NORDIQC DATA FOR BREAST MARKERS

Antibody selection, protocols and controls

NordiQC Seminar, September 17th - 19th 2025

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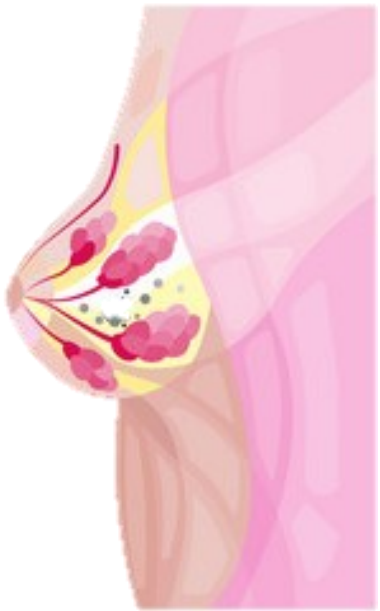
NORDIQC EQA DATA FOR IHC BREAST MARKERS

		Purpose	Last run	Pass rate	No of labs
Type I IHC tests	GATA3	Breast vs non-breast	Run 73, 2025	81%	422
	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 74, 2025	76%	430
	TRPS1	Breast vs non-breast	Run 72, 2024	73%	89
Type II IHC tests	KI67	PI index	Run 72, 2024	90%	443
	ER	Predictive for Tamoxifen	Run B39, 2025	77%	430
	PR	Predictive for Tamoxifen	Run B39, 2025	98%	427
	HER2 IHC	Predictive for Herceptin	Run B39, 2025	92%	418
	HER2 BRISH	Predictive for Herceptin	Run H27, 2025	85%	174
	PD-L1 IC	Predictive for Tecentriq	Run C16, 2024	71%	141
	PD-L1 TPS/CPS	Predictive for Keytruda	Run C17, 2025	76%	273



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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	mAb 36B5, rmAb EP700Y
TRPS1	rmAb ZR382	rmAb EP392, pAbs
KI67	mAbs MIB-1 & K2, rmAbs 30.9 & SP6	
ER	rmAbs SP1 & EP1, mAb 6F11	-
PR	mAbs 16 & PgR1294, rmAb 1E2	-
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*

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.
Mammaglobin	Skin: Epithelial cells of eccrine sweat glands.	Tonsil: All cell types.
GCDFP15	Skin: Epithelial cells of eccrine sweat glands.	Tonsil: All cell types.
CK5	Pancreas: Scattered epithelial cells of intercalated ducts.	Liver. All cell types.
SMH	Tonsil: Follicular dendritic cells in germinal centers.	Tonsil: Epithelial cells.
p63	Placenta: Cytotrophoblastic cells.	Appendix: Epithelial- and smooth muscle cells.
E-Cadherin	Liver: Hepatocytes.	Appendix: Stromal cells, smooth muscle cells, endothelial cells.
TRPS1	Cervix: Suprabasal squamous epithelial cells.	Appendix: Epithelial cells
KI67	Tonsil: B-cells in the light zones of the germinal centers.	Liver: Hepatocytes
ER	Tonsil: Squamous epithelial cells, T-cells in germinal centres.	Tonsil: B-cells in mantle zone and germinal centres.
PR	Cervix: Basal squamous epithelial cells.	Tonsil: All cells types (especially focus on lymphocytes in germinal centres).
PD-L1 IC	Tonsil: T-cells and macrophages in germinal centres.	Tonsil: Normal squamous epithelial cells, lymphocytes.
PD-L1 TPS/CPS	Tonsil: Germinal center macrophages and T-cells.	Tonsil: Stratified normal squamous epithelial cells and vast majority of lymphocytes.

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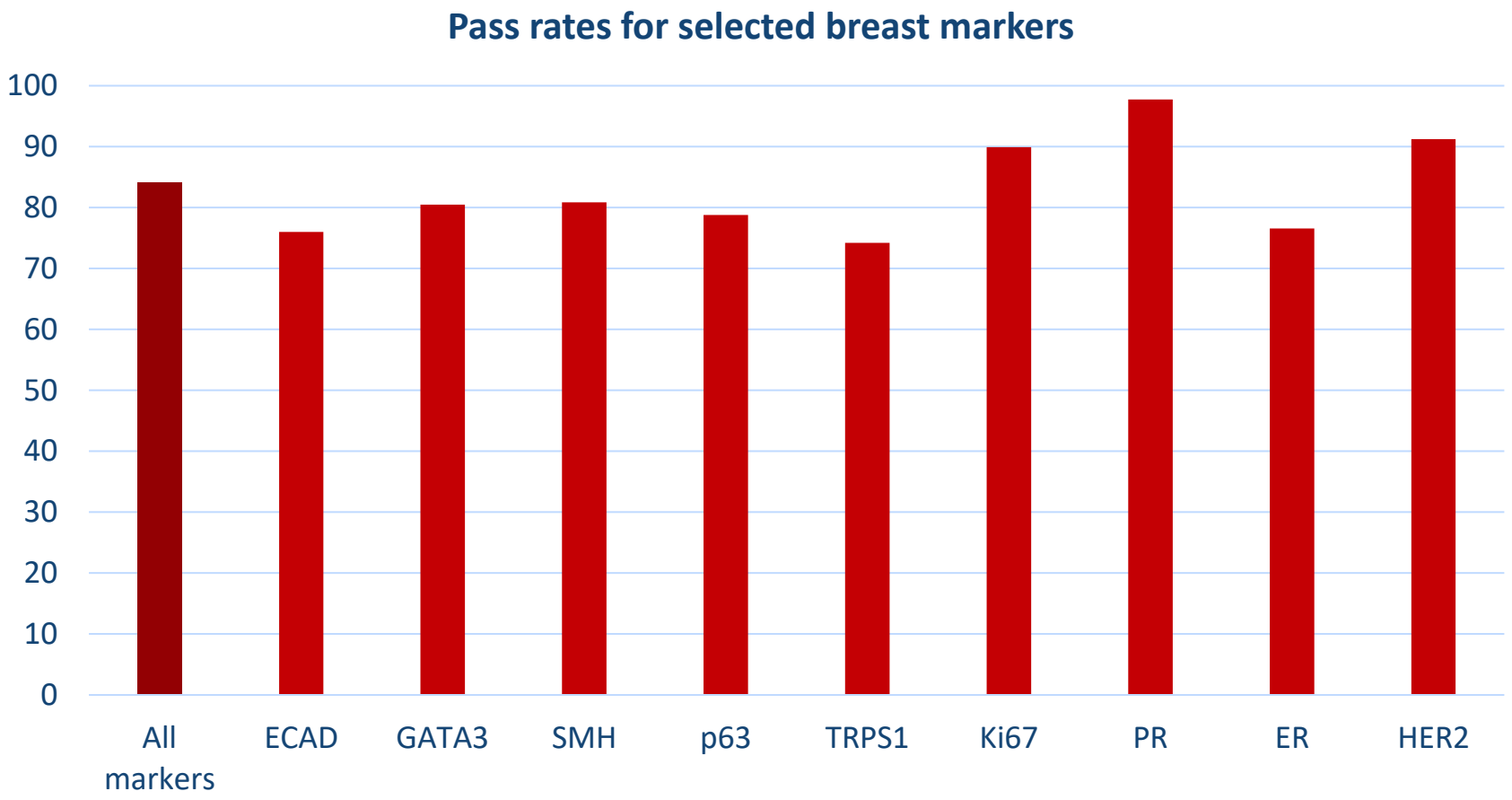
KEY-POINTS FOR BEST PROTOCOLS



Selected breast markers:

Overall pass rate: **84%** (2.763/3.287), ranging from 74% for TRPS1 till 98% for PR.

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KEY-POINTS FOR BEST PROTOCOLS

Breast markers:

Overall pass rate: **83%**

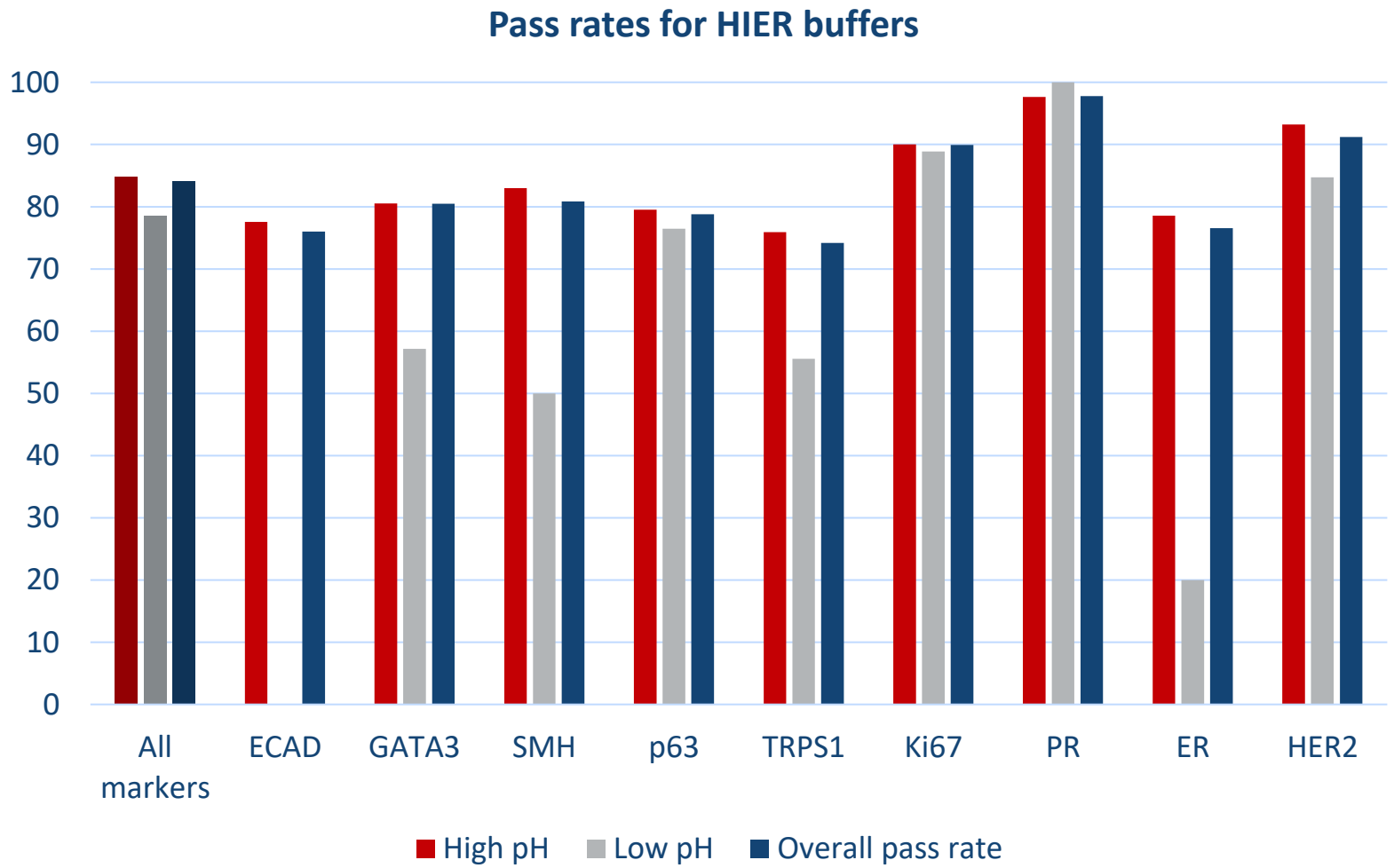
HIER in High pH: 84%

Ranging from 76% for TRPS1 till 98% for PR

HIER in Low pH: 72%

*Ranging from 0% for ECAD till 100% for PR**

**10/10 using BERS1*



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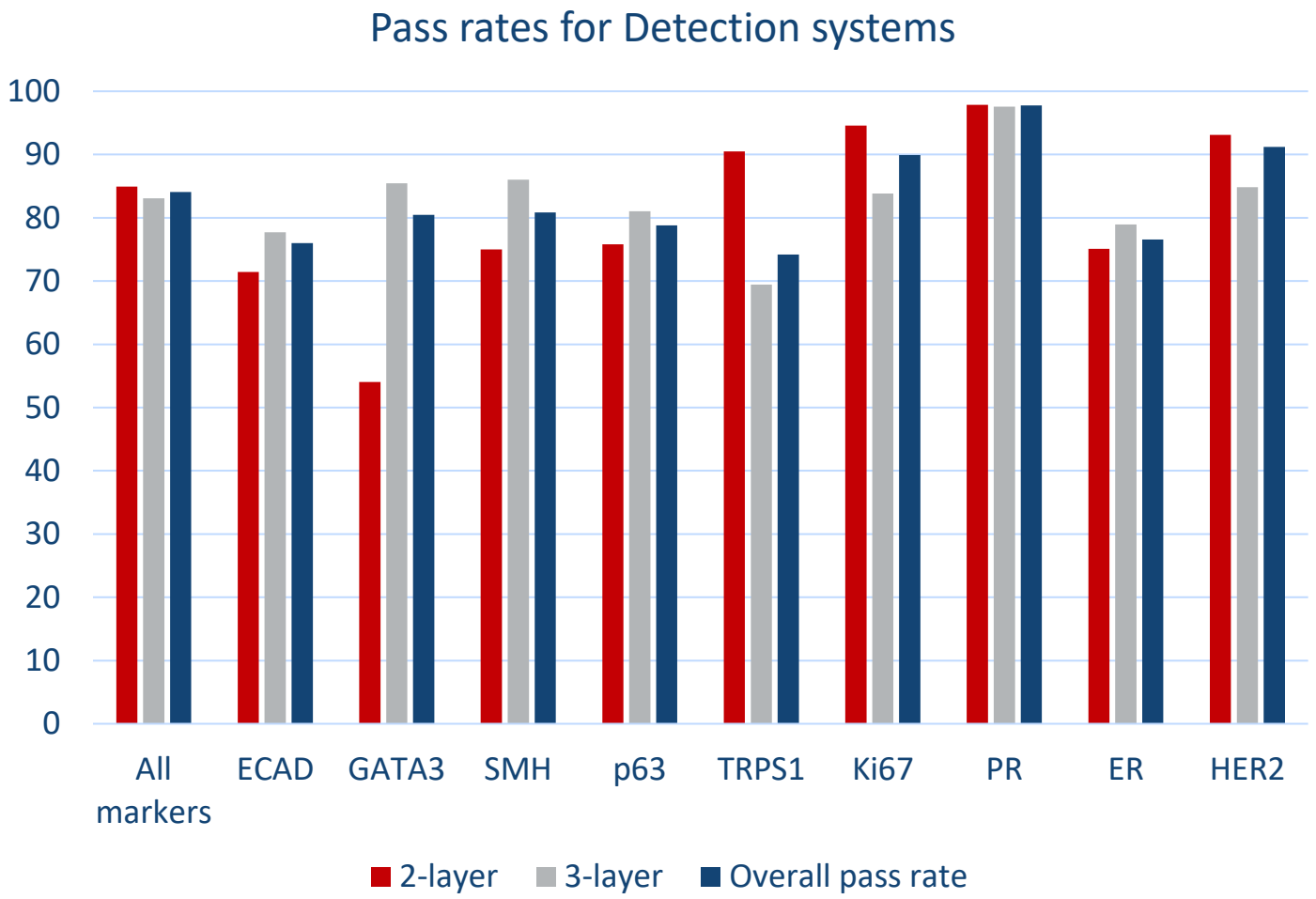
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 85%

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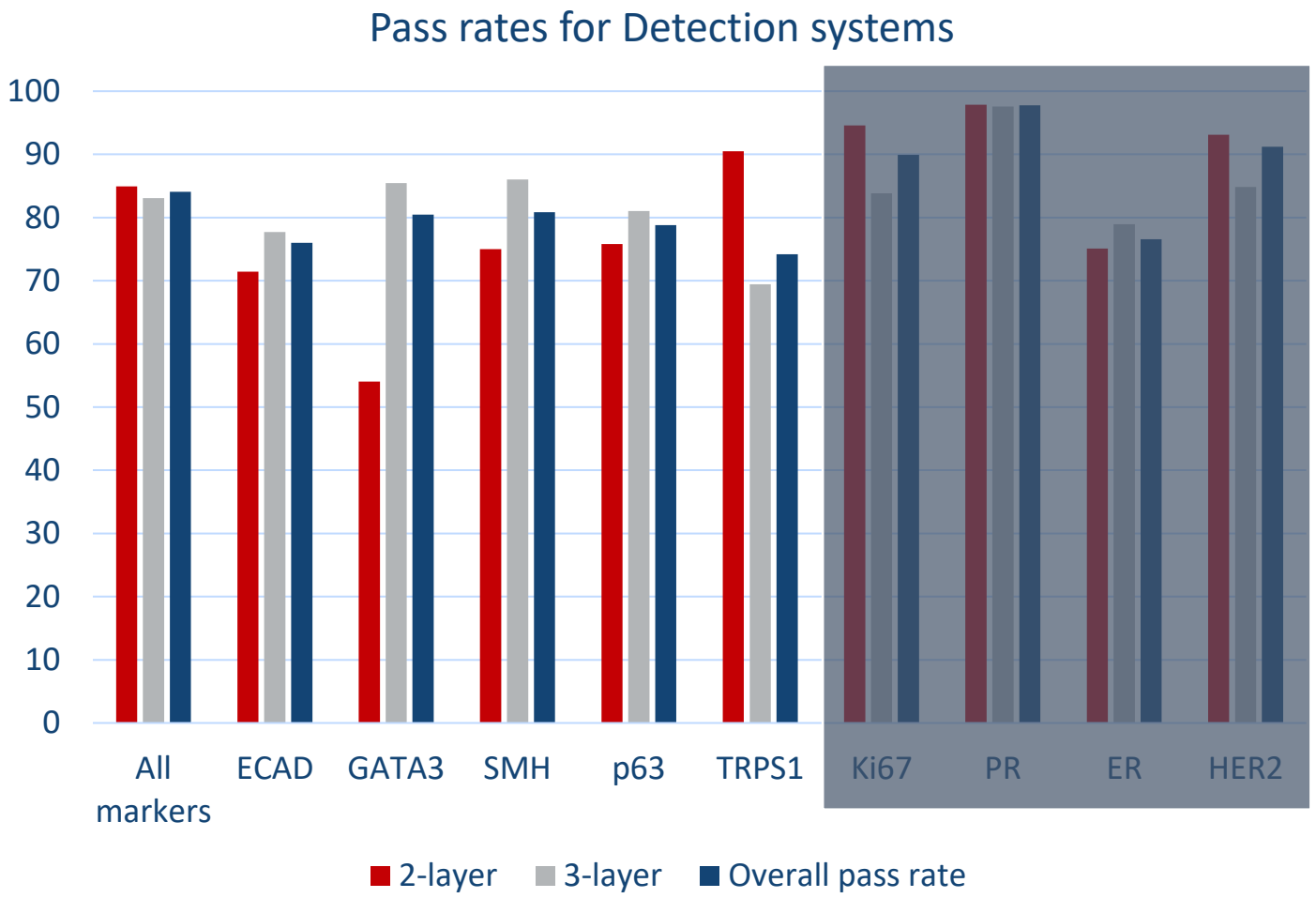
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 85%

Type I tests

3-layer detection system: 80%

2-layer detection system: 73%

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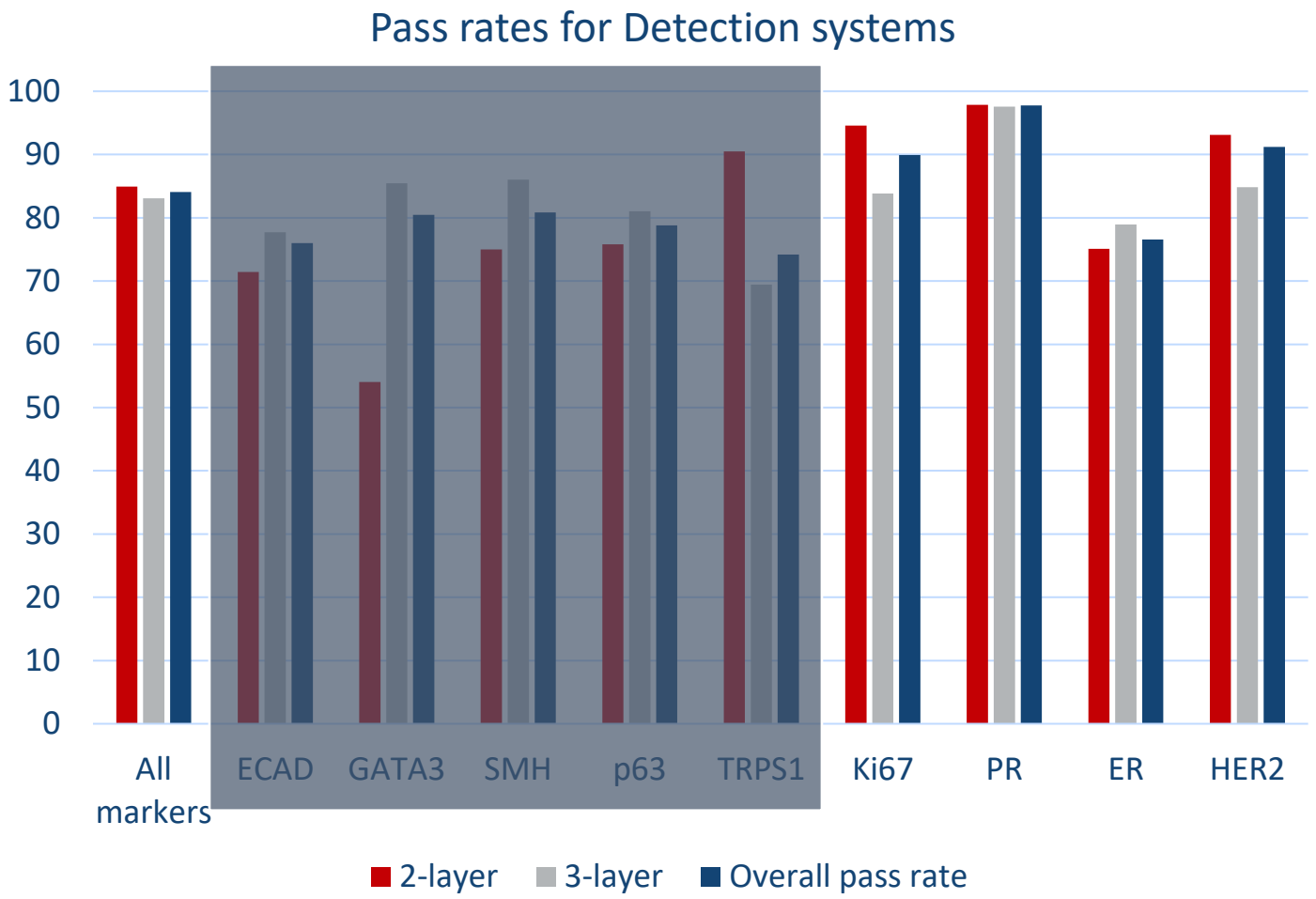
KEY-POINTS FOR BEST PROTOCOLS



Breast markers:

3-layer detection system: 83%

2-layer detection system: 85%

Type I tests

3-layer detection system: 80%

2-layer detection system: 73%

Type II test

3-layer detection system: 86%

2-layer detection system: 90%

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NOW IT'S TIME TO LOOK AT SOME SPECIFIC MARKERS

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TRPS1 – NEW MARKER IN NORDIQC

Table 1a. **Overall results for TRPS1, run 72**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	59	22	20	12	5	71%	37%
Ready-To-Use antibodies	30	13	10	2	5	76%	43%
Total	89	35	30	14	10		
Proportion		39%	34%	16%	11%	73%	

Table 1c. **Ready-To-Use antibodies and assessment marks for TRPS1, run 72**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP392** **BSB-3790-x**	7	Bio SB	1	1	1	4	29%	14%
rmAb clone **EPR16171** **GT245402**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **MXR063** **RMA-1141**	1	Fuzhou Maixin	-	1	-	-	-	-
rmAb clone **ZR382** **8359-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone **ZR382** **Z2673RP**	18	Zeta Corporation	9	8	-	1	94%	50%
Ab clone **506I3C7** **PA588**	1	abcarta	-	-	1	-	-	-
Ab clone **BY193** **BFM-0560**	1	Bioin Biotechnology	1	-	-	-	-	-
Total	30		13	10	2	5		
Proportion			43%	33%	7%	17%	76%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Sufficient results were all based on HIER in High pH buffer:
Autostainer 2/2, Omnis 2/2, Bond 2/2
BenchMark 11/12 (insuff due to contamination)

Table 2. **Proportion of optimal results for TRPS1 for the most commonly used antibody concentrates on the four main IHC systems***

Concentrated antibodies	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
rmAb clone **EP392**	2/3**	-	4/6 (67%)	-	0/2	-	1/7 (14%)	-
rmAb clone **ZR382**	-	-	1/1	-	10/13 (77%)	-	1/2	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 RTUs from the main vendors.
The most widely used conc. Abs could provide optimal results on the main platforms.

The RTU from Zeta Corp., was also successful on all main platforms.

GATA3 – POINTS OF ATTENTION

Table 1a. **Overall results for GATA3, run 73**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	133	60	38	25	10	74%	45%
Ready-To-Use antibodies	289	168	75	44	2	84%	58%
Total	422	228	113	69	12		
Proportion		54%	27%	16%	3%	81%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for GATA3, run 73**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823**	81	Cell Marque	40	22	16	3	75%	44%
	18	BioCare	6	5	5	2		
	15	Diagnostic Biosystem	6	6	-	3		
	4	Bio SB	1	2	1	-		
	1	BD Pharmingen	-	-	1	-		
	1	Zytomed Systems	-	1	-	-		
	1	Gennova	-	1	-	-		
	1	Immunologic	1	-	-	-		
	1	Master Diagnostica	-	1	-	-		
	1	Nordic Biosite	1	-	-	-		
mAb clone **HG3-31**	2	Santa Cruz	-	-	1	1	-	-
rmAb clone **EP368**	3	Cell Marque	4	-	-	-	100%	100%
	1	Epitomic						
rmAb clone **ZR358**	2	Zeta Corporation	-	-	1	1	-	-
rmAb clone **QR018**	1	Quartett	1	-	-	-	-	-
Total	133		60	38	25	10		
Proportion			45%	28%	19%	8%	74%	

Table 1c. **Ready-To-Use antibodies and assessment marks for GATA3, run 73**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L50-823 760-4897 (VRPS)**[3]	25	Ventana/Roche **UltraView**, 760-500	-	13	12	-	52%	0%
mAb clone **L50-823 760-4897 (VRPS)**[3]	58	Ventana/Roche **OptiView**, 760-700	48	9	1	-	98%	83%
mAb clone **L50-823 760-4897 (LMPS)**[4]	33	Ventana/Roche **UltraView**	12	14	7	-	79%	36%
mAb clone **L50-823 760-4897(LMPS)**[4]	73	Ventana/Roche **OptiView**	53	14	5	1	92%	73%
mAb clone **L50-823 760-4897**[5]	8	Ventana/Roche Other	5	2	1	-	88%	63%
mAb clone **L50-823 390M-17,-18,-10**	59	Cell Marque	37	14	8	-	86%	63%
mAb clone **L50-823 PM 405AA**	13	BioCare Medical	8	3	2	-	85%	62%

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**	3/10** (30%)	-	23/44 (52%)	1/3	23/38 (61%)	-	5/26 (19%)	-
rmAb clone **EP368**	1/1	-	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 titre differs between vendors

Mean titre for sufficient results
Biocare: 1:100
Cell Marque: 1:175

Recommended protocol settings for L50-823 as a concentrate:

- HIER in an alkaline buffer
- 22% pass rate for 2-step detection systems (2/9)
- 78% pass rate for 3-step detection systems (90/115)

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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 d… incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval durati… ation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrat…

Most common and successful modifications:
Prolong HIER and use of OptiView

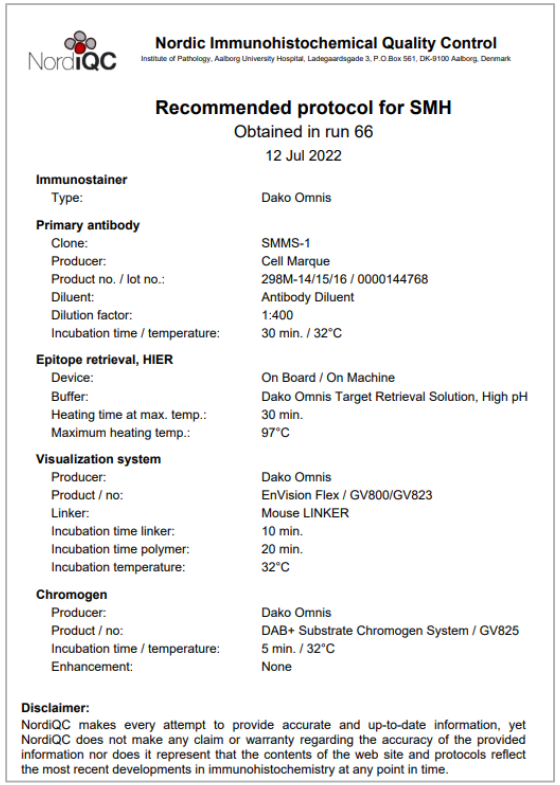
Table 2. **Proportion of optimal results for SMH for the most commonly used antibody as concentrate on the 4 main IHC systems***

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:
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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.
In run 48 (2016), 2/8 obtained an optimal result with 4A4.

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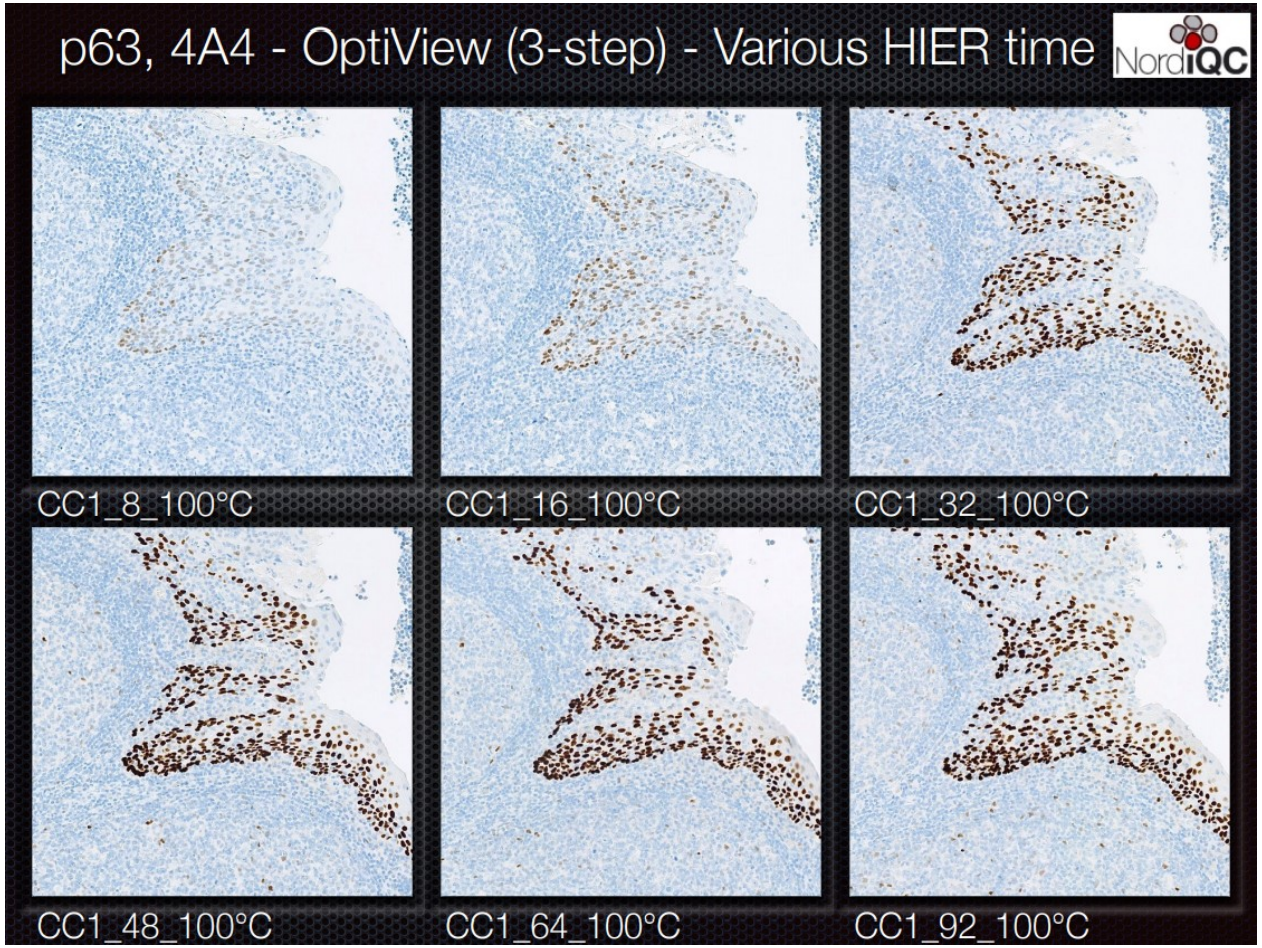
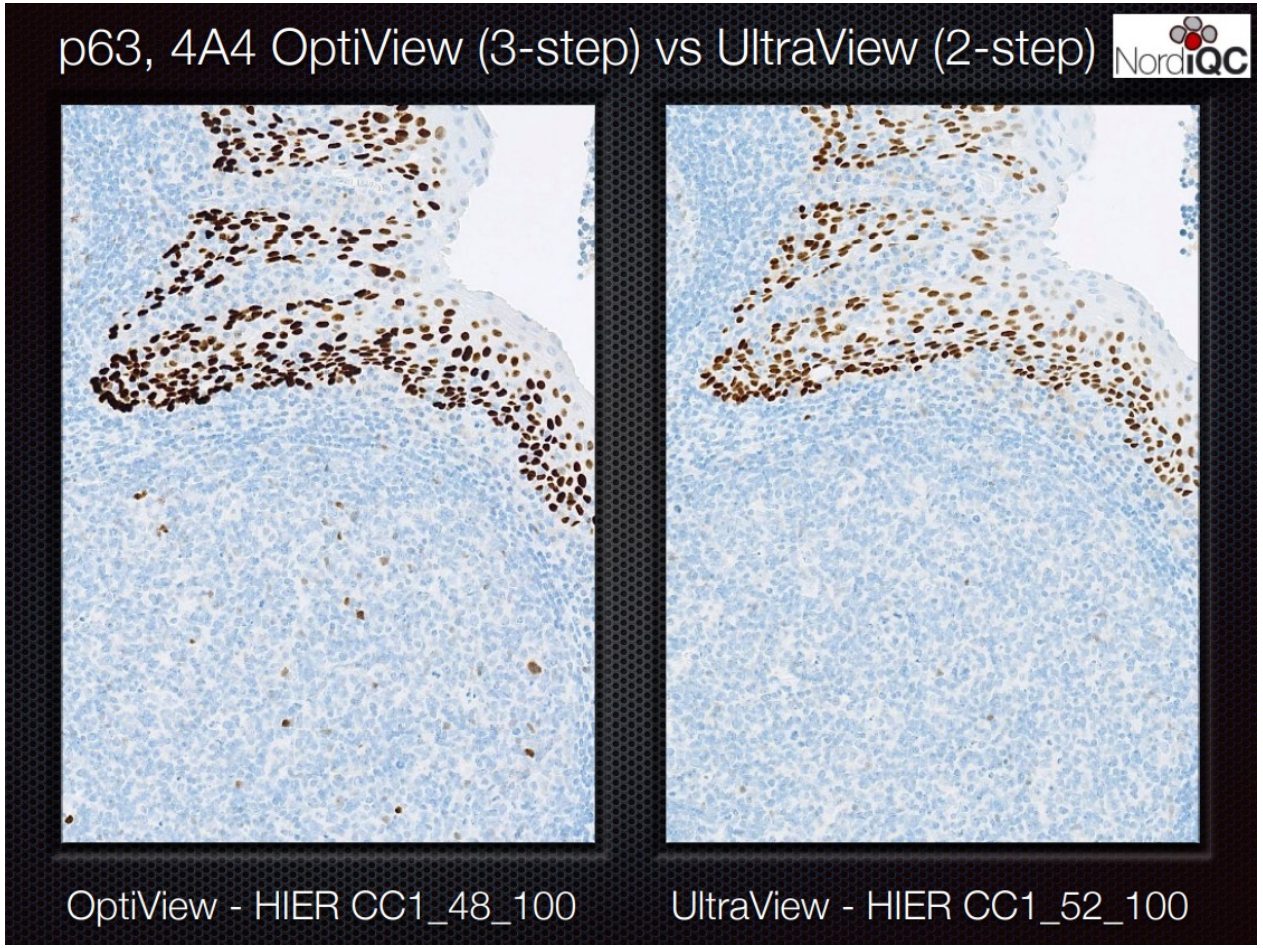
P63 - POINTS OF ATTENTION





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

ECAD - POINTS OF ATTENTION

Table 1c. **Ready-To-Use antibodies and assessment marks for ECAD, run 74**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **36 790-4497 (VRPS)**[3]	34	Roche/Ventana	25	8	1	0	97%	71%
mAb clone **36 790-4497 (LMPS)**[4]	105	Roche/Ventana	64	28	11	2	88%	61%
mAb clone **ZM63 8269-C010**	3	Sakura Finetek	3	0	0	0	-	-
mAb clone **NCH-38 GA059 (VRPS)**[3]	61	Dako/Agilent	56	3	1	1	97%	92%
mAb clone **NCH-38 GA059 (LMPS)**[4]	39	Dako/Agilent	36	3	0	0	100%	92%
mAb clone **NCH-38 IS/IR059 (VRPS)**[3]	11	Dako/Agilent	9	2	0	0	100%	82%
mAb clone **NCH-38 IS/IR059 (LMPS)**[4]	13	Dako/Agilent	9	3	1	0	92%	69%
mAb clone **MX020 MAB-0738**	1	Fuzhou Maixin	0	1	0	0	-	-
mAb clone **SPM471 PDM182**	1	Diagnostic Biosystems	1	0	0	0	-	-
mAb clone **HECD-1 MAD-000761QD**	1	Master Diagnostica	0	0	1	0	-	-
mAb clone **35B5 PA0387 (VRPS)**[3]	22	Leica Biosystems	0	1	21	0	5%	0%
mAb clone **35B5 PA0387 (LMPS)**[4]	9	Leica Biosystems	0	0	9	0	0%	0%
mAb clone **EP6 API3012**	1	Biocare Medical	0	0	1	0	-	-
mAb clone **IHC564 IHC564**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **EP700Y 760-4440 (VRPS)**[3]	7	Roche/Ventana	0	0	7	0	0%	0%
rmAb clone **EP700Y 760-4440 (LMPS)**[4]	19	Roche/Ventana	2	1	15	1	16%	11%
rmAb clone **EP700Y 246R-18**	2	Cell Marque	0	0	1	1	-	-
Ab clone **499C4F1 PA073**	1	Abcarta	1	0	0	0	-	-
Ab clone **BY149 BFM-0155**	1	Bioin Biotechnology	1	0	0	0	-	-
Ab clone **C12A15 CER-0022**	1	Celnovte	1	0	0	0	-	-
Ab clone **GR111 GT2107**	1	Gene Tech	0	0	1	0	-	-
Total	334		208	51	70	5		
Proportion			62%	15%	21%	2%	77%	

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

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

Concentrated antibodies	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 **NCH-38**	¾**	-	6/6 (100%)	-	22/40 (55%)	-	7/16 (44%)	-

* 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

Leica users can choose NCH-38, with HIER in BERS2 → 100% pass rate (16/16)

Table 3. **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% (11/11)	82% (9/11)	83% (5/6)	67% (4/6)
Dako Omnis mAb NCH-38 **GA059**	97% (59/61)	92% (56/61)	100% (33/33)	94% (31/33)
VMS XT/GX/Ultra/Ultra Plus mAb 36 **790-4497**	97% (33/34)	74% (25/34)	88% (91/103)	62% (64/103)
VMS Ultra/Ultra Plus rmAb EP700Y **760-4440**	0/7 (0%)	0/7 (0%)	3/19 (16%)	2/19 (11%)
Leica Bond III/Prime mAb 36B5 **PA0387**	1/22 (5%)	0/22 (0%)	0/8 (0%)	0/8 (0%)

RTU assays work as "plug-and-play" products.

– except assays based on EP700Y and 36B5

NordiQC

KI67 - POINTS OF ATTENTION

Table 1a. **Overall results for Ki67, run 72**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	106	63	25	16	2	83%	59%
Ready-To-Use antibodies	337	241	70	22	4	92%	71%
Total	443	304	95	38	6		
Proportion		69%	21%	9%	1%	90%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 2. **Proportion of optimal results for Ki67 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibodies	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 **MIB-1**	2/2**	-	4/4	-	33/47 (70%)	-	6/19 (32%)	-
rmAb clone **SP6**	1/1	-	1/1	-	4/4	-	5/8 (63%)	-

* 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)

Table 3. **Proportion of sufficient and optimal results for Ki67 in the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako Autostainer mAb MIB-1 **IR/IS626**	(4/4)	(4/4)	91% (10/11)	64% (7/11)
Dako Omnis mAb MIB-1 **GA626**	90% (57/63)	46% (29/63)	88% (23/26)	77% (20/26)
Leica Bond mAb MM1 **PA0118/PA0410**	22% (2/9)	0% (0/9)	80% (4/5)	0% (0/5)
Leica Bond mAb K2 **PA0230**	(4/4)	(3/4)	(4/4)	(3/4)
Ventana BenchMark rmAb 30.9 **790-4286 UltraView**	100% (49/49)	94% (46/49)	99% (71/72)	85% (61/72)
Ventana BenchMark rmAb 30.9 **790-4286 OptiView**	88% (7/8)	63% (5/8)	96% (47/49)	88% (43/49)

* 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 were included.

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*†*

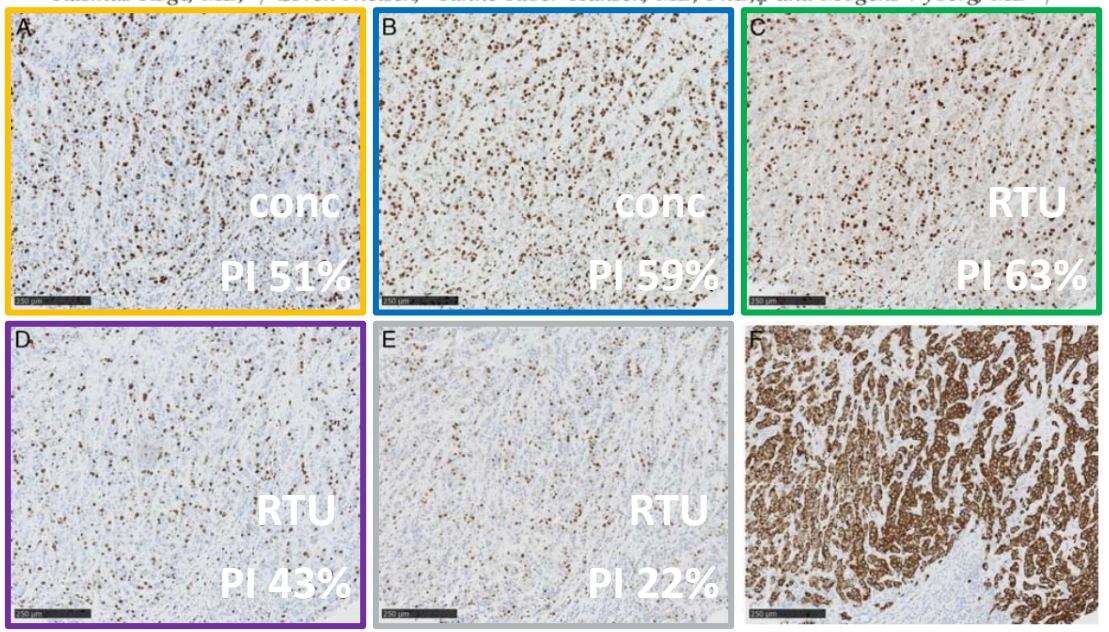


TABLE 2. Immunohistochemical Staining Protocols

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.

ER – POINTS OF ATTENTION

Table 1a. **Overall results for ER, run B39**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	30	5	15	4	6	67%	17%
Ready-To-Use antibodies	400	104	205	72	19	77%	26%
Total	430	109	220	76	25		
Proportion		25%	51%	18%	6%	77%	

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	0/2	13/16 (81%)	5/16 (31%)
Dako Omnis rmAb EP1 **GA084**	39/42 (93%)	18/42 (43%)	24/27 (89%)	10/27 (37%)
Leica Bond III/Prime mAb 6F11 **PA0009/PA0151**	0/3	0/3	9/16 (56%)	2/16 (13%)
VMS Ultra/XT/Ultra Plus rmAb SP1 **790-4324/4325**	57/75 (76%)	8/75 (11%)	135/175 (77%)	49/175 (28%)

* 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.

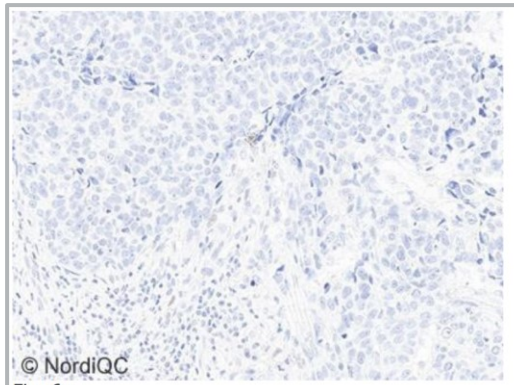


Fig. 6a
Optimal ER staining of the breast carcinoma no. 5 expected to be negative using same protocol as in Figs. 1a – 5a. No nuclear staining reaction is seen and a high signal-to-noise ratio is observed.

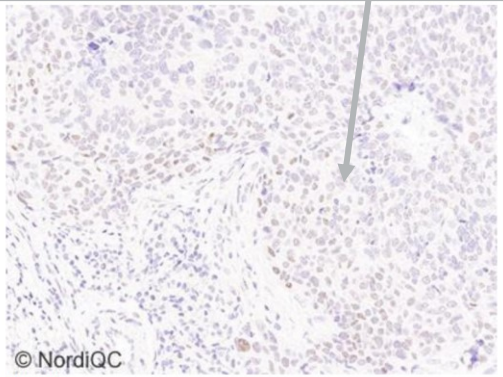


Fig. 6b
Insufficient ER staining of the breast carcinoma no 5 with no ER expression. A weak but distinct nuclear staining reaction is seen in >10% of the neoplastic cells. The insufficient result was only seen for the mAb clone 6F11 and likely was caused by performing HIER in an alkaline buffer in combination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

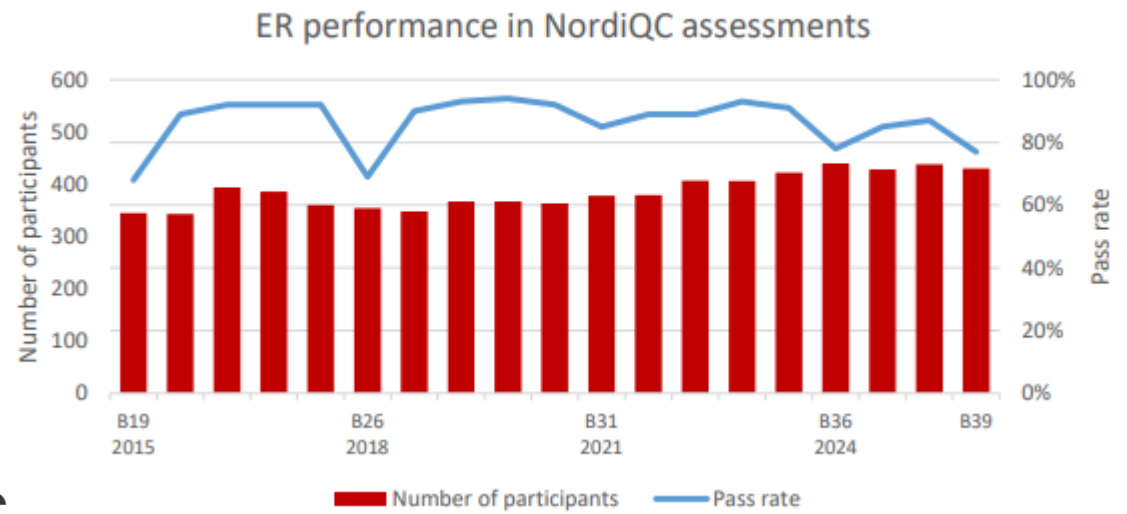
Photos from run B35

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 - 2025**



PR

Table 1a. **Overall results for PR, run B39**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	41	31	9	-	1	98%	76%
Ready-To-Use antibodies	386	298	80	8	-	98%	77%
Total	427	329	89	8	1	-	
Proportion		77%	21%	1,8%	0,2%	98%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1c. **Ready-To-Use antibodies and assessment marks for PR, run B39**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **1E2 790-2223/4296 (VRPS[3])**	91	Ventana/Roche	73	17	1	-	99%	80%
rmAb clone **1E2 790-2223/4296 (LMPS[4])**	153	Ventana/Roche	116	32	5	-	97%	76%
mAb **PgR 1294 GA090 (VRPS[3])**	40	Dako/Agilent	32	8	-	-	100%	80%
mAb **PgR 1294 GA090 (LMPS[4])**	36	Dako/Agilent	25	10	1	-	97%	69%
mAb **PgR 636 IR/IS068 (VRPS[3])**	10	Dako/Agilent	10	-	-	-	100%	100%
mAb **PgR 636 IR/IS068 (LMPS[4])**	7	Dako/Agilent	3	4	-	-	100%	43%
mAb clone **16 PA0312 (VRPS[3])**	21	Leica Biosystems	19	2	-	-	100%	90%
mAb clone **16 PA0312 (LMPS[4])**	14	Leica Biosystems	13	1	-	-	100%	93%
mAb clone **PgR 636 PM343**	1	Biocare Medical	-	1	-	-	-	-
rmAb clone **YR85 8360-C010**	2	Sakura Finetek	2	-	-	-	-	-
mAb clone **16 MAD-000670QD**	3	Master Diagnostica/Vitro	1	1	1	-	-	-
mAb clone **MXR008 MAB-0854**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **278G8D6 PA246**	1	Abcarta	1	-	-	-	-	-
rmAb clone **QR014 P006-30**	1	Quartett	-	1	-	-	-	-
rmAb clone **EP2 AN711-5M**	1	BioGenex	1	-	-	-	-	-
rmAb clone **SP2 GT205702**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **SP2 RMPD002**	1	Diagnostic Biosystems	-	1	-	-	-	-
Ab clone **DY49836 4911422**	1	Dakewe	-	1	-	-	-	-
Ab clone **MSVA-570 MAD-001370QD**	1	Master Diagnostica	-	1	-	-	-	-
Total	386		298	80	8	-	-	
Proportion			77%	21%	2%	0%	98%	

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

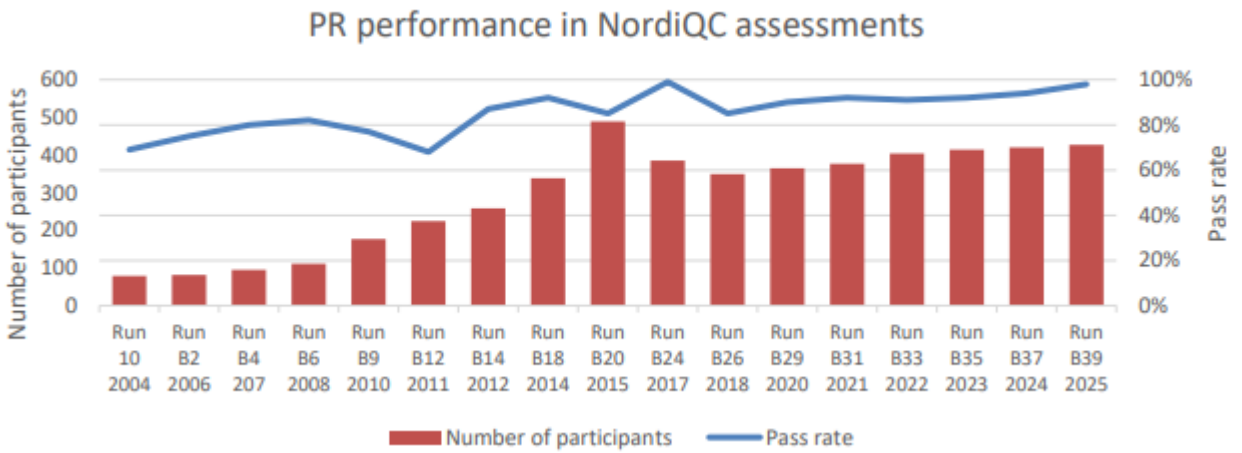


Table 3. **Proportion of sufficient and optimal results for PR for the most commonly used RTU IHC systems**

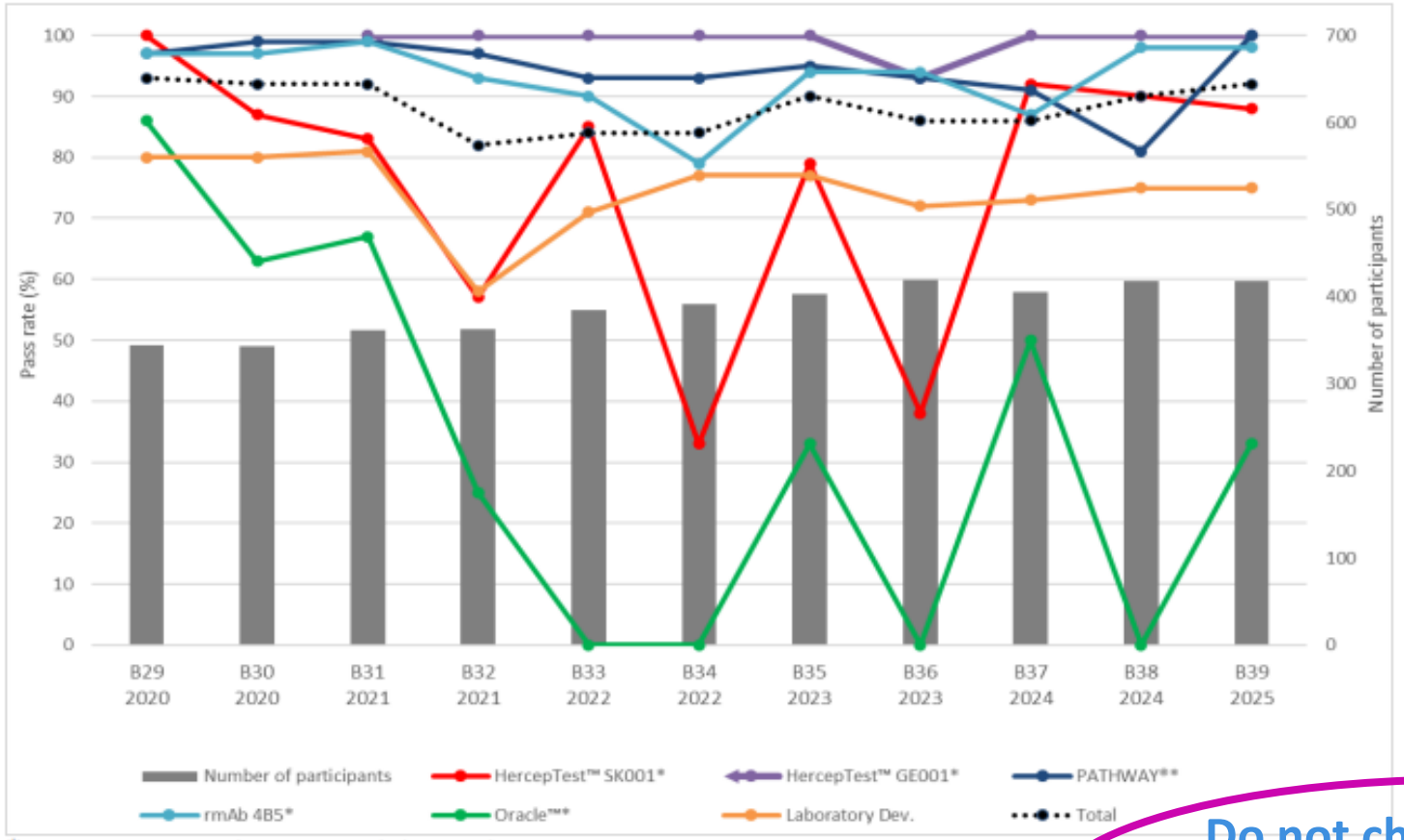
RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark rmAb 1E2 **790-2223/790-4296**	99% (90/91)	80% (73/91)	97% (148/153)	76% (116/153)
Dako Omnis mAb PgR 1294 **GA090**	100% (40/40)	80% (32/40)	97% (34/35)	71% (25/35)
Dako Autotstainer mAb PgR 636 **IR068/IS068**	100% (10/10)	100% (10/10)	100% (6/6)	33% (2/6)
Leica Bond mAb 16 **PA0312**	100% (21/21)	90% (19/21)	100% (12/12)	92% (11/12)

* 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.

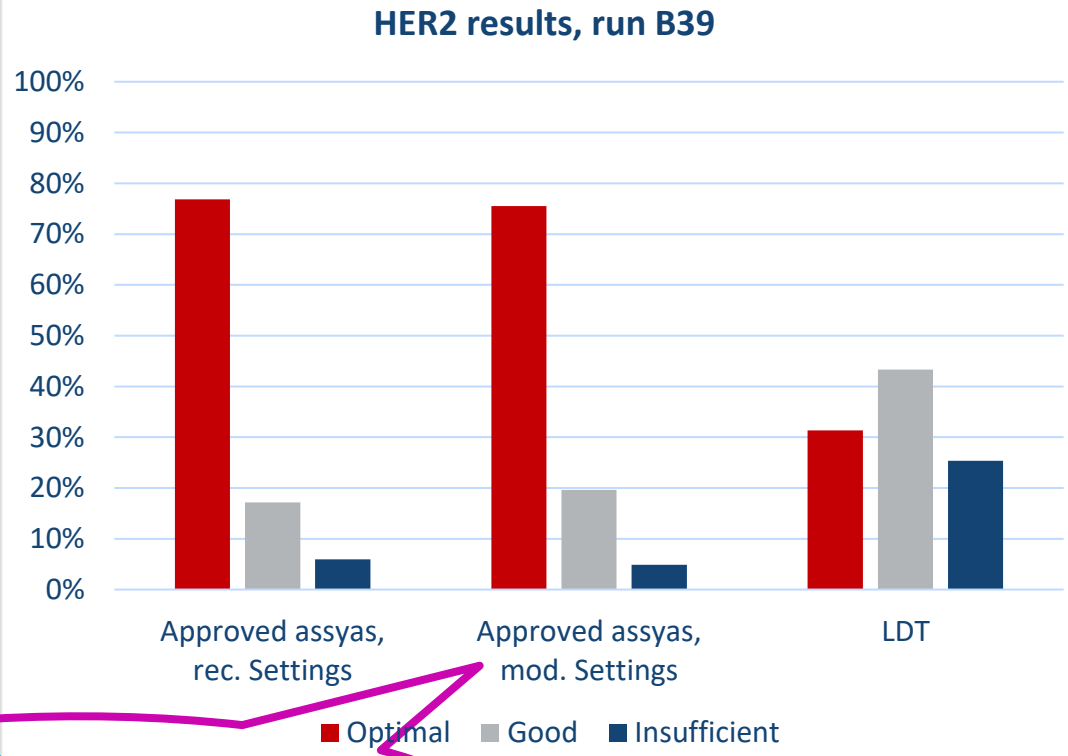
NordiQC

HER2 – POINTS OF ATTENTION

Graph 1. **Pass rates of the HER2 IHC assessments in the NordiQC breast cancer module 2020-2025**



* pass rates using vendor recommended protocol settings



Do not change to a more sensitive detection system!

PD-L1 IC – POINTS OF ATTENTION

Table 2a. **Overall results for PD-L1 IC, run C16**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved PD-L1 assays	68	39	11	9	9	74%	58%
Antibodies for laboratory developed PD-L1 assays, based on concentrated antibodies	9	0	1	5	3	11%	0%
Ready-To-Use antibodies	64	32	17	9	6	77%	50%
Total	141	71	29	23	18		
Proportion		50%	21%	16%	13%	71%	

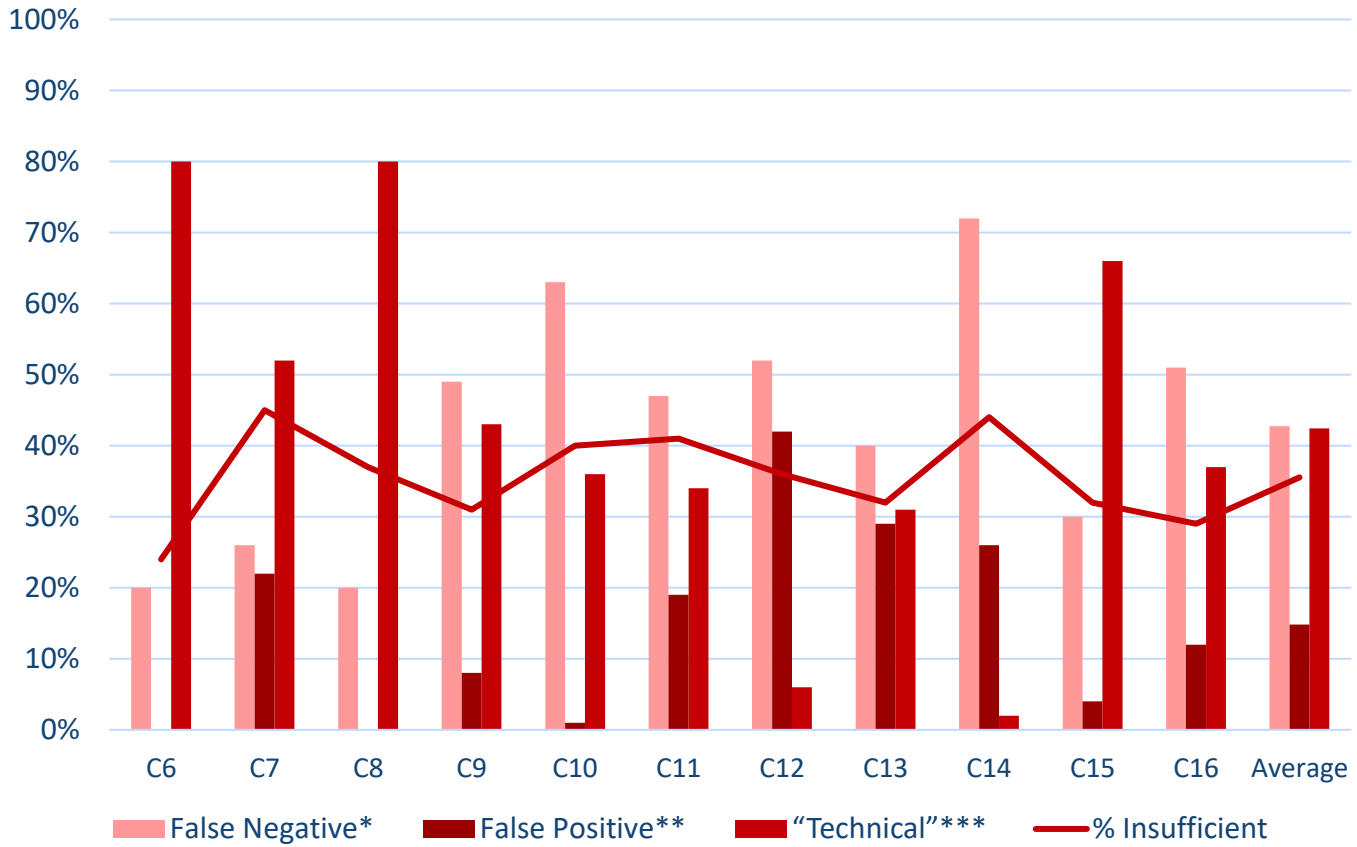
Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 IC, run C16**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **741-4860**[3]	54	Ventana/Roche	39	10	2	3	91%	72%
rmAb clone SP263, **741-4905**[3]	8	Ventana/Roche	0	1	3	4	13%	0%
rmAb clone 28-8 pharmDX, **SK005**	1	Dako/Agilent	0	0	1	0	-	-
mAb clone 22C3 pharmDX, **SK006**	1	Dako/Agilent	0	0	0	1	-	-
mAb clone 22C3 pharmDX, **GE006**	4	Dako/Agilent	0	0	3	1	-	-
Total	68		39	11	9	9		
Proportion			58%	16%	13%	13%	74%	

Table 2d. **Assessment marks for Ready-To-Use antibodies[8] for PD-L1 IC, run C16**

Ready-To-Use antibodies[8]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP142, **790-4860 (VRPS)**[5]	20	Ventana/Roche	14	3	2	1	85%	70%
rmAb clone SP142, **790-4860 (LMPS)**[6]	38	Ventana/Roche	18	13	4	3	82%	47%
rmAb clone SP263, **790-4905/740-4907**	3	Ventana/Roche	0	0	3	0	-	-
rmAb clone SP263, **790-4905**[4]	1	Ventana/Roche	0	0	0	1	-	-
rmAb clone SP142, **RMA-0724**	1	Fuzhou Maixin	0	1	0	0	-	-
rmAb clone **CAL10**, **API3171**	1	Biocare Medical	0	0	0	1	-	-
Total	64		32	17	9	6		
Proportion			50%	27%	14%	9%	77%	

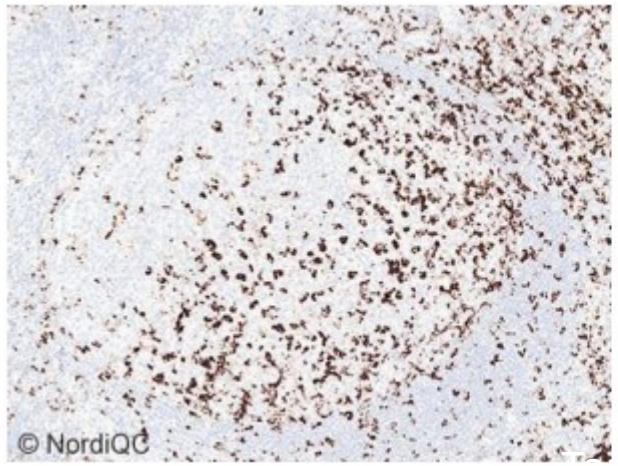
Characteristics of insufficient results in the NordiQC PD-L1 IC assessments.

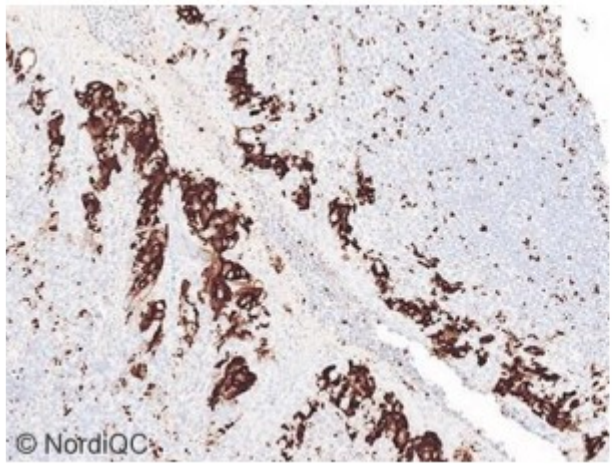


NordiQC

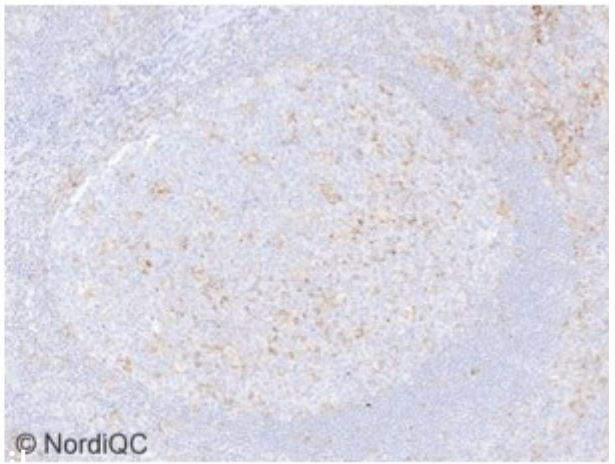
PD-L1 IC – ICAPS

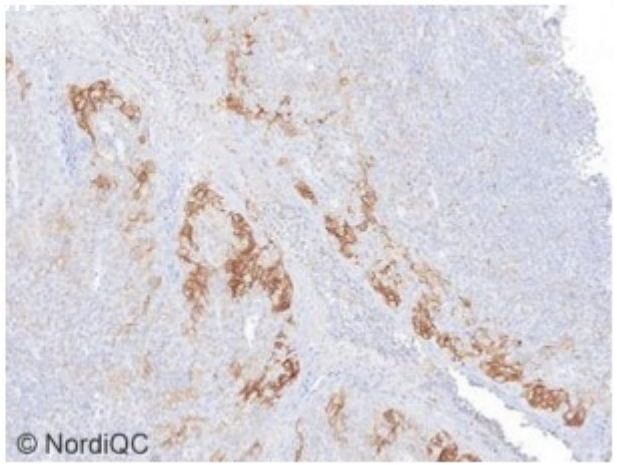
rmAb SP142



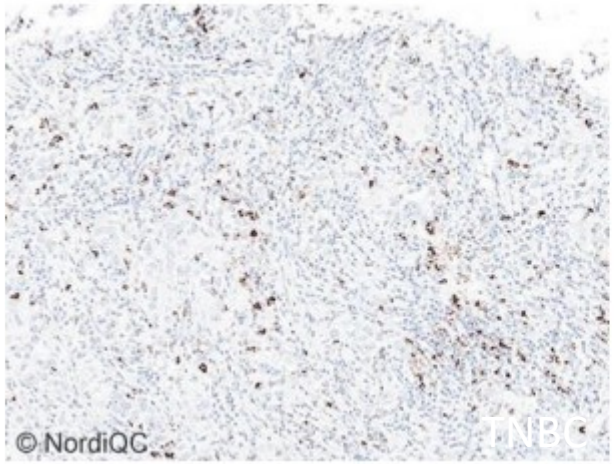


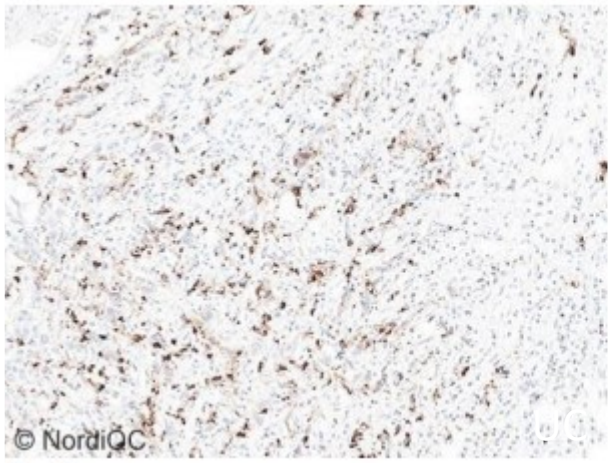
mAb 22C3



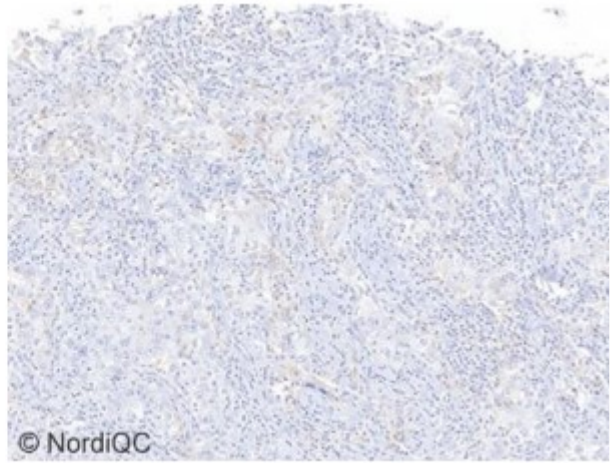


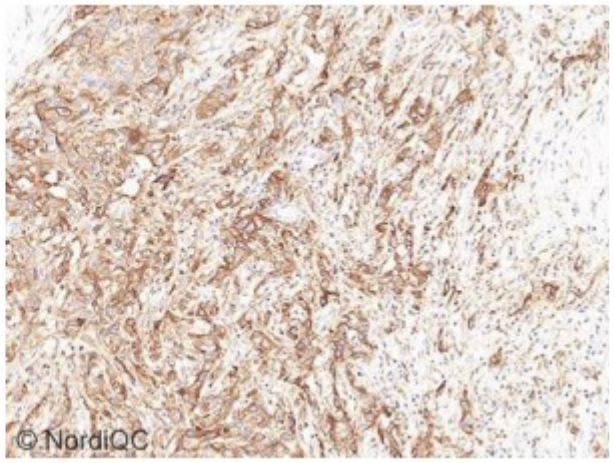
rmAb SP142





mAb 22C3





rmAb SP142

rmAb ZR3

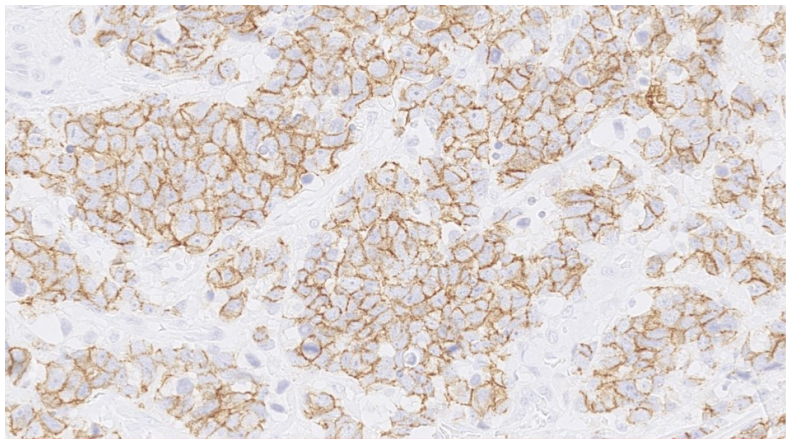
NordiQC

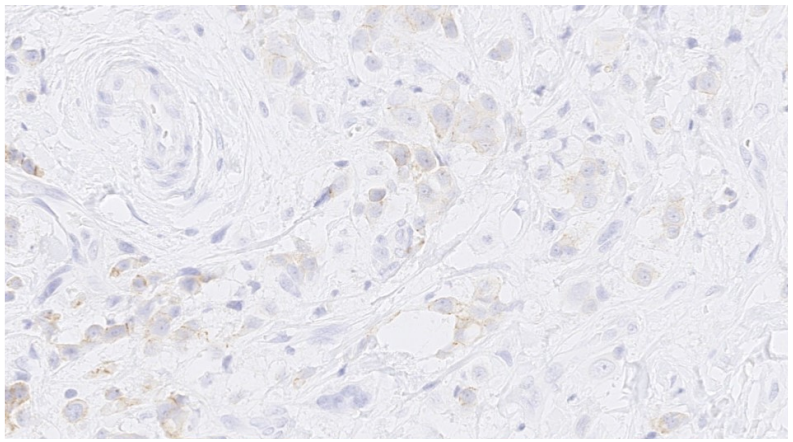
…YOU SURVIVED! THANK YOU FOR YOUR ATTENTION

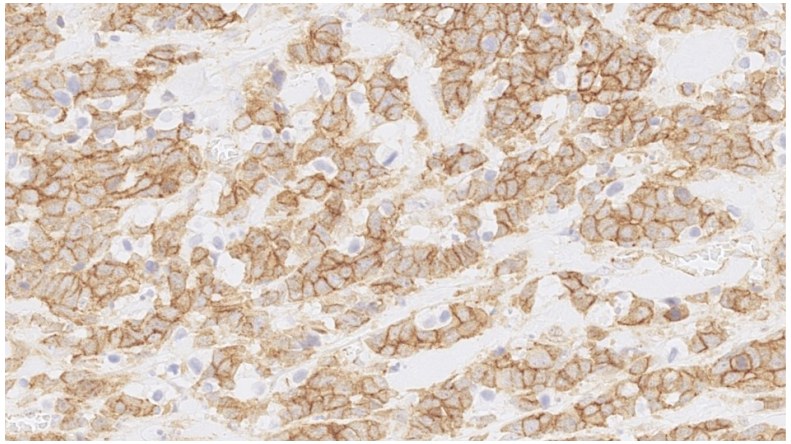
NordiQC

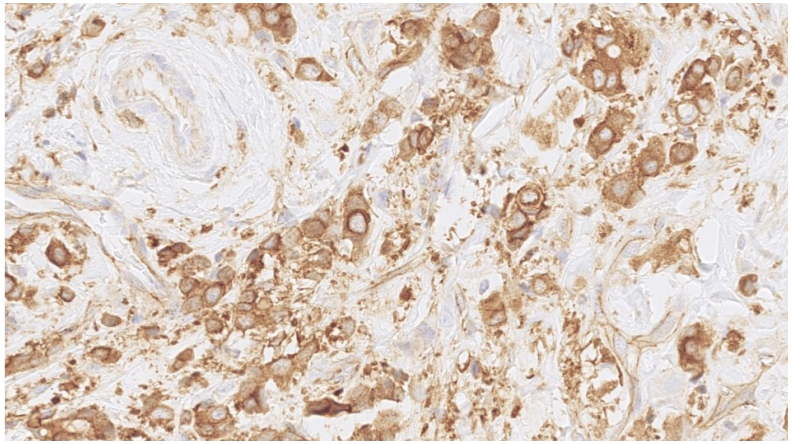
BONUS – P120

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

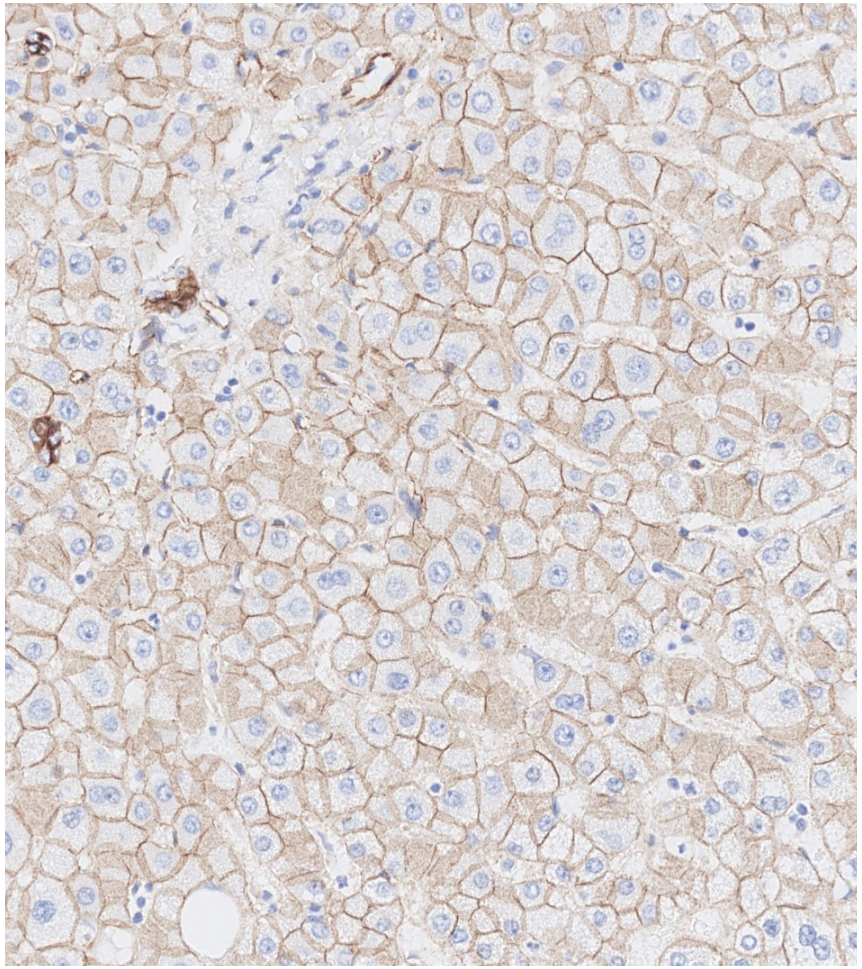
E-CAD: membranous staining reaction in (most) ductal breast carcinomas, negative in (most) lobular breast carcinomas.

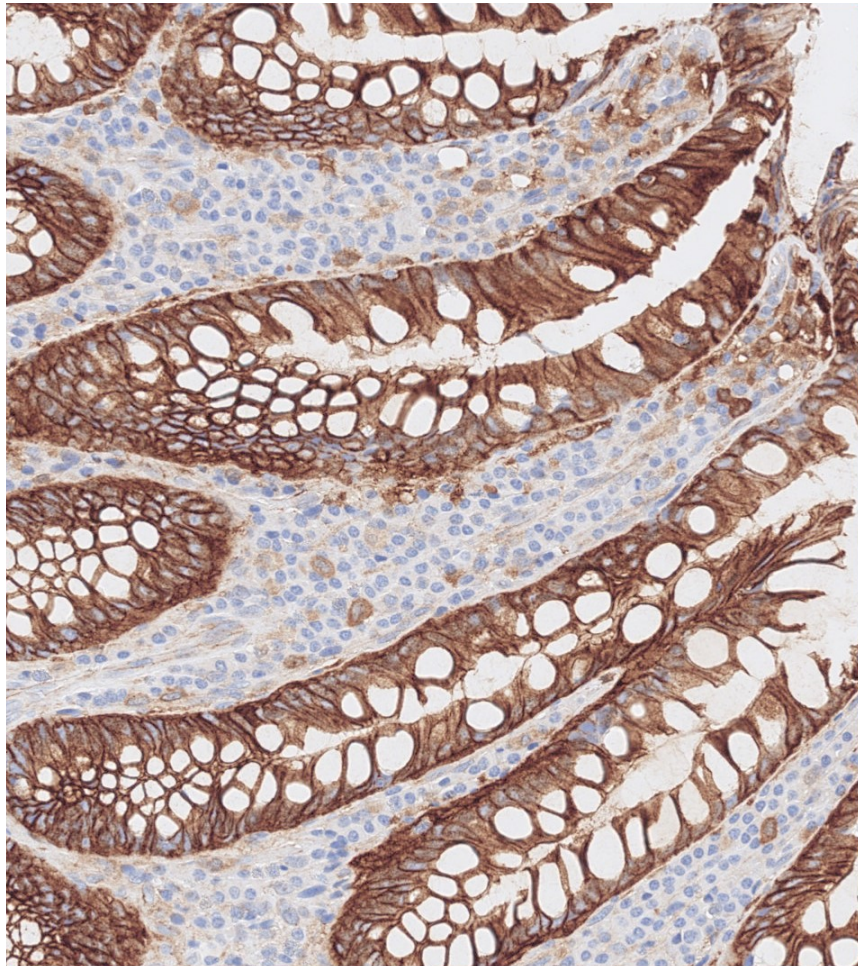
p120: membranous staining reaction in (most) ductal breast carcinomas, cytoplasmic staining reaction in (most) lobular breast carcinomas.

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.