NORDIQC DATA FOR BREAST MARKERS

Antibody selection, protocols and controls

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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 70, 2024	65%	390
	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 66, 2022	81%	152
	p63	CIS vs invasive	Run 61, 2021	79%	324
	E-Cadherin	Ductal vs lobular	Run 53, 2018	89%	298
	TRPS1	Breast vs non-breast	Coming run 72	?	*~100*
Type II IHC tests	KI67	PI index	Run B22, 2016	93%	409
	ER	Predictive for Tamoxifen	Run B37, 2024	85%	428
	PR	Predictive for Tamoxifen	Run B37, 2024	94%	420
	HER2 IHC	Predictive for Herceptin	Run B37, 2024	89%	405
	HER2 BRISH	Predictive for Herceptin	Run H25, 2024	85%	161
	PD-L1 IC	Predictive for Tecentriq	Run C15, 2024	68%	145
	PD-L1 TPS/CPS	Predictive for Keytruda	Run C15, 2024	88%	255

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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

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CLONE PERFORMANCE FOR SELECTED BREAST MARKERS

Marker	Successful clones	Less successful clones
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*

*see ppt for lung-markers

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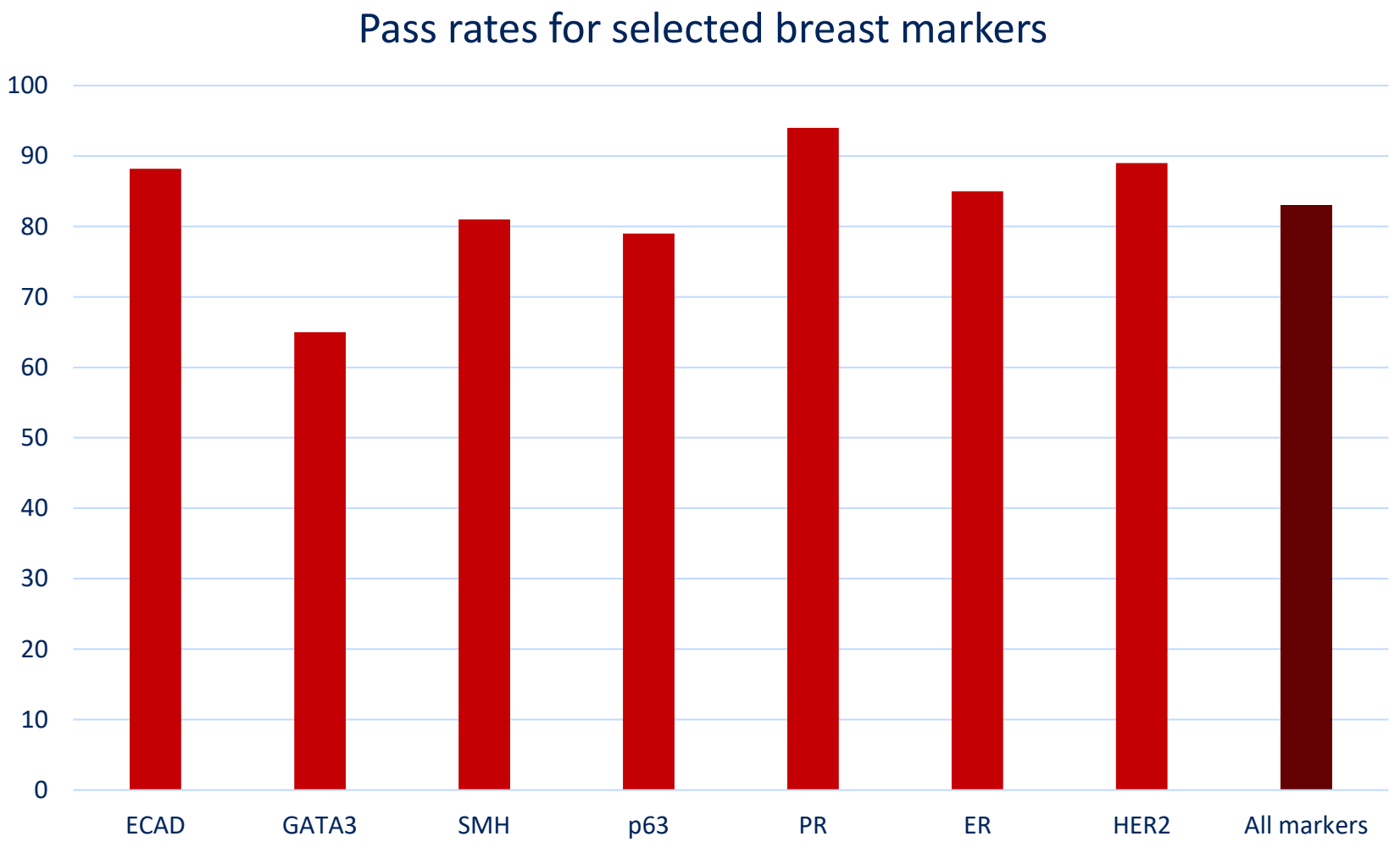
ICAPS FOR SELECTED BREAST MARKERS

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

KEY-POINTS FOR BEST PROTOCOLS



Selected breast markers:

Overall pass rate: **83%** (2.099/2.531), ranging from 65% for GATA3 till 94% for PR.

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KEY-POINTS FOR BEST PROTOCOLS

Breast markers:

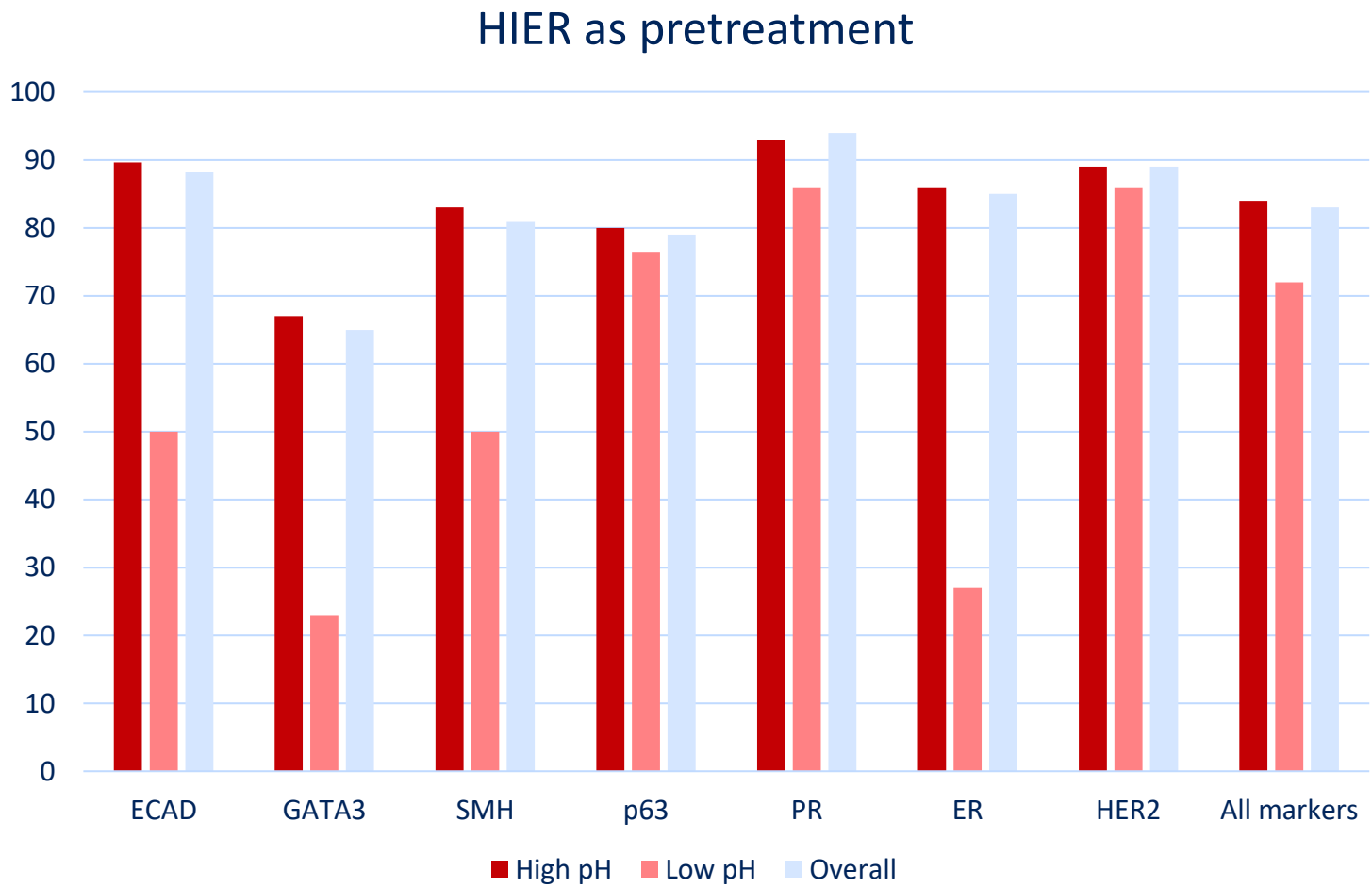
Overall pass rate: **83%**

HIER in High pH: 84%

Ranging from 67% for GATA3 till 93% for PR

HIER in Low pH: 72%

Ranging from 23% for GATA3 till 86% for PR and HER2



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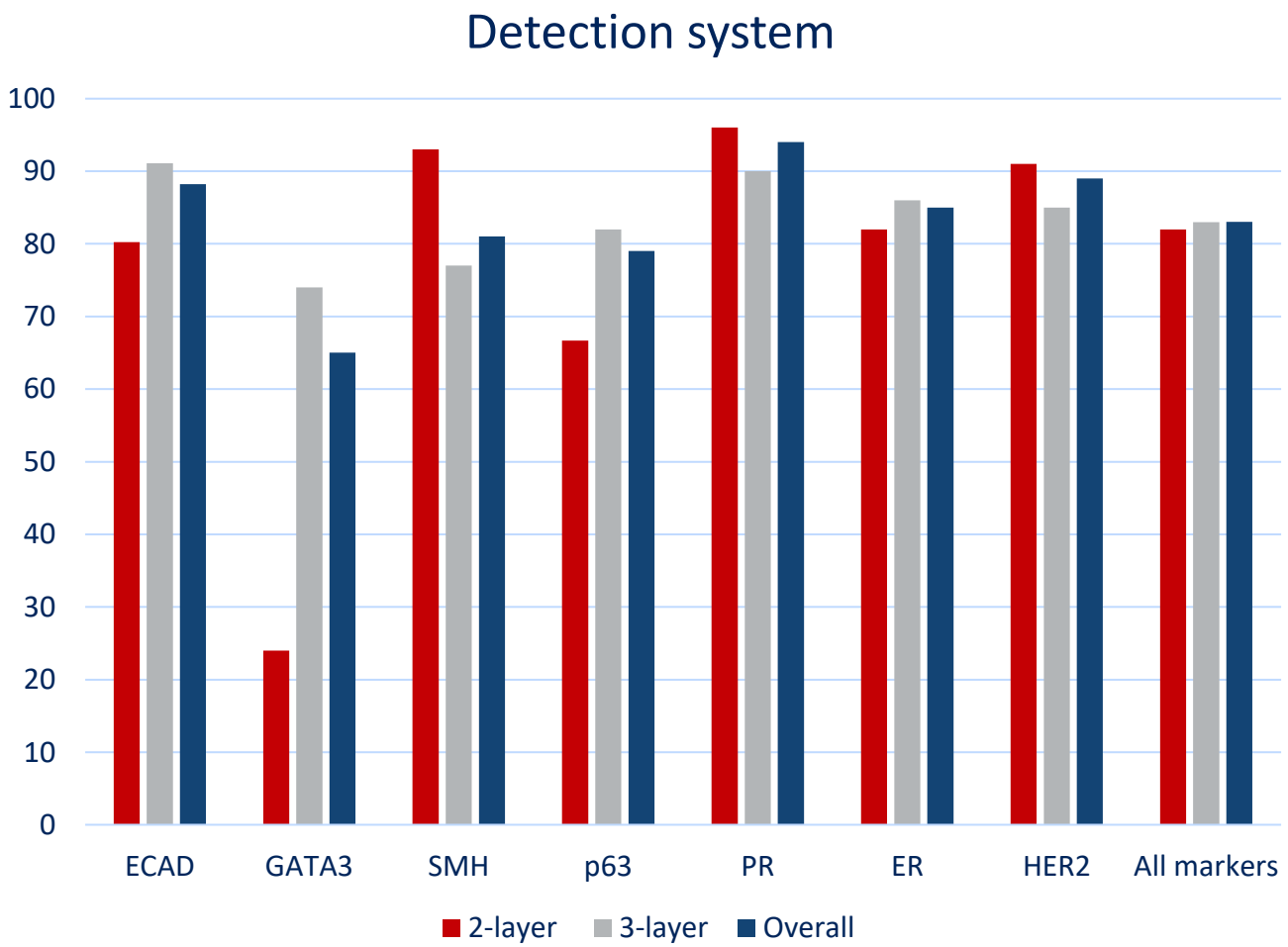
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 82%

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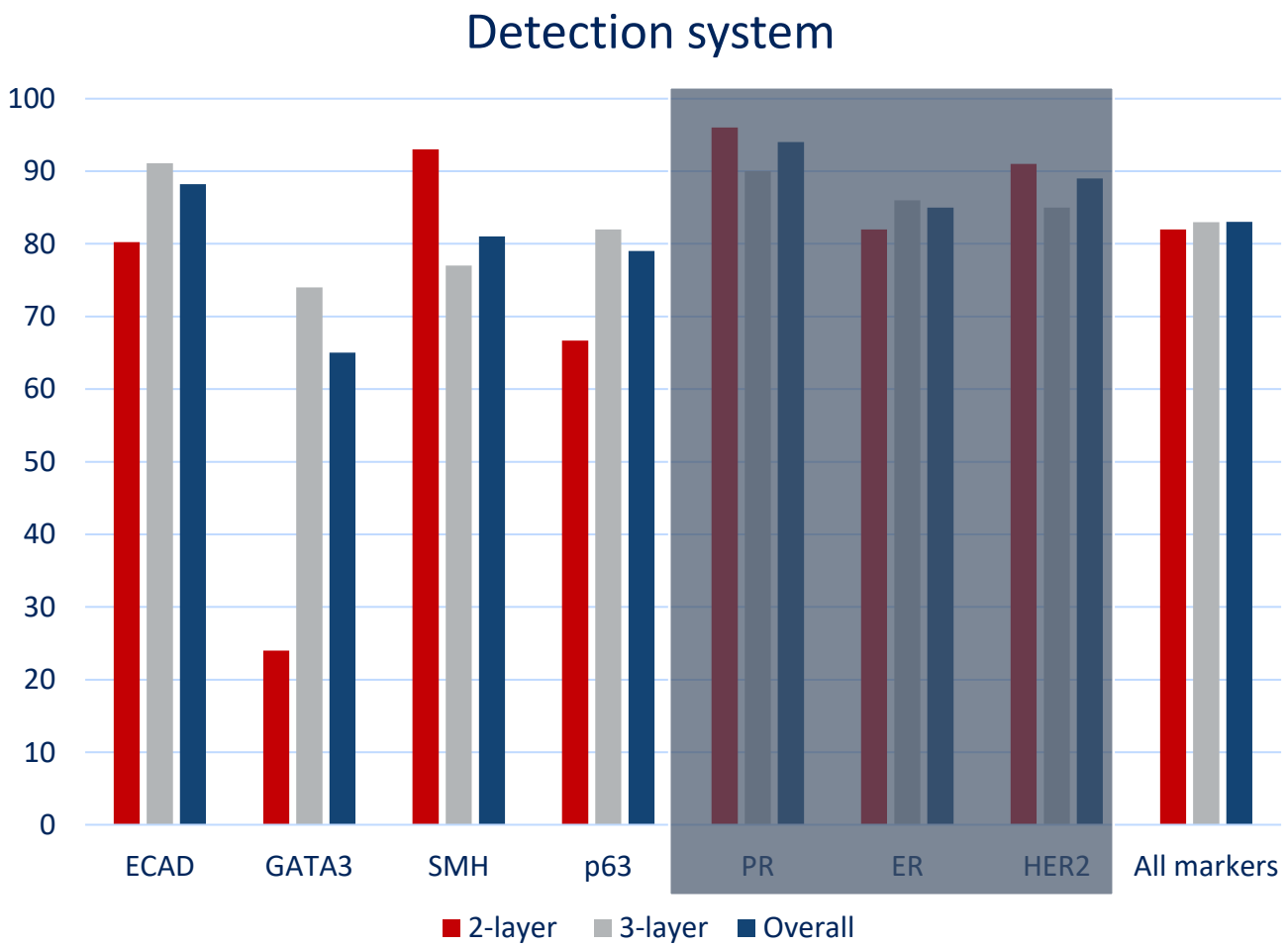
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 82%

Type I tests

3-layer detection system: 81%

2-layer detection system: 66%

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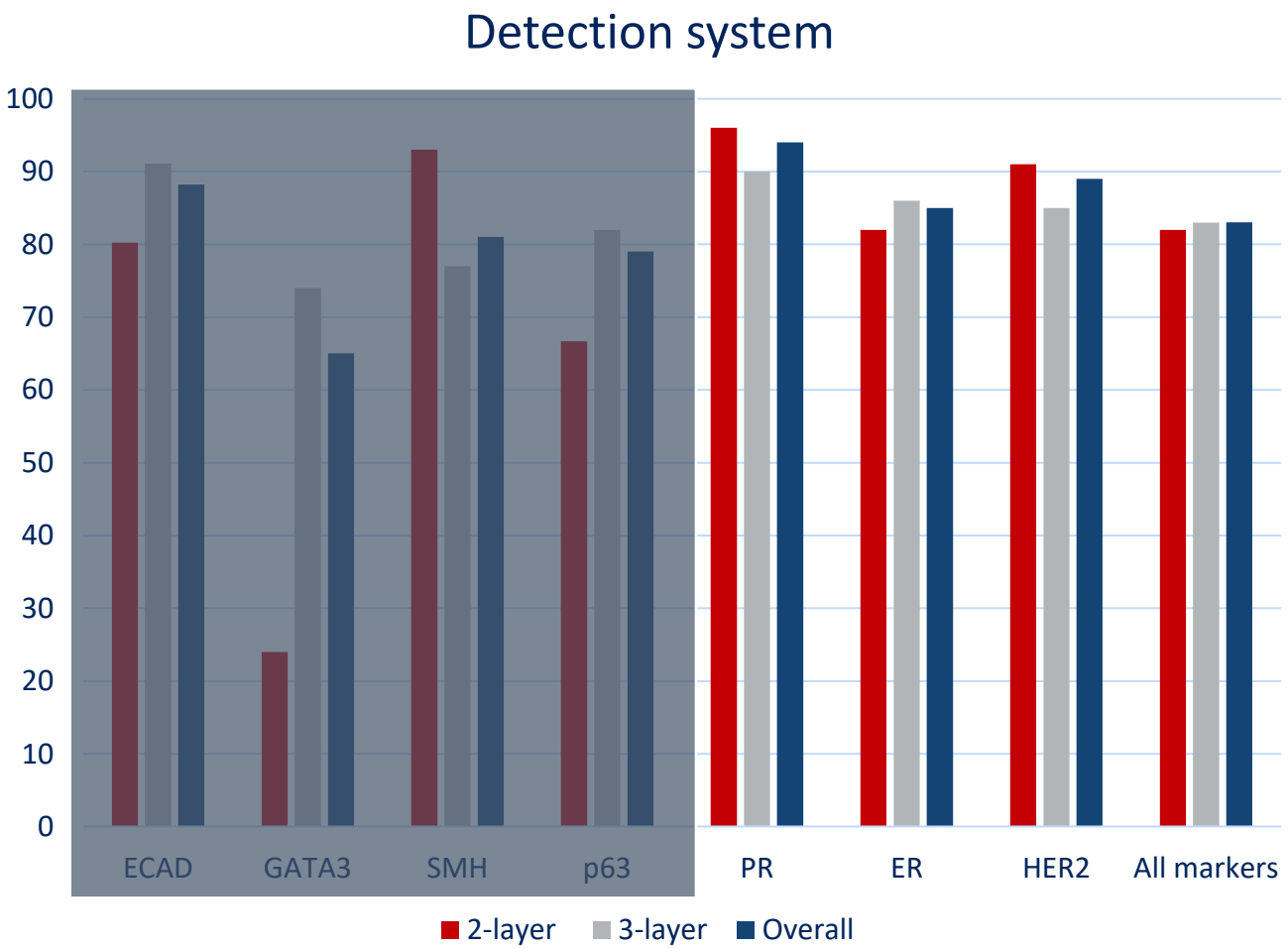
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 82%

Type I tests

3-layer detection system: 81%

2-layer detection system: 66%

Type II test

3-layer detection system: 87%

2-layer detection system: 90%

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NOW IT'S TIME TO LOOK AT SOME SPECIFIC MARKERS

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Table 1a. **Overall results for GATA3, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	135	35	47	40	13	60%	26%
Ready-To-Use antibodies	255	120	52	72	11	68%	47%
Total	390	155	99	112	24		
Proportion		40%	25%	29%	6%	65%	

Table 1b. **Concentrated antibodies and assessment marks for GATA3, run 70**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823**	87 21 3 1 4 2 2 1 6	Cell Marque BioCare Bio SB BD Pharmingen Zytomed Systems Gennova Immunologic Diagnostic Bio DBS	30	46	39	12	60%	24%
mAb clone **HG3-31**	1	Santa Cruz	-	-	-	1	-	-
rmAb clone **EP368**	4 1	Cell Marque Quartett	4	1	-	-	100%	80%
rmAb clone **ZR358**	1	Thermo Fisher Scientific	-	-	1	-	-	-
rmAb clone **QR018**	1	Quartett	1	-	-	-	-	-
Total	135		35	47	40	13		
Proportion			26%	35%	30%	9%	60%	

Table 1c. **Ready-To-Use antibodies and assessment marks for GATA3, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823 760-4897**[3]	30	Ventana/Roche **UltraView**, 760-500	1	3	26	-	13%	3%
mAb clone **L50-823 760-4897**[3]	44	Ventana/Roche **OptiView**, 760-700	34	7	2	1	93%	77%
mAb clone **L50-823 760-4897**[4]	33	Ventana/Roche **UltraView**	8	6	18	1	42%	24%
mAb clone **L50-823 760-4897**[4]	58	Ventana/Roche **OptiView**	43	9	5	1	90%	74%
mAb clone **L50-823 760-4897**	4	Ventana/Roche Other platform	-	1	3	-	-	-
mAb clone **L50-823 390M-17,18,10**	57	Cell Marque	24	16	13	4	70%	42%
mAb clone **L50-823 PM 405AA**	11	BioCare Medical	5	4	2	-	82%	46%
mAb clone **L50-823 MAD-000632QD**	6	Master Diagnostica Vitro SA	1	4	-	1	83%	17%
mAb clone **L50-823 HAM199**	1	Path N Situ	-	-	1	-	-	-
rmAb clone **QR018, 8357-C010**	1	Sakura	1	-	-	-	-	-
mAb clone **L50-823, BMS054**	5	Zytomed systems	-	-	2	3	0%	0%
mAb clone **DA060, RMB1A070**	1	Dartmon	1	-	-	-	-	-
rmAb clone **2555B6B8 PA077**	1	Abcarta	-	1	-	-	-	-
rmAb clone **EP368, RMA-1067**	2	Fuzhou Maixin	1	1	-	-	-	-
rmAb clone **EP368, I12012E-05**	1	BioLynx Biotechnology	1	-	-	-	-	-
Total	255		120	52	72	11		
Proportion			47%	20%	28%	4%	68%	

GATA3 – POINTS OF ATTENTION

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[1]		Dako/Agilent Omnis		Ventana/Roche BenchMark[2]		Leica Biosystems Bond[3]	
	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**	1/11** (9%)	0/2	5/37 (14%)	0/3	14/41 (34%)	-	10/26 (39%)	0/4
rmAb clone **EP368**	0/1	-	3/3	-	-	-	-	-

* 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.

No RTU products for Dako and Leica users.

Use of conc. format of mAb L50-823 can obtain optimal results.

- The performance seems to be influenced by the vendor of the clone

Pass rate:
Biocare: 38%
Cell Marque: 67%

Recommended protocol settings for L50-823 as a concentrate:

- HIER in an alkaline buffer
- *(Low pH diluent)*
- 0% pass rate for 2-step detection systems (0/4)
- 63% pass rate for 3-step detection systems (65/104)

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SMH - POINTS OF ATTENTION

Table 3. **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**	73% (8/11)	18% (2/11)	86% (6/7)	57% (4/7)
Leica BOND mAb S131 **PA0493**	100% (7/7)	100% (7/7)	3/3	2/3
VMS Ultra/XT mAb SMMS-1 **760-2704**	87% (13/15)	67% (10/15)	95% (38/40)	60% (24/40)

* 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.

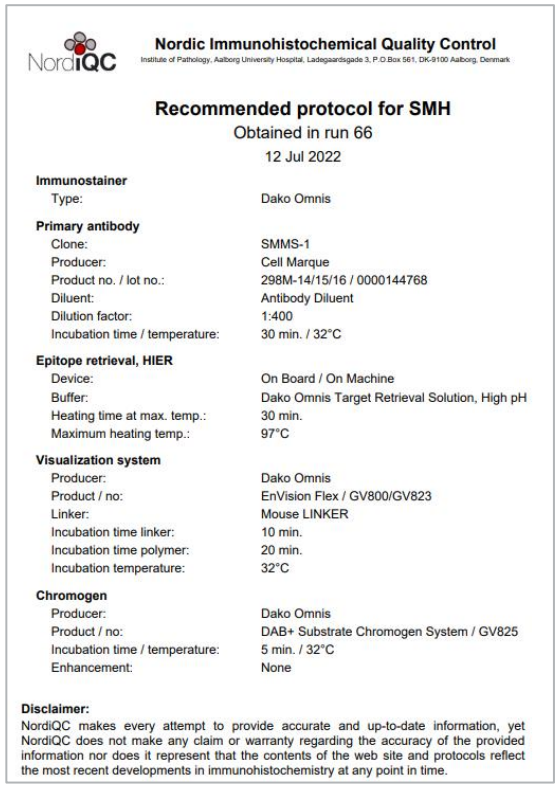
Table 2. **Proportion of optimal results for SMH for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	Dako/Agilent Autostainer Link / Classic		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / 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	ER2 pH 9.0	ER1 pH 6.0
mAb **SMMS-1**	0/1**	-	6/7 (86%)	0/1	8/12 (67%)	-	6/8 (75%)	(1/1)

* 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 for Omnis is available. 13 laboratories used the Autostainer RTU on the Omnis; 15% pass rate, none optimal.

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



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

Recommended protocol for SMH
Obtained in run 66
12 Jul 2022

Immunostainer
Type: Dako Omnis

Primary antibody
Clone: SMMS-1
Producer: Cell Marque
Product no. / lot no.: 298M-14/15/16 / 0000144768
Diluent: Antibody Diluent
Dilution factor: 1:400
Incubation time / temperature: 30 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.

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P63 - 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.

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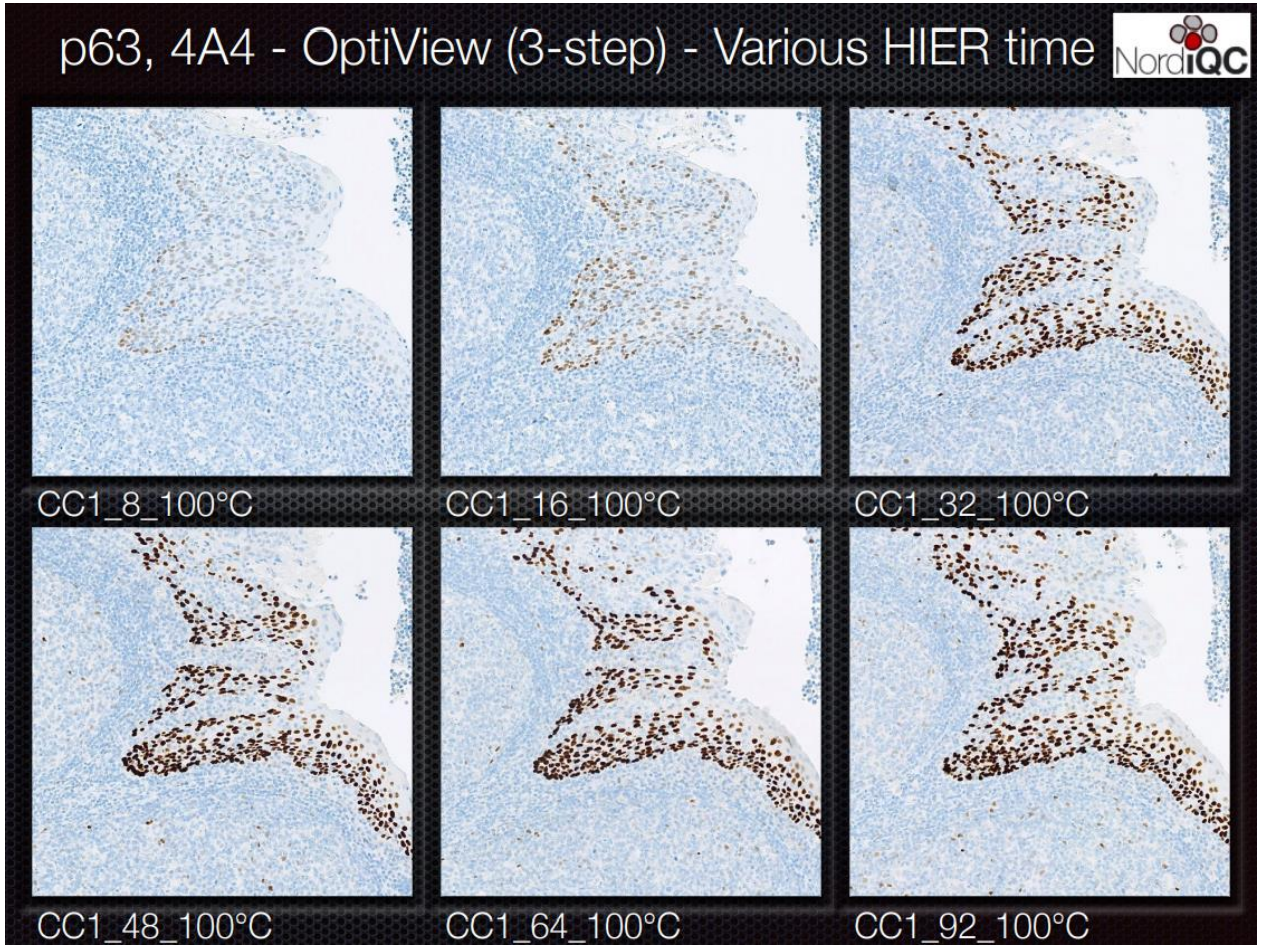
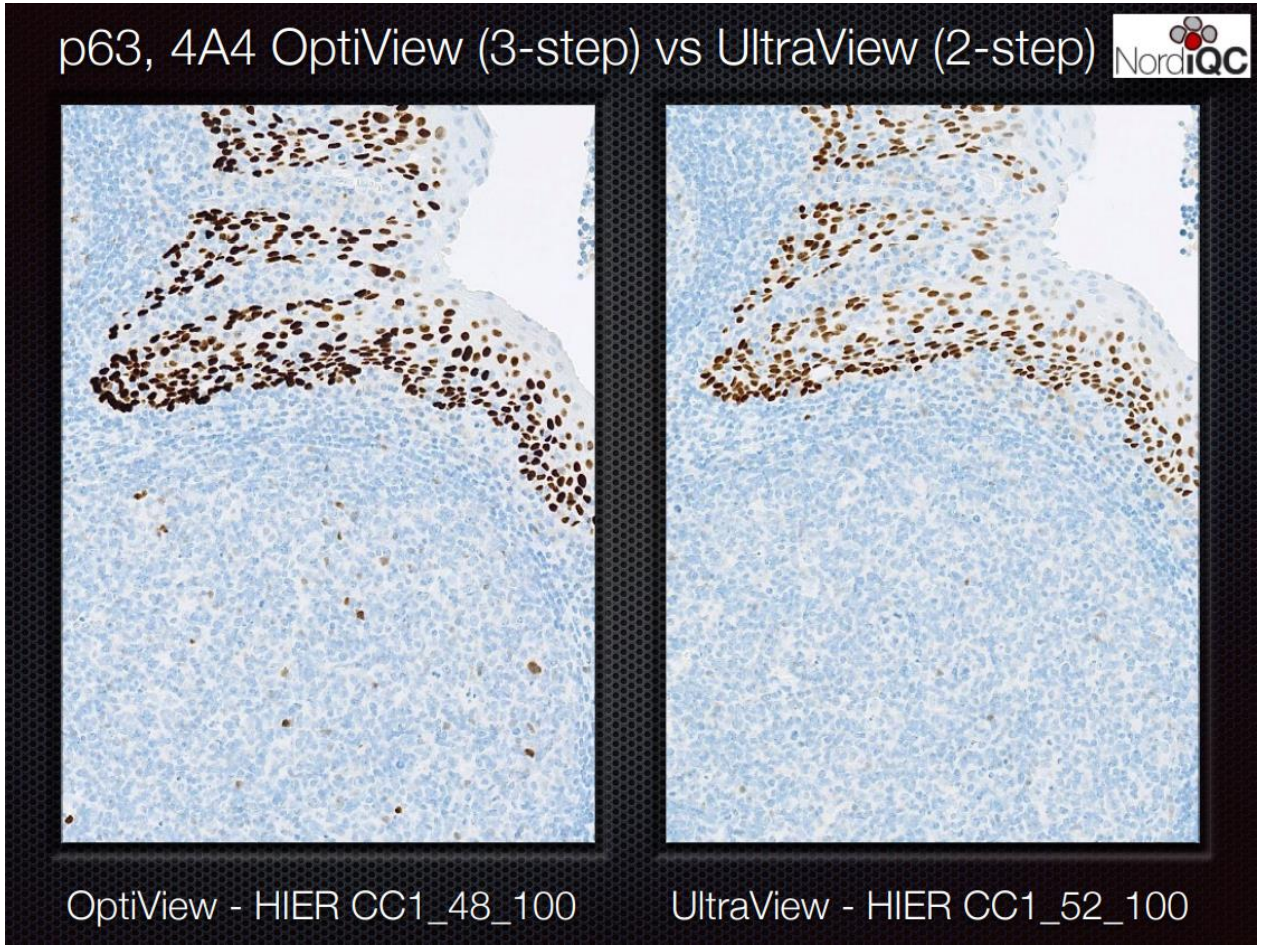
P63 - POINTS OF ATTENTION





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

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

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TRPS1 in Breast Cancer:
A comparative study of five different IHC assays

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Disclosures: Birgit Truumees: None; Mia Korsdal Stensballe: None; Rasmus Røge: None; Søren Nielsen: None.

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AALBORG UNIVERSITY HOSPITAL

Background & Objective

Immunohistochemistry (IHC) for TRPS1 (Trichorhinophalangeal syndrome 1) is a novel biomarker for breast cancer (BC), especially triple negative breast cancer (TNBC). The aim of the study was to compare the staining patterns and diagnostic sensitivity and specificity of five different TRPS1 IHC assays.

Methods

Five commercially available antibodies for TRPS1 were optimized on TMAs with a range of normal tissues and BCs. The antibodies were extensively tested by various protocol settings including adjustments of antibody concentrations, diluents and type of IHC platform to achieve best analytical performance (data not shown). Subsequently each IHC assay was validated on TMAs comprising of 135 breast carcinomas (BC), including 64 TNBCs, and 74 various neoplasias. A positive cut-off at ≥10% of neoplastic cells (NCs) with nuclear TRPS1 expression was applied to determine the diagnostic sensitivity and specificity.

Table 1. The five TRPS1 IHC assays applied

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Antibody clone / vendor	EP392 / BioSB	polyclonal / Invitrogene	MSVA-512R / MS validated	8131R / NeoBiotechnologies	ZR382 / Zeta corporation
Titer / incubation time	1:40 / 30 min	1:100 / 32 min	1:200 / 32 min	1:1000 / 32 min	1:1000 / 32 min
Antibody diluent	Background sniper, Biocare Medical	Background sniper, Biocare Medical	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent
HIER / incubation time	TRS High, pH 9,0 / 20 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min
Detection system	EnVision Flex	OptiView DAB	OptiView DAB	OptiView DAB	OptiView DAB
IHC platform	Dako Omnis	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra

Results

The five different and individually optimized IHC assays provided a fully comparable staining pattern (Figure 1) and level of diagnostic sensitivity (Graph 1) and specificity. All assays reached an overall diagnostic sensitivity of 97% in BCs (Table 1). 100% of luminal BCs and 94% of TNBCs were scored as TRPS1 positive. The general diagnostic specificity was in the range of 91-93% (Graph 2). Positive TRPS1 staining in ≥10% of neoplastic cells was seen in a subset of lung, gynecological and urothelial carcinomas with all assays. In addition, two assays also labelled melanomas (20%). If a positive cut-off was changed to ≥1%, TRPS1 was observed in more neoplasias (Table 2) and the diagnostic specificity was reduced to 82-85% (Graph 2).

Graph 1. Diagnostic sensitivity (reaction in breast carcinomas) of 5 TRPS1 IHC assays

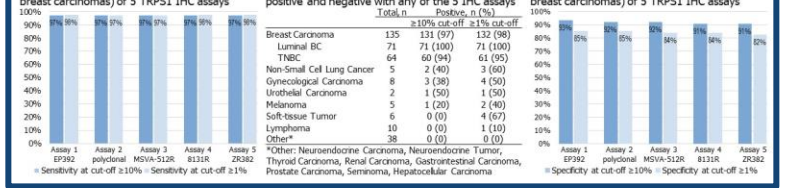


Table 2. Tumors tested and reported TRPS1 positive and negative with any of the 5 IHC assays

	Total, n	Positive, n (%)	
		≥10% cut-off	≥1% cut-off
Breast Carcinoma	135	131 (97)	132 (98)
Luminal BC	71	71 (100)	71 (100)
TNBC	64	60 (94)	61 (95)
Non-Small Cell Lung Cancer	5	2 (40)	3 (60)
Gynecological Carcinoma	8	3 (38)	4 (50)
Urothelial Carcinoma	2	1 (50)	1 (50)
Melanoma	5	1 (20)	2 (40)
Soft-tissue Tumor	6	0 (0)	4 (67)
Lymphoma	10	0 (0)	1 (10)
Other*	38	0 (0)	0 (0)

*Other: Neuroendocrine Carcinoma, Neuroendocrine Tumor, Thyroid Carcinoma, Renal Carcinoma, Gastrointestinal Carcinoma, Prostate Carcinoma, Seminoma, Hepatocellular Carcinoma

Graph 2. Diagnostic specificity (reaction in non-breast carcinomas) of 5 TRPS1 IHC assays

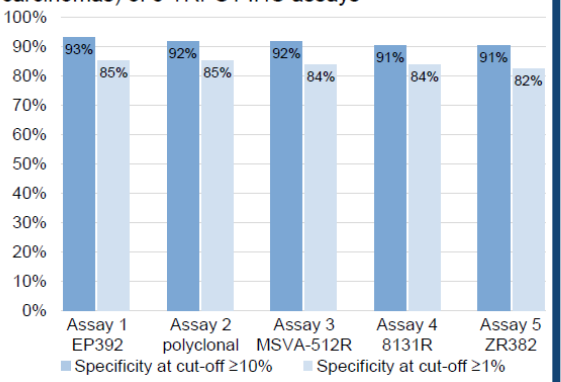
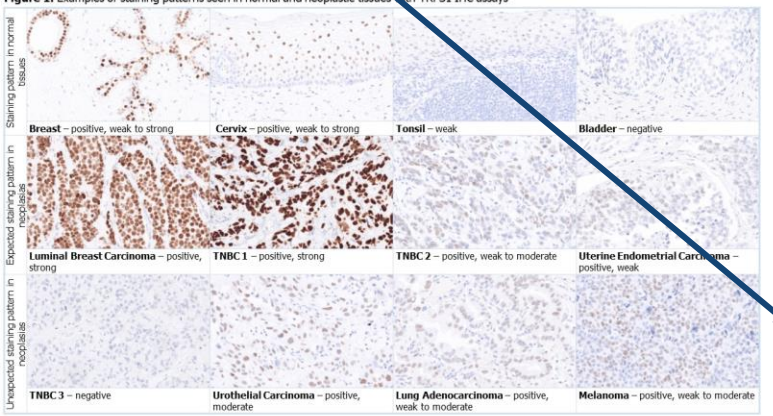


Figure 1. Examples of staining patterns seen in normal and neoplastic tissues with TRPS1 IHC assays



Conclusion

All five tested TRPS1 assays exhibited comparable staining patterns in both normal and neoplastic tissues. However, implementing TRPS1 IHC as diagnostic tool for BCs requires rigorous optimization and validation to ensure appropriate diagnostic sensitivity and specificity of the assay, as some antibodies might require special technical assay conditions. This study presents potential diagnostic pitfalls as TRPS1 expression is not restricted to BCs since it can be seen in other neoplasias that are relevant differential diagnoses. This underlines the need for further studies on larger cohorts of these neoplasias.

← **Download the poster**

References

- Ai, Di, et al. "TRPS1: a highly sensitive and specific marker for breast carcinoma, especially for triple-negative breast cancer." Modern Pathology 34.4 (2021): 710-719.
- Rammal, Rayan, et al. "Utility of TRPS1 immunohistochemistry in confirming breast carcinoma: Emphasis on staining in triple-negative breast cancers and gynecologic tumors." American Journal of Clinical Pathology 160.4 (2023): 425-434.
- Lennartz, Maximilian, et al. "TRPS1 is a Highly Sensitive Marker for Breast Cancer: A Tissue Microarray Study Evaluating More Than 19,000 Tumors From 152 Different Tumor Entities." The American Journal of Surgical Pathology 48.6 (2024): 637-651.

TRPS1 – NEW MARKER IN NORDIQC

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Antibody clone / vendor	EP392 / BioSB	polyclonal / Invitrogene	MSVA-512R / MS validated	8131R / NeoBiotechnologies	ZR382 / Zeta corporation
Titer / incubation time	1:40 / 30 min	1:100 / 32 min	1:200 / 32 min	1:1000 / 32 min	1:1000 / 32 min
Antibody diluent	Background sniper, Biocare Medical	Background sniper, Biocare Medical	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent	Antibody diluent, Dako/Agilent
HIER / incubation time	TRS High, pH 9,0 / 20 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min	CC1 pH 8,5 / 48 min
Detection system	EnVision Flex	OptiView DAB	OptiView DAB	OptiView DAB	OptiView DAB
IHC platform	Dako Omnis	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra	Ventana Benchmark Ultra

Graph 1. Diagnostic sensitivity (reaction in breast carcinomas) of 5 TRPS1 IHC assays

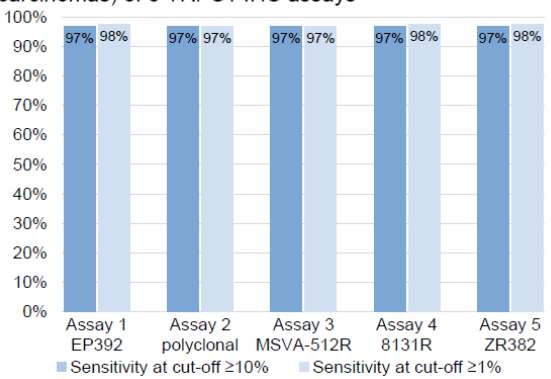


Table 2. Tumors tested and reported TRPS1 positive and negative with any of the 5 IHC assays

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Lymphoma	10	0 (0)	1 (10)
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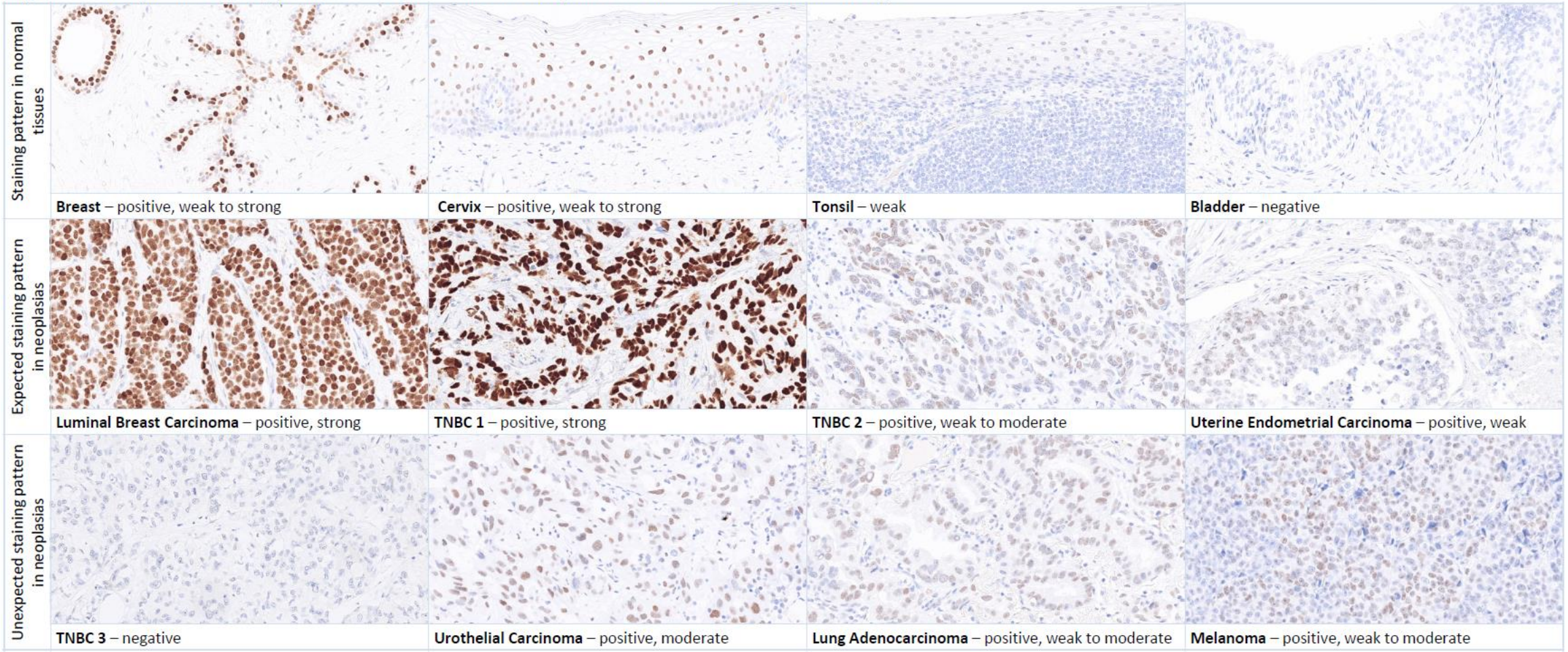
*Other: Neuroendocrine Carcinoma, Neuroendocrine Tumor, Thyroid Carcinoma, Renal Carcinoma, Gastrointestinal Carcinoma, Prostate Carcinoma, Seminoma, Hepatocellular Carcinoma

Graph 2. Diagnostic specificity (reaction in non-breast carcinomas) of 5 TRPS1 IHC assays

NordiQC

TRPS1 – NEW MARKER IN NORDIQC

Figure 1. Examples of staining patterns seen in normal and neoplastic tissues with TRPS1 IHC assays



Enlarged photos from the poster presented at ECP 2024;
TRPS1 in Breast Cancer: A comparative study of five different IHC assays

ER – 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**	2/2	1/2	17/18 (94%)	8/18 (44%)
Dako Omnis rmAb EP1 **GA084**	42/44 (95%)	28/44 (64%)	25/25 (100%)	14/32 (56%)
Leica Bond III mAb 6F11 **PA009/PA0151**	0/3	0/3	7/10 (70%)	1/10 (10%)
VMS Ultra/XT rmAb SP1 **790-4324/4325**	51/61 (84%)	5/61 (8%)	144/172 (84%)	53/172 (31%)

* 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, detection kit and use of amplification. Only protocols performed on the specified vendor IHC stainer are included.

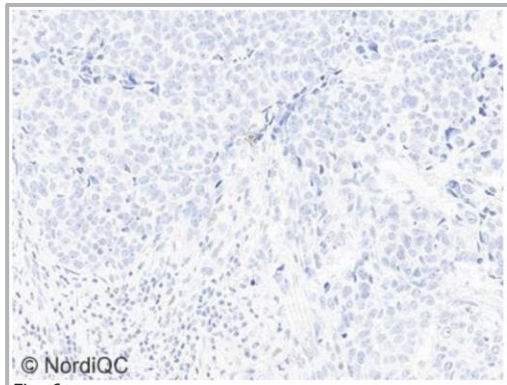


Fig. 6a
Optimal ER staining of the breast carcinoma no. 5 expected to be negative using same protocol as in Figs. 1a – 5a. No nuclear staining reaction is seen and a high signal-to-noise ratio is observed.

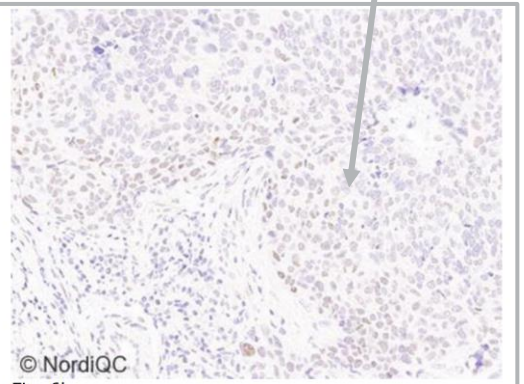


Fig. 6b
Insufficient ER staining of the breast carcinoma no 5 with no ER expression. A weak but distinct nuclear staining reaction is seen in >10% of the neoplastic cells. The insufficient result was only seen for the mAb clone 6F11 and likely was caused by performing HIER in an alkaline buffer in combination with other protocol settings inducing a too high level of technical/analytical IHC sensitivity compromising the diagnostic specificity.

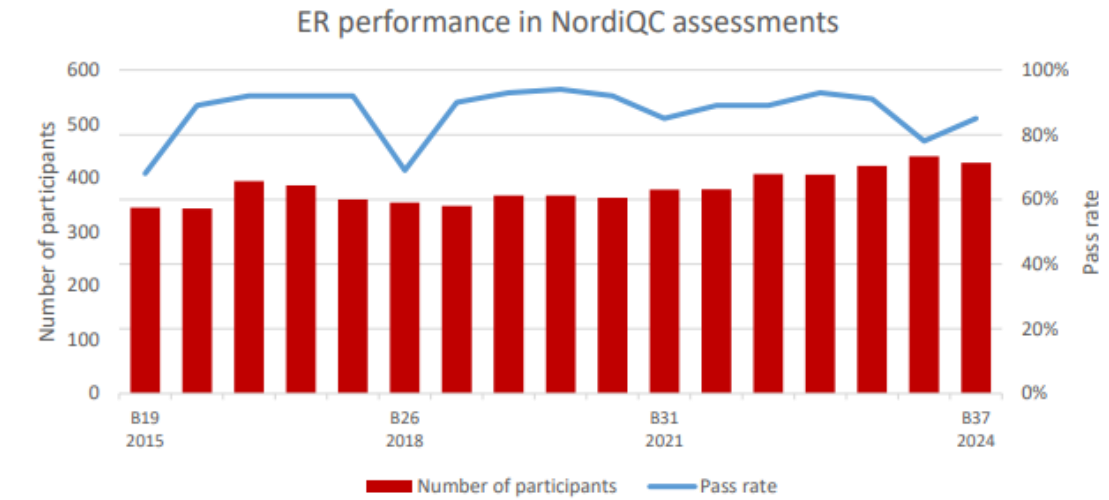
Photos from run B35

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 2015 - 2024**



PR – POINTS OF ATTENTION

Table 1a. **Overall results for PR, run B37**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	51	32	11	7	1	84%	63%
Ready-To-Use antibodies	369	265	84	19	1	95%	72%
Total	420	297	95	26	2	-	
Proportion		71%	23%	6%	0%	94%	

Table 1b. **Concentrated antibodies and assessment marks for PR, run B37**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16**	30 1 1	Leica Biosystems Monosan DCS	20	7	4	1	84%	63%
mAb clone cocktail **16 + SAN27**	2	Leica Biosystems	1	0	1	0	-	-
mAb clone **1A6**	1	Leica Biosystems	0	1	0	0	-	-
mAb clone **PgR 636**	8	Dako/Agilent	4	2	2	0	75%	50%
mAb clone **PgR 1294**	6	Dako/Agilent	5	1	0	0	100%	83%
rmAb clone **BP6081**	1	Biolynx	1	0	0	0	-	-
rmAb clone **QR014**	1	Quartett	1	0	0	0	-	-
Total	51		32	11	7	1		
Proportion			63%	21%	14%	2%	84%	

Table 1c. **Ready-To-Use antibodies and assessment marks for PR, run 37**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16 PA0312 (VRPS[3])**	16	Leica Biosystems	15	1	0	0	100%	94%
mAb clone **16 PA0312 (LMPS[4])**	18	Leica Biosystems	15	2	1	0	94%	83%
mAb clone **16 MAD-000670QD**	2	Master Diagnostica/Vitro	1	0	1	0	-	-
mAb **PgR 636 IR/IS068 (VRPS[3])**	12	Dako/Agilent	11	1	0	0	100%	92%
mAb **PgR 636 IR/IS068 (LMPS[4])**	12	Dako/Agilent	11	1	0	0	100%	92%
mAb **PgR 1294 GA090 (VRPS[3])**	43	Dako/Agilent	17	17	9	0	79%	40%
mAb **PgR 1294 GA090 (LMPS[4])**	29	Dako/Agilent	13	14	2	0	93%	45%
mAb clone **C4D10 CPM-0365**	1	Celnovte	1	0	0	0	-	-
mAb clone **MXR008 MAB-0854**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **1E2 790-2223/4296 (VRPS[3])**	90	Ventana/Roche	73	17	0	0	100%	81%
rmAb clone **1E2 790-2223/4296 (LMPS[4])**	138	Ventana/Roche	103	28	6	1	95%	75%
rmAb clone **278G8D6 PA246**	1	Abcarta	0	1	0	0	-	-
rmAb clone **SP2 GT205702**	1	GeneTech	1	0	0	0	-	-
rmAb clone **YR85 8360-C010**	2	Sakura Finetek	2	0	0	0	-	-
Ab clone **DA201 DRMD0249**	1	Dartmon	0	1	0	0	-	-
Ab clone **MSUA-570R MAD-000670QD**	1	Master Diagnostica/Vitro	0	1	0	0	-	-
Total	369		265	84	19	1		
Proportion			72%	23%	5%	0%	95%	

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

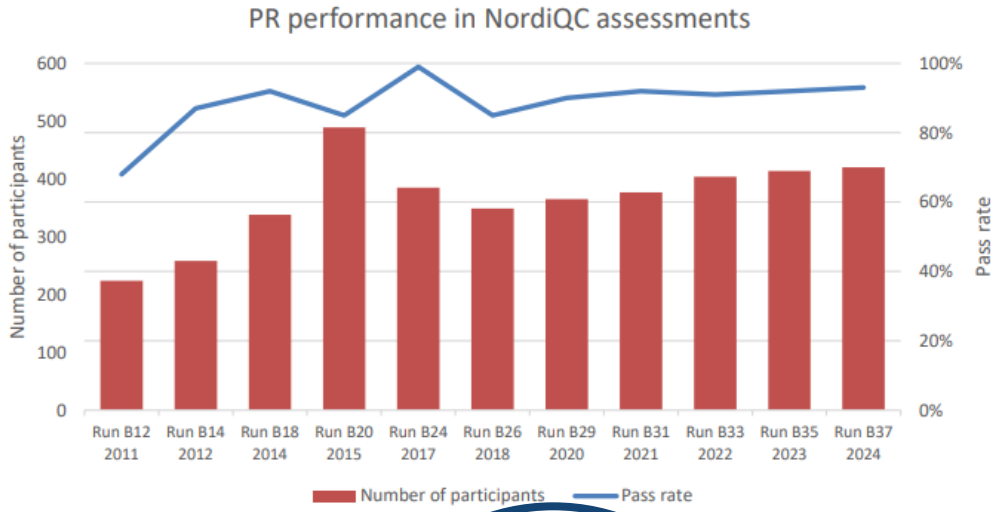


Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

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%)

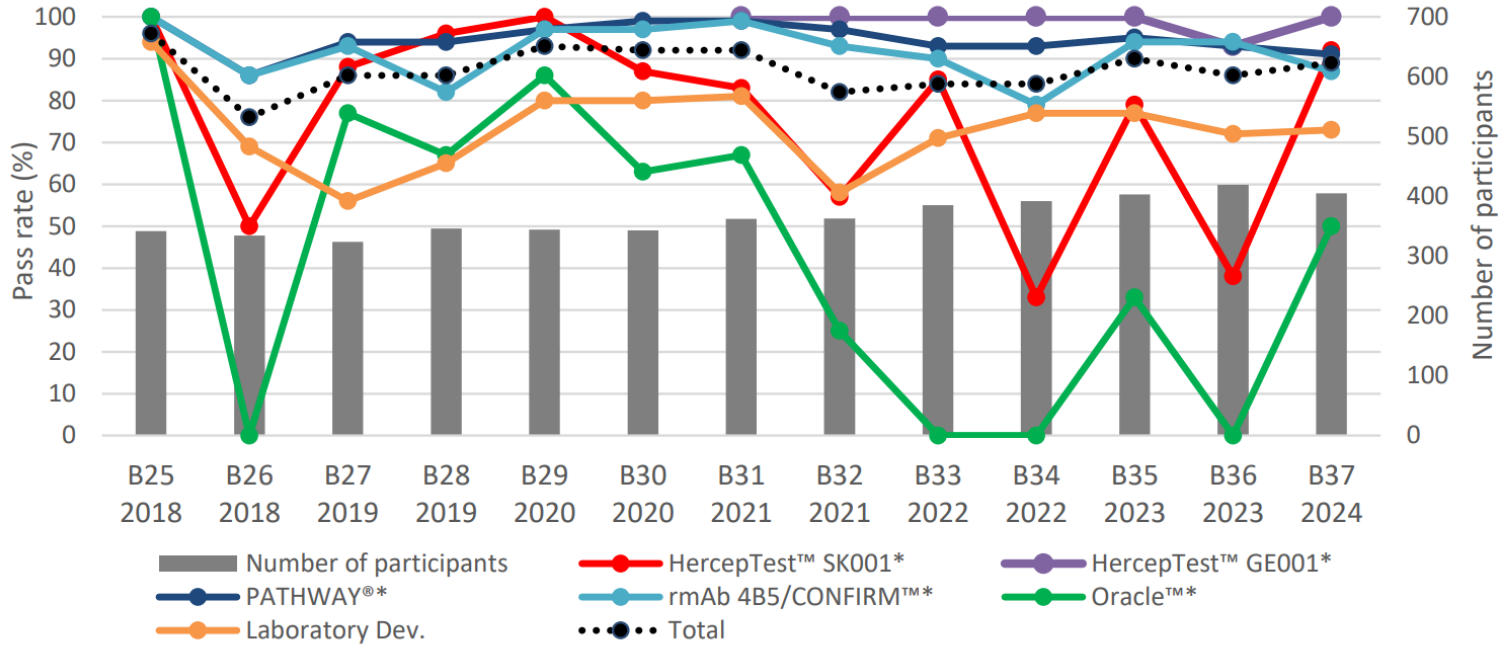
* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor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

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

NordiQC

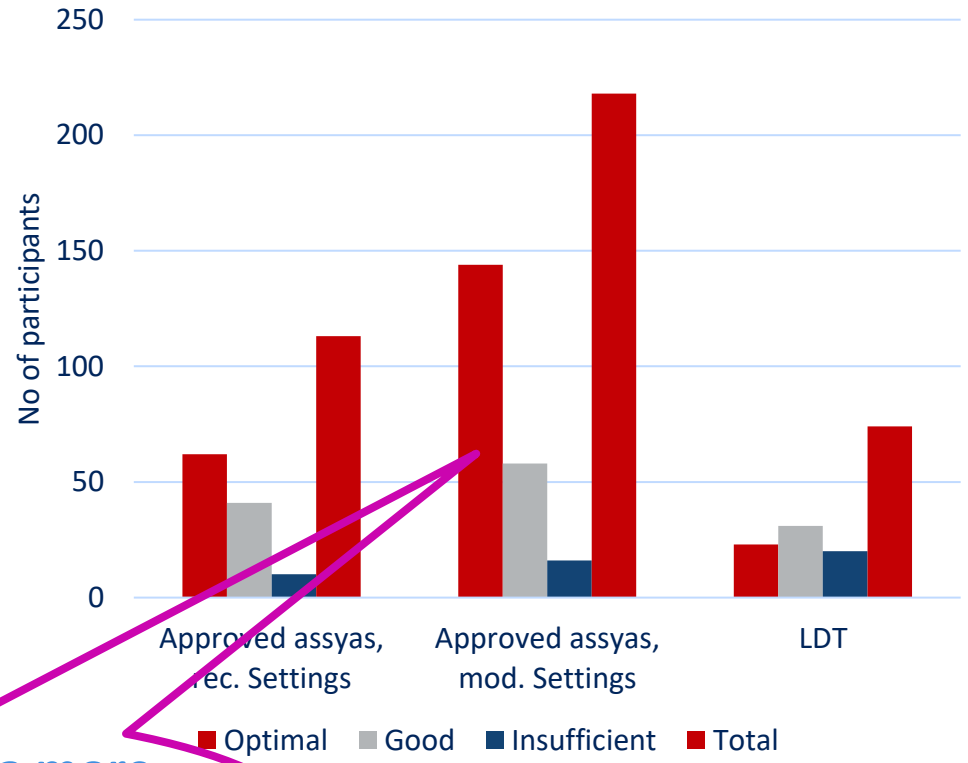
HER2 – POINTS OF ATTENTION

Graph 1. **Pass rates of the HER2 IHC assessments in the NordiQC breast cancer module 2018-2024**



* pass rates using vendor recommended protocol settings

HER2 results, run B37



Do not change to a more sensitive detection system!

NordiQC

PD-L1 IC – POINTS OF ATTENTION

Table 2a. **Overall results for PD-L1 IC, run C15**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved PD-L1 assays	66	40	8	6	12	73%	61%
Antibodies for laboratory developed PD-L1 assays, based on concentrated antibodies	10	-	1	1	8	10%	0%
Ready-To-Use antibodies	69	30	19	6	14	71%	43%
Total	145	70	28	13	34		
Proportion		48%	19%	9%	23%	68%	

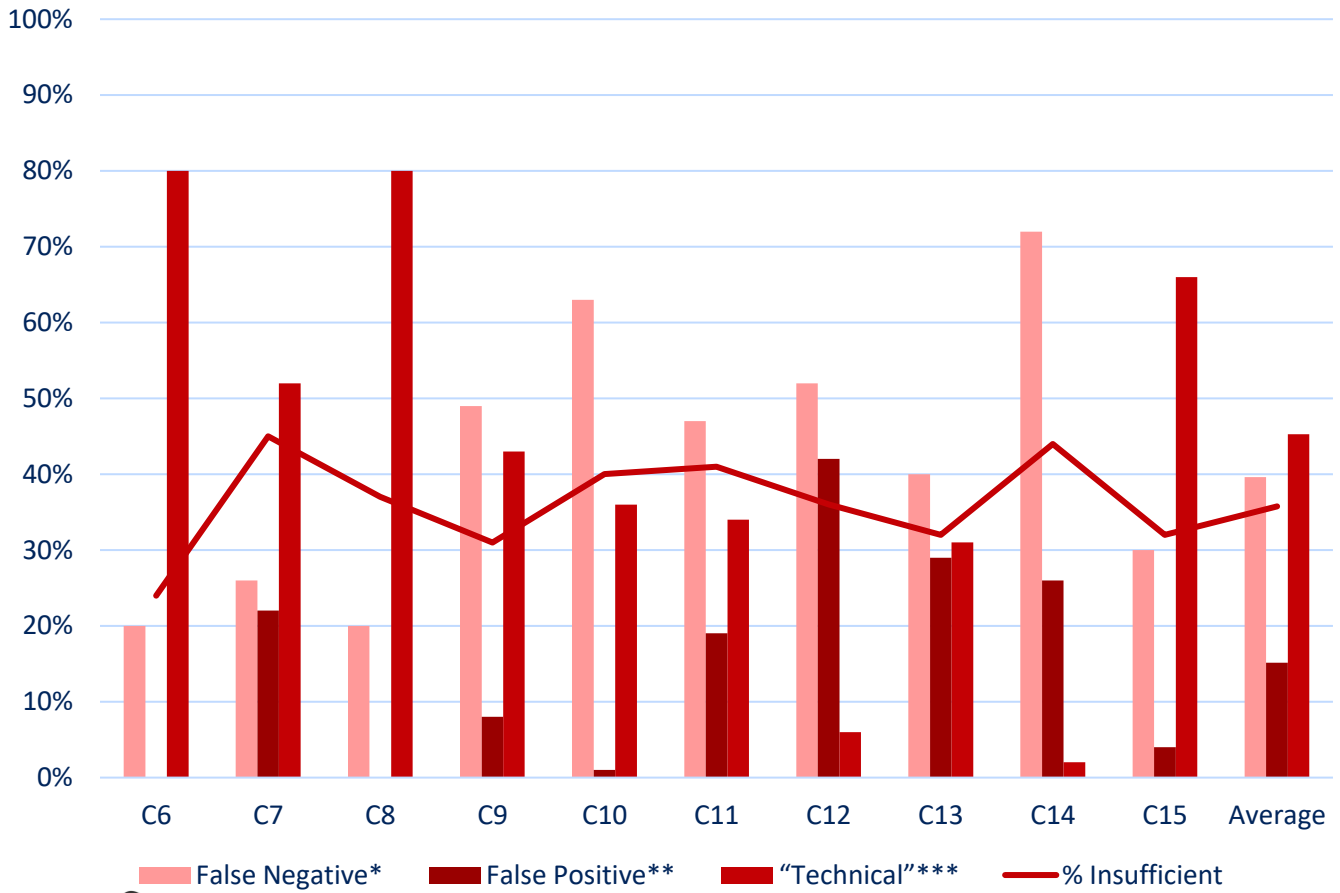
Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 IC, run C15**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **741-4860**[3]	51	Ventana/Roche	39	8	2	2	92%	76%
rmAb clone SP142, **741-4860**[4]	1	Ventana/Roche	1	-	-	-	-	-
rmAb clone SP263, **741-4905**[3]	6	Ventana/Roche	-	-	2	4	0%	0%
rmAb clone SP263, **741-4905**[4]	2	Ventana/Roche	-	-	1	1	-	-
mAb clone 22C3 pharmDX, **SK006**	2	Dako/Agilent	-	-	-	2	-	-
mAb clone 22C3 pharmDX, **GE006**	4	Dako/Agilent	-	-	1	3	-	-
Total	66		40	8	6	12		
Proportion			61%	12%	9%	18%	73%	

Table 2d. **Assessment marks for Ready-To-Use antibodies[8] for PD-L1 IC, run C15**

Ready-To-Use antibodies[8]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **790-4860 (VRPS)**[5]	22	Ventana/Roche	17	4	1	-	95%	77%
rmAb clone SP142, **790-4860 (LMPS)**[6]	33	Ventana/Roche	13	15	3	2	85%	39%
rmAb clone SP263, **790-4905/740-4907**	10	Ventana/Roche	-	-	1	9	0%	0%
rmAb clone SP142, **RMA-0724**	1	Fuzhou Maixin	-	-	-	1	-	-
rmAb clone MSVA-711R, **MSVA-711R**	1	MS Validated Antibodies	-	-	1	-	-	-
rmAb clone GR110, **GT256202**	1	Gene Tech	-	-	-	1	-	-
rmAb clone E1L3N **P06B01**	1	MEDx Translational Medicine	-	-	-	1	-	-
Total	69		30	19	6	14		
Proportion			43%	28%	9%	20%	71%	

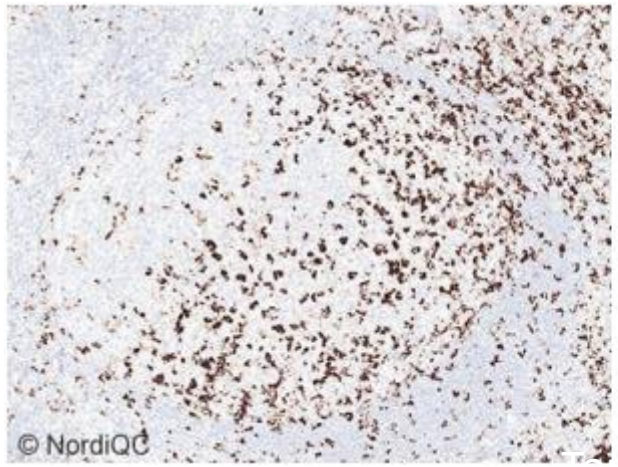
Characteristics of insufficient results in the NordiQC PD-L1 IC assessments.



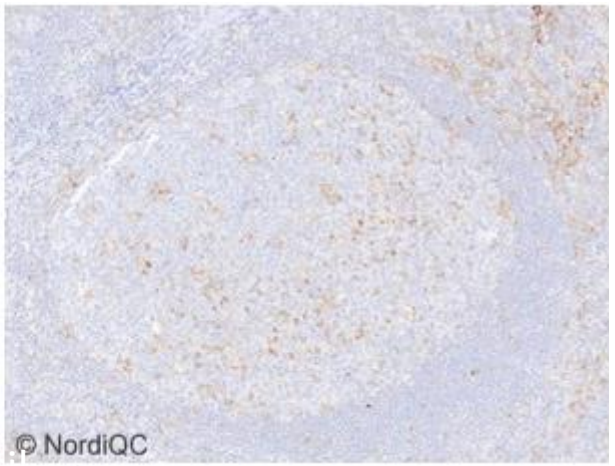
NordiQC

PD-L1 IC – ICAPS

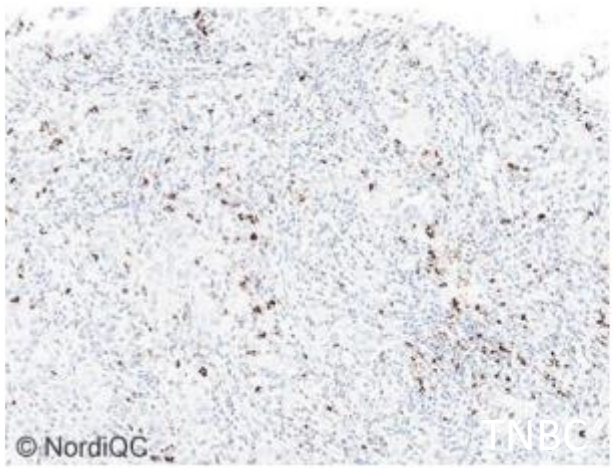
rmAb SP142



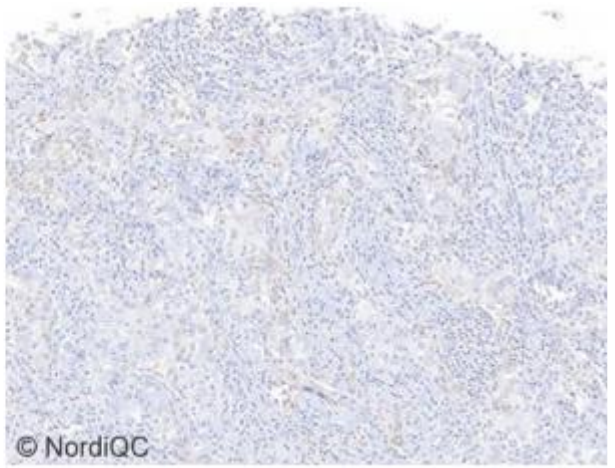
mAb 22C3

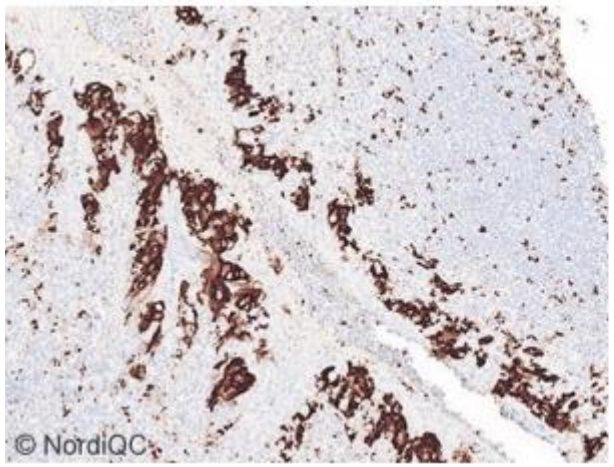


rmAb SP142

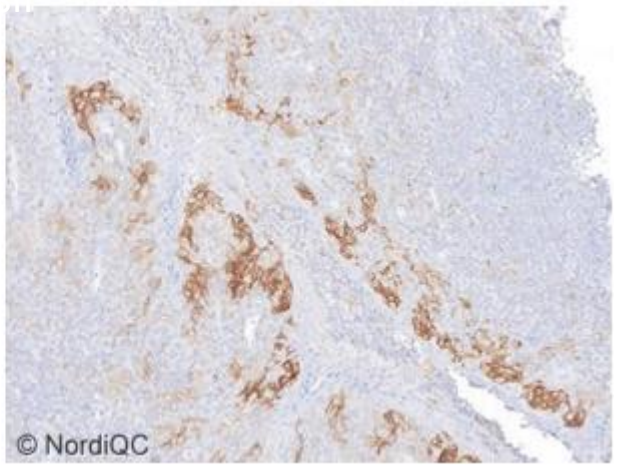


mAb 22C3

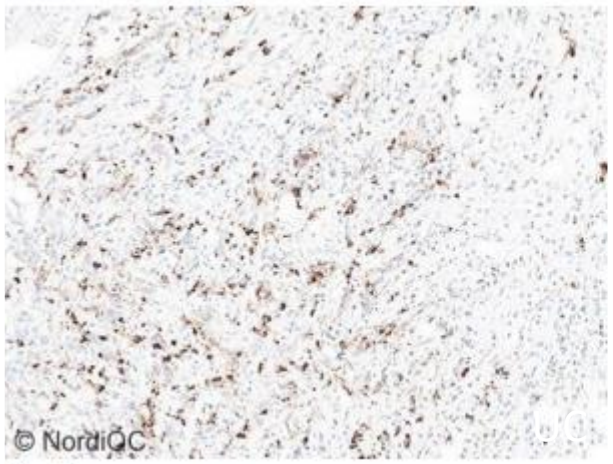




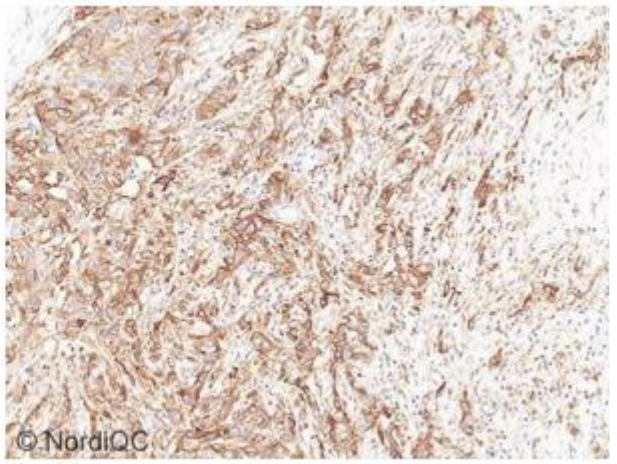
rmAb SP142



mAb 22C3



rmAb SP142



rmAb ZR3

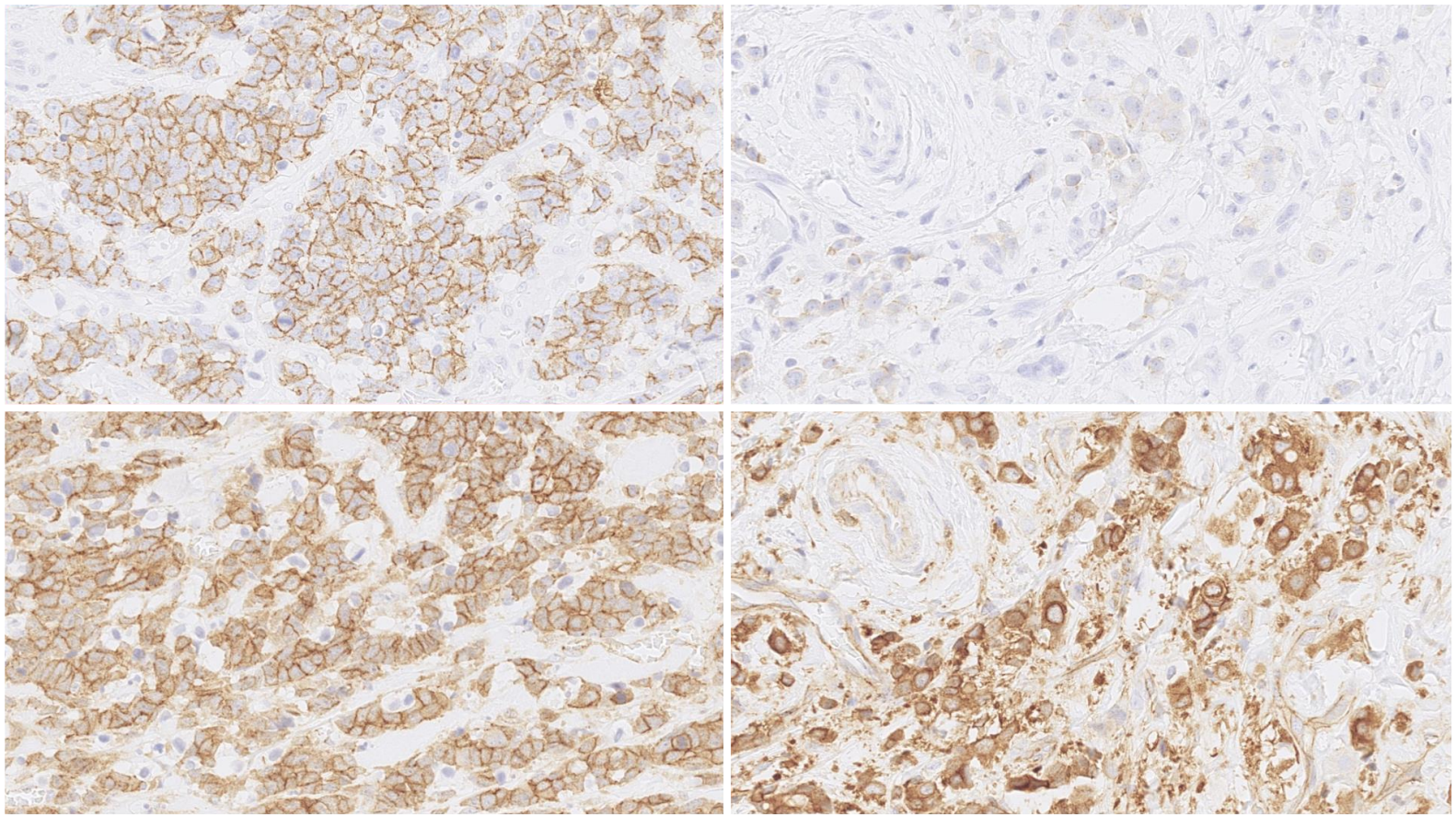
NordiQC

…YOU SURVIVED! THANK YOU FOR YOUR ATTENTION

NordiQC

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

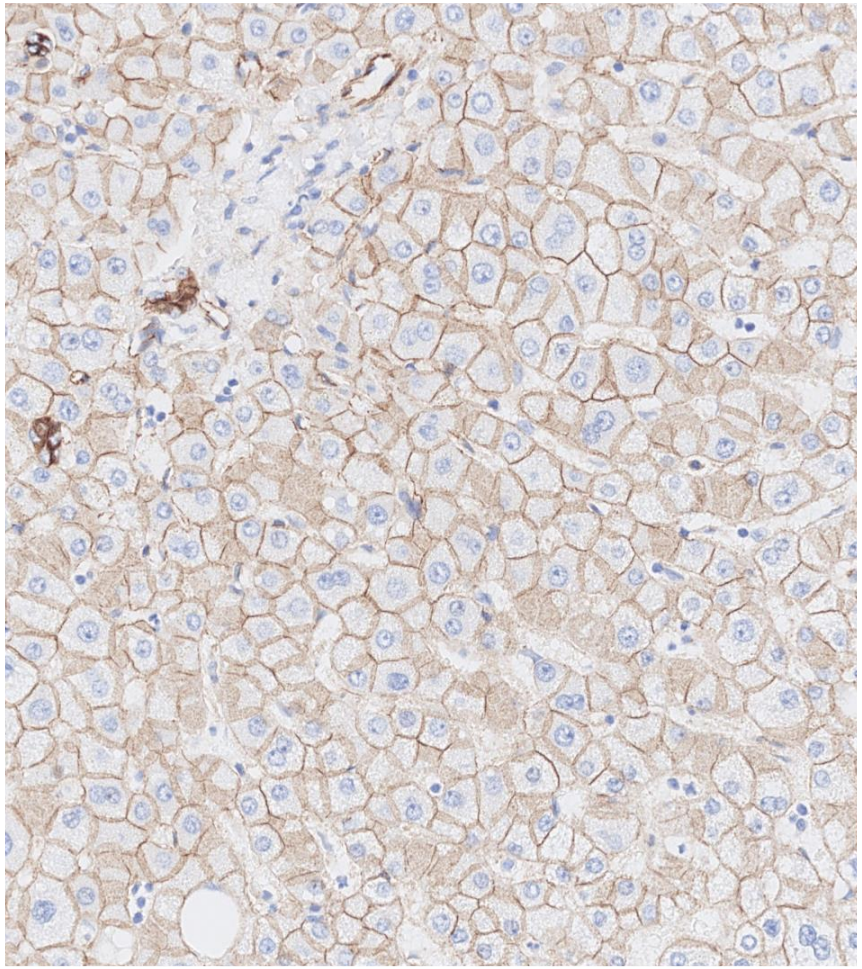
E-CAD: membranous staining reaction in (most) ductal breast carcinomas, negative in (most) lobular breast carcinomas.

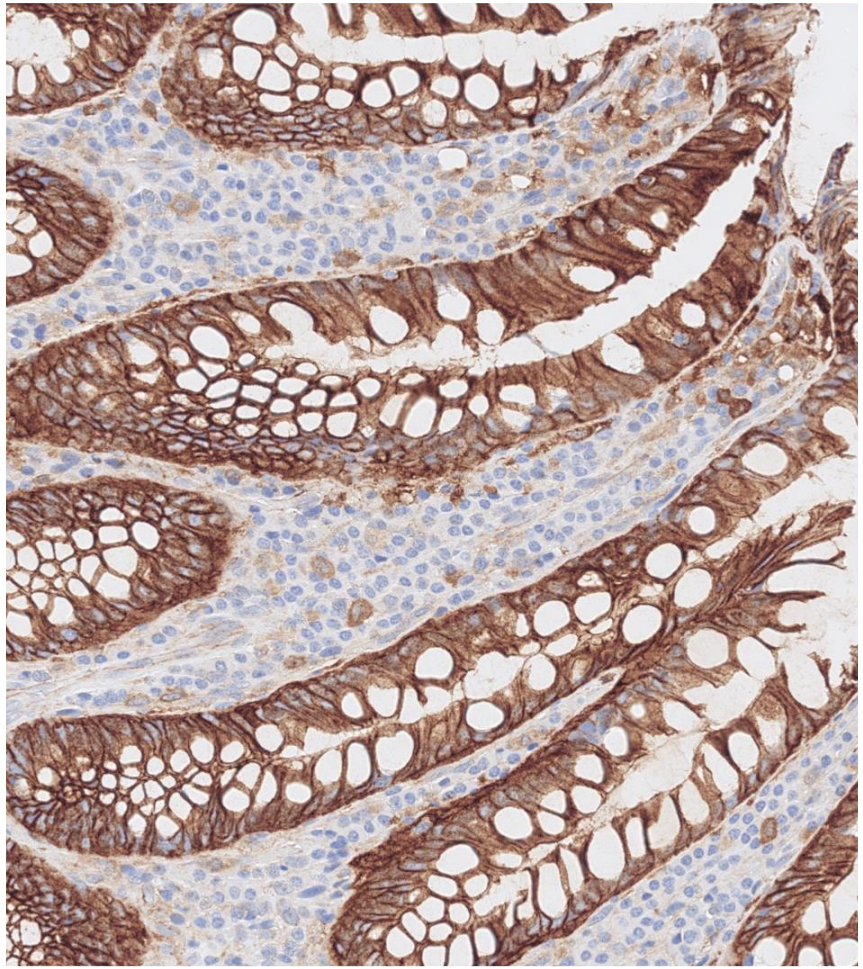
p120: membranous staining reaction in (most) ductal breast carcinomas, cytoplasmic staining reaction in (most) lobular breast carcinomas.

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.