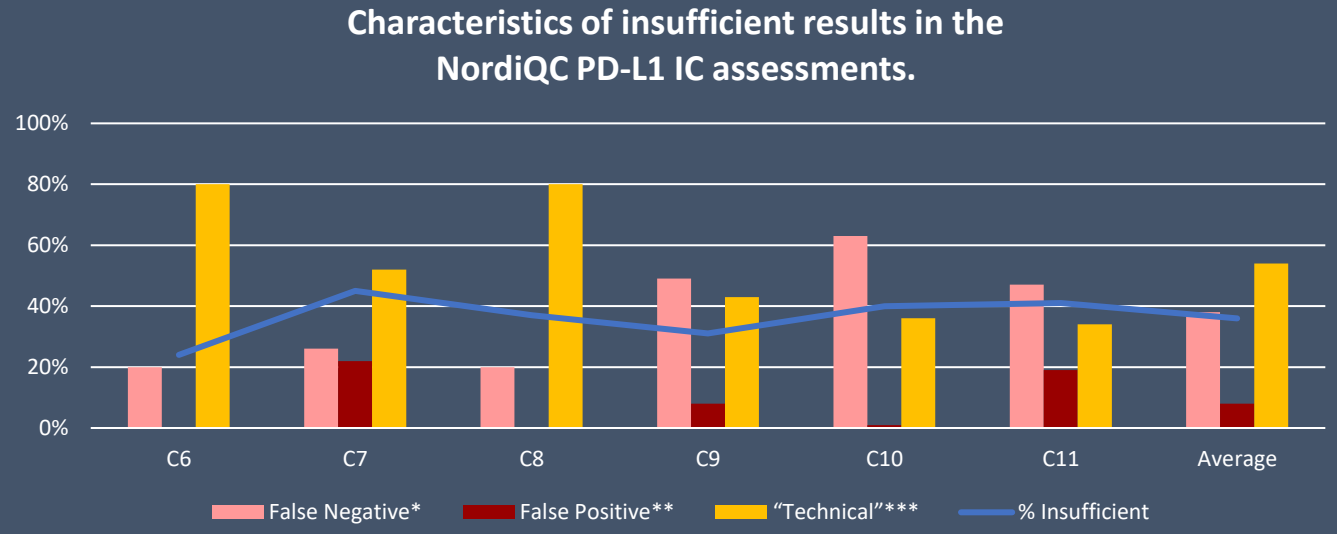
Do not change to a more sensitive detection system!

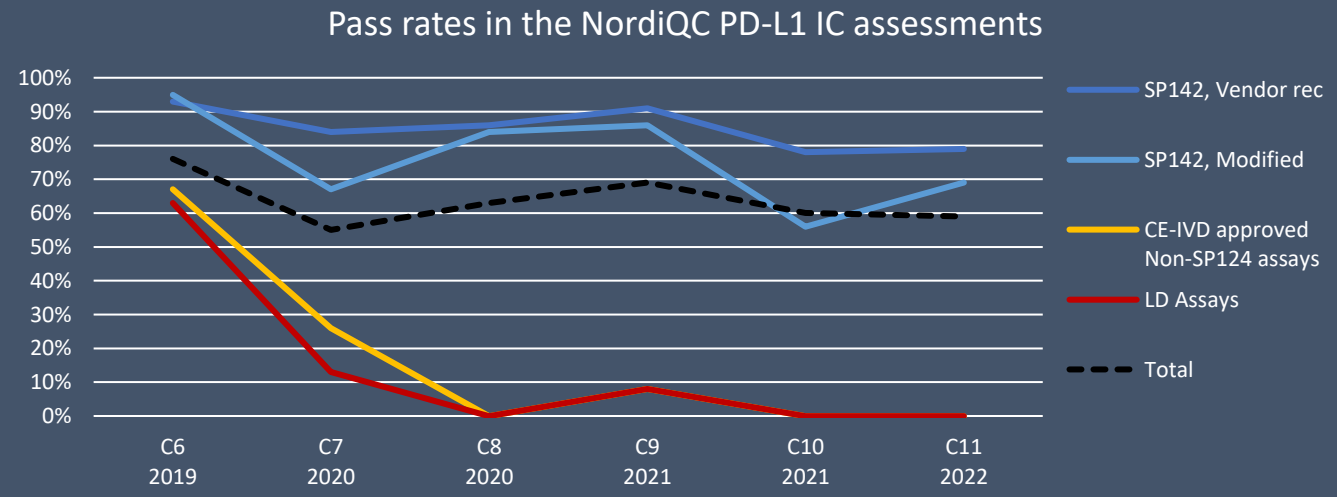
PD-L1 IC – PITFALLS/POINTS OF ATTENTION

Table 2. **Assessment marks for IHC assays and antibodies run C11, PD-L1 IC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **741-4860**[3]	61	Ventana/Roche	31	17	7	6	79%	51%
rmAb clone SP142, **741-4860**[4]	1	Ventana/Roche	0	0	1	0	-	-
rmAb clone SP263, **741-4905**[3]	3	Ventana/Roche	0	0	3	0	-	-
rmAb clone SP263, **740-4907**[3]	1	Ventana/Roche	0	0	1	0	-	-
mAb clone 22C3 pharmDX, **SK006**	3	Dako/Agilent	0	0	3	0	-	-
mAb clone 22C3 pharmDX, **GE006**	6	Dako/Agilent	0	0	6	0	0%	0%
Antibodies[7] for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **22C3**	6	Dako/Agilent	0	0	4	2	0%	0%
mAb clone **E1L3N**	2	Cell Signaling	0	0	2	0	-	-
rmAb clone **CAL10**	4	Zytomed	0	0	2	2	-	-
rmAb clone **QR001**	1	Quartett	0	0	1	0	-	-
rmAb clone **SP142**	1	Abcam	0	0	1	0	-	-
Ready-To-Use antibodies[8]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **790-4860 (VRPS)**[5]	16	Ventana/Roche	7	6	3	0	81%	44%
rmAb clone SP142, **790-4860 (LMPS)**[6]	32	Ventana/Roche	10	12	6	4	69%	31%
rmAb clone SP263, **790-4905**	2	Ventana/Roche	0	0	2	0	-	-
rmAb clone AC37, **AD80167**	1	Abcarta	0	0	1	0	-	-
mAb clone C9C9 **CPM-0278**	1	Celnovte	0	0	1	0	-	-
Total	141		48	35	44	14		
Proportion			34%	25%	31%	10%	59%	

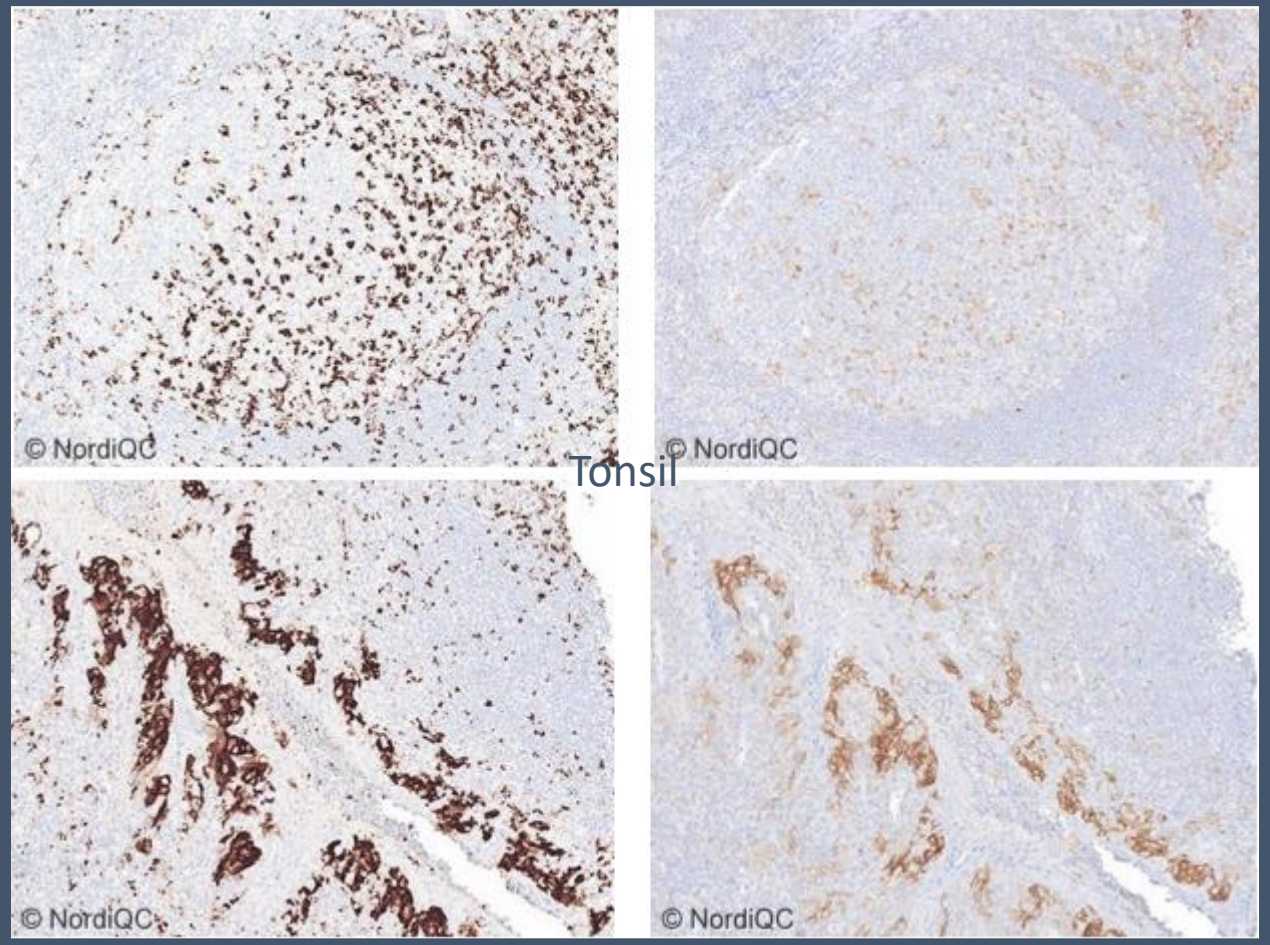
1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) This product has a locked protocol on all BenchMark platforms and cannot be changed.
4) RTU product applied on another platform than developed for.
5) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
6) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
7) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
8) Ready-To-Use antibodies without predictive claim.

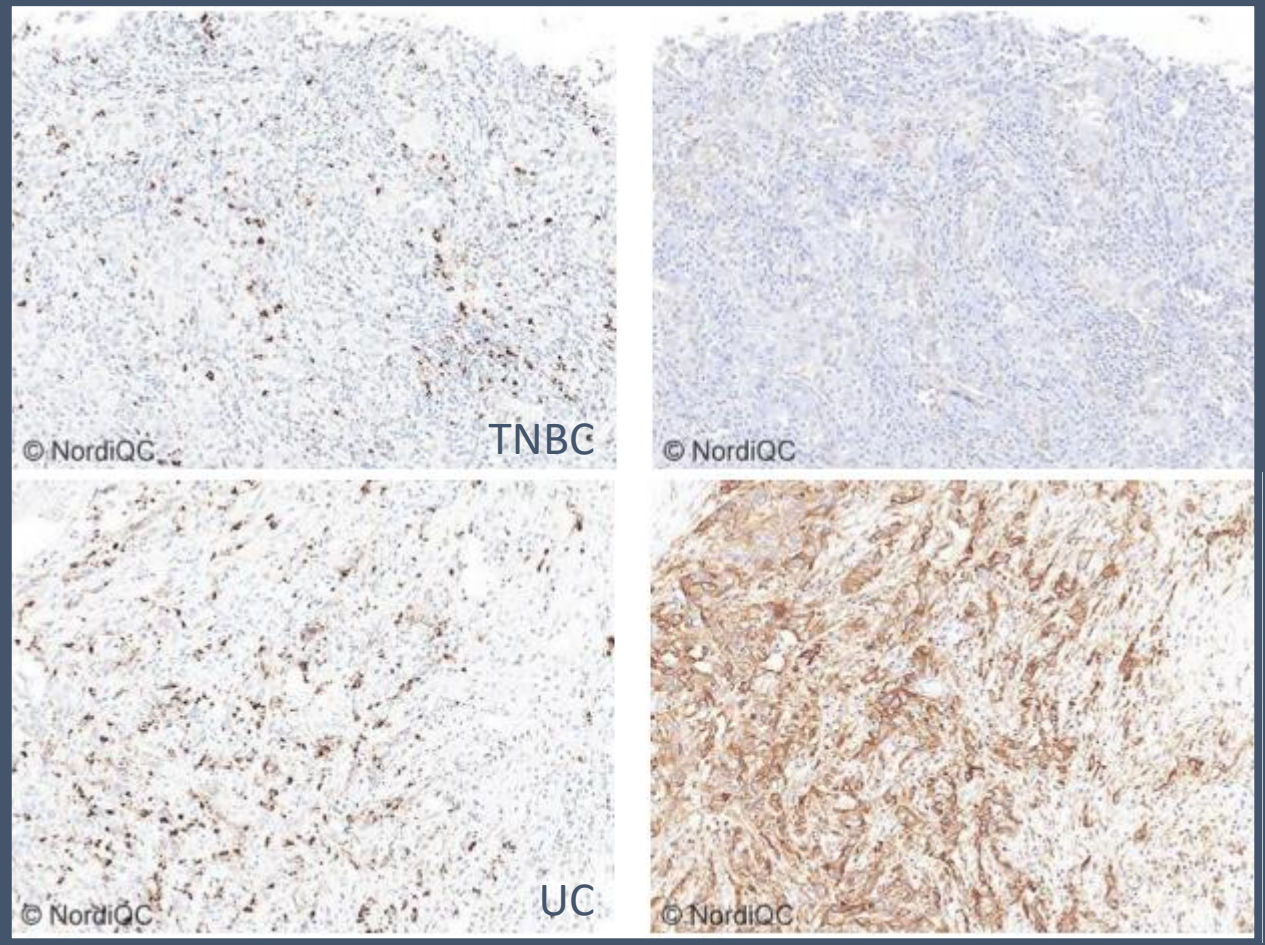




PD-L1 IC – ICAPS

rmAb SP142 | mAb 22C3 | rmAb SP142 | mAb 22C3



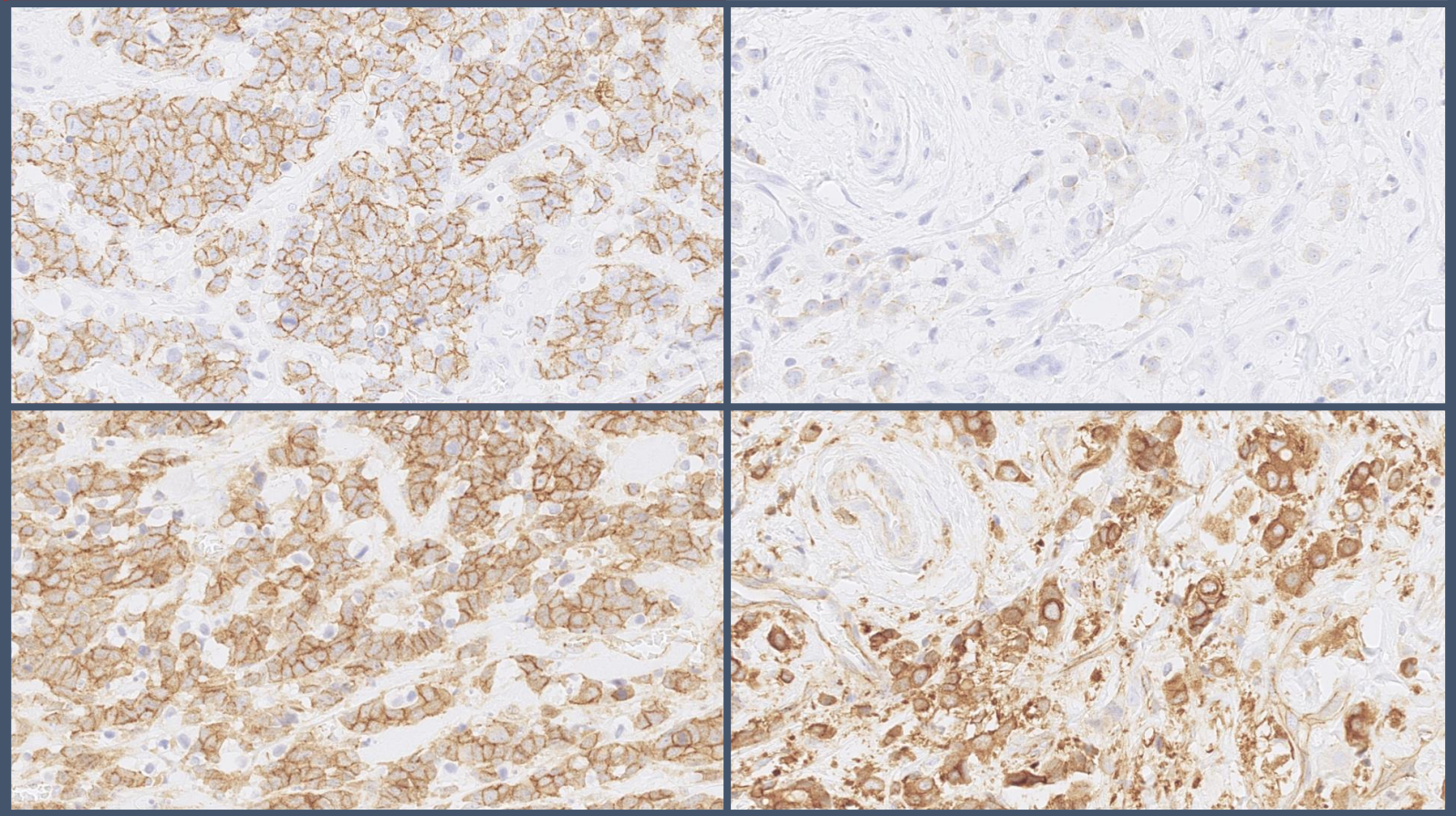


rmAb SP142 | mAb 22C3 | rmAb SP142 | rmAb ZR3

…YOU SURVIVED! THANK YOU FOR YOUR ATTENTION

BONUS – P120

No NordiQC data available for p120 Catenin.
For the p120 stains below, a concentrated format of the mAb clone MRQ-5 is used.



Ductal breast carcinoma

Lobular breast carcinoma

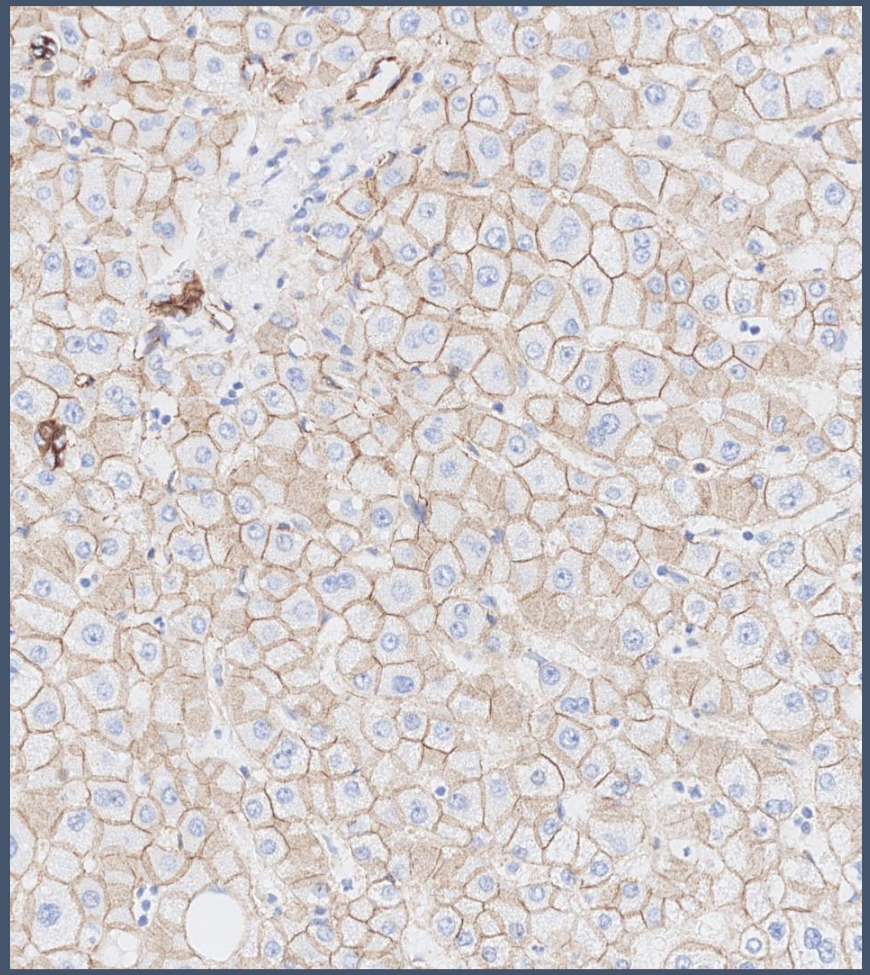
E-CAD: membranous staining reaction in (most) ductal breast carcinomas, negative in (most) lobular breast carcinomas.

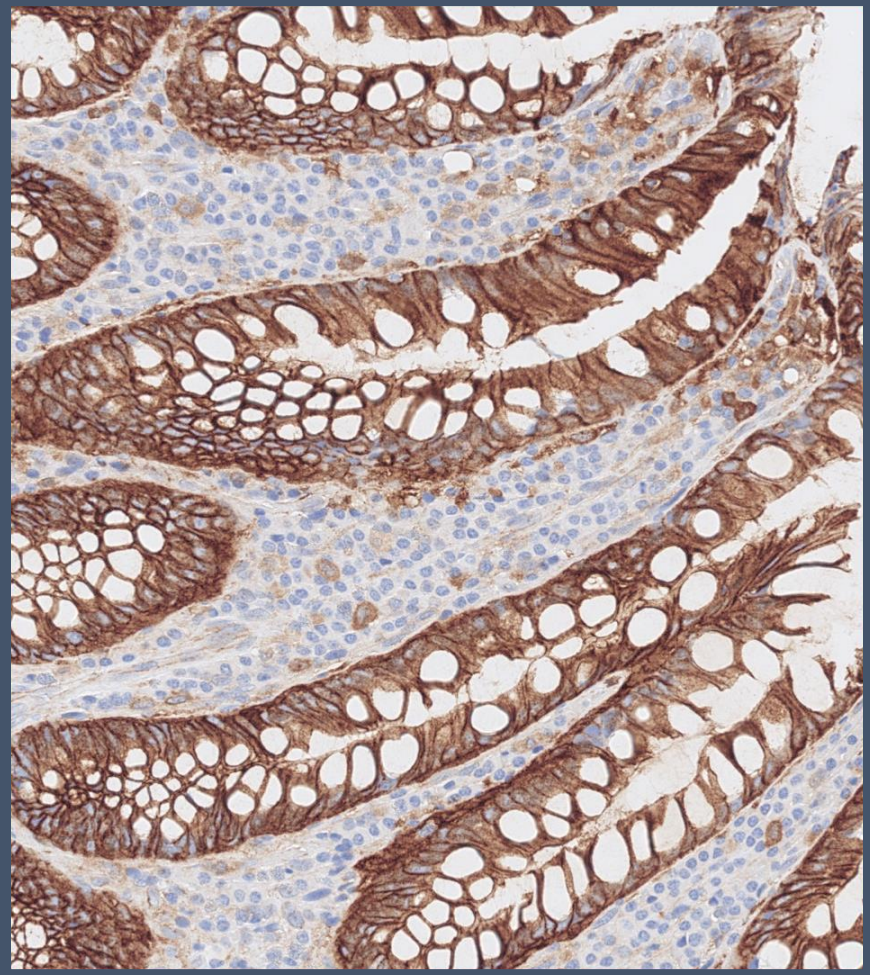
p120: membranous staining reaction in (most) ductal breast carcinomas, cytoplasmic staining reaction in (most) lobular breast carcinomas.

BONUS – P120 ICAPS

No NordiQC data available for p120 Catenin.
For the p120 stains below, a concentrated format of the mAb clone MRQ-5 is used.

Liver:
Hepatocytes must show a weak to moderate membranous staining





Appendix:
Columnar epithelial cells must show a strong membranous staining.