

NordiQC data: Antibody selection, protocols and controls

The general module

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Primary panel for the unknown primary tumour

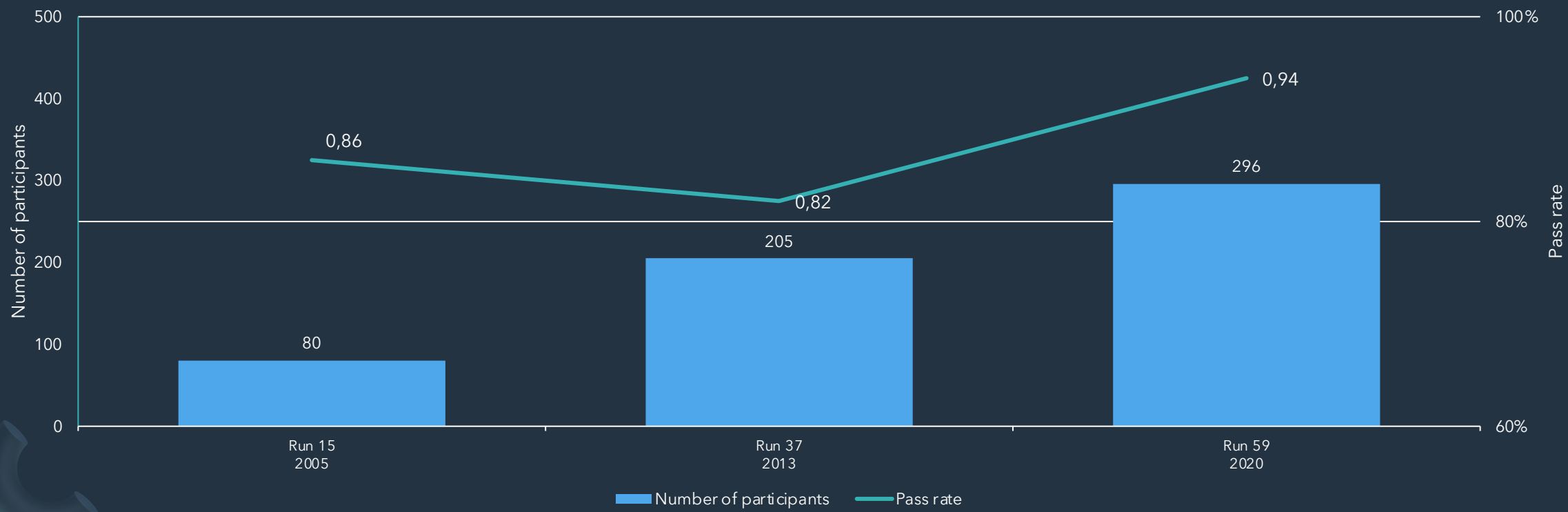


Is it as easy as it looks?!

	CD45	Pan-CK	S100	Vimentin
Haematolymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
Mesothelial neoplasms	-	+	-	+
mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
non-neuronal neuroepithelial neoplasms	-	-/(+)	+	+
Germ cell neoplasms	-	-/+	-/+	+

CD45

CD45 performance in NordiQC assessments



76% are using the mAb clone
2B11+PD7/26

And it is a real Ready-to-use!!

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clones 2B11+PD7/26 GA751 (VRPS) ³	23	Agilent/Dako	23	0	0	0	100%	100%
mAb clones 2B11+PD7/26 GA751 (LMPS) ⁴	27	Agilent/Dako	23	4	0	0	100%	85%
mAb clones 2B11+PD7/26 IR/IS751 (VRPS) ³	6	Agilent/Dako	6	0	0	0	100%	100%
mAb clones 2B11+PD7/26 IR/IS751 (LMPS) ⁴	18	Agilent/Dako	17	0	0	1	94%	94%
mAb clones 2B11+PD7/26 760-4279 (VRPS) ³	7	Ventana/Roche	7	0	0	0	100%	100%
mAb clones 2B11+PD7/26 760-4279 (LMPS) ⁴	36	Ventana/Roche	32	4	0	0	100%	89%
mAb clone X16/99 PA0042 (VRPS) ³	5	Leica Biosystems	3	1	1	0	80%	60%
mAb clone X16/99 PA0042 (LMPS) ⁴	4	Leica Biosystems	1	3	0	0	-	-
mAb clone RP2/18 760-2505 (VRPS) ³	3	Ventana/Roche	0	0	2	1	-	-
mAb clone RP2/18 760-2505 (LMPS) ⁴	45	Ventana/Roche	36	6	3	0	93%	80%
Total	296		232	45	15	4	-	
Proportion			79%	15%	5%	1%	94%	

Only a cut out of table 1

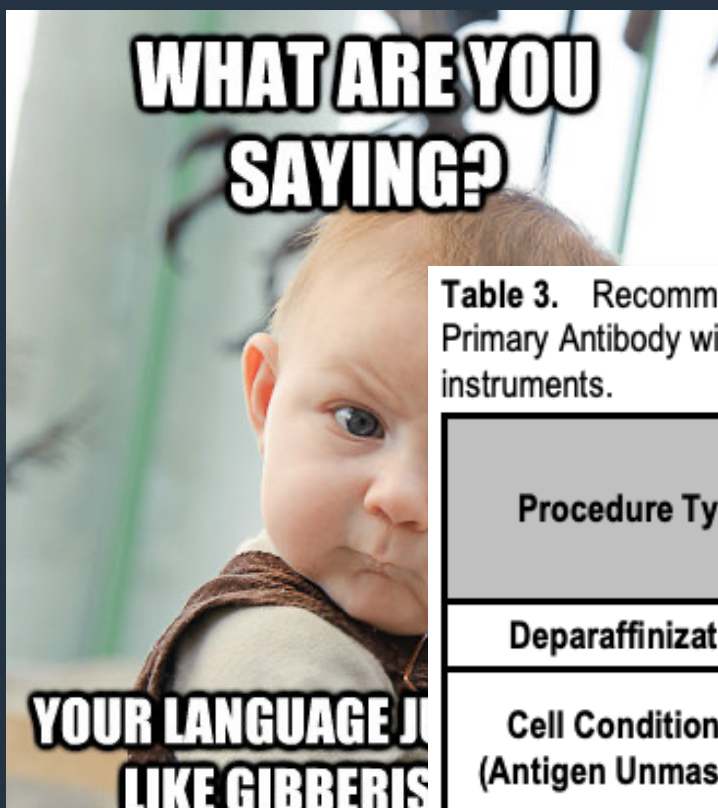


Table 1. Recommended Staining Protocols for CONFIRM anti-CD45, LCA (RP2/18)

Procedure Type	Platform or Method	
	NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected

Table 3. Recommended staining protocol for CONFIRM anti-CD45, LCA (RP2/18) Primary Antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method			
	GX	XT	ULTRA or ULTRA PLUS ^a	
Deparaffinization	Selected	Selected	Selected	None required
Cell Conditioning (Antigen Unmasking)	CC1, 16 minutes	CC1, 24 minutes	ULTRA CC1, 24 minutes, 100°C	None required
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected	Approximately 16 minutes, 37° C
Antibody (Primary)	4 minutes, 37°C	4 minutes, 37°C	4 minutes, 36°C	Optional
Counterstain	Hematoxylin II, 4 minutes			Optional
Post Counterstain	Bluing, 4 minutes			Hematoxylin II, 2 to 4 minutes

mAb clone **RP2/18 760-2505 (VRPS)**³

3

Ventana/Roc

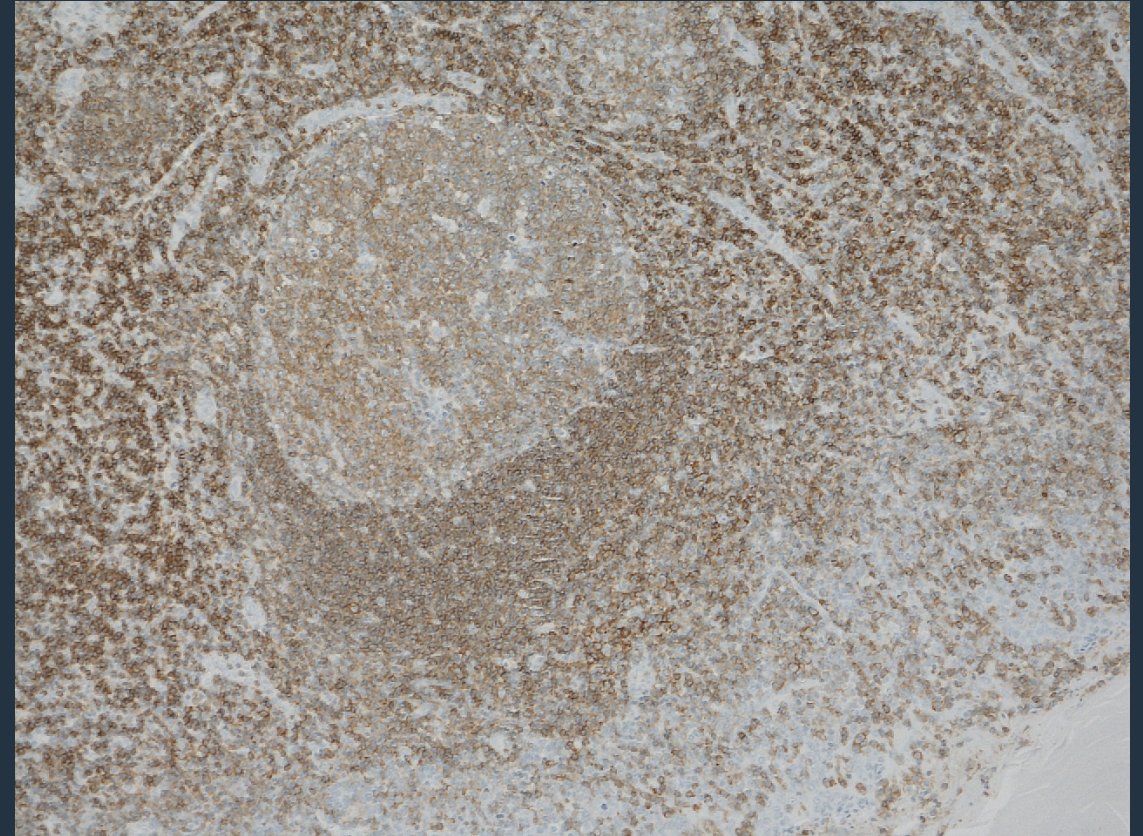
mAb clone **RP2/18 760-2505 (LMPS)**⁴

45

Ventana/Roc

Controls - Tonsil

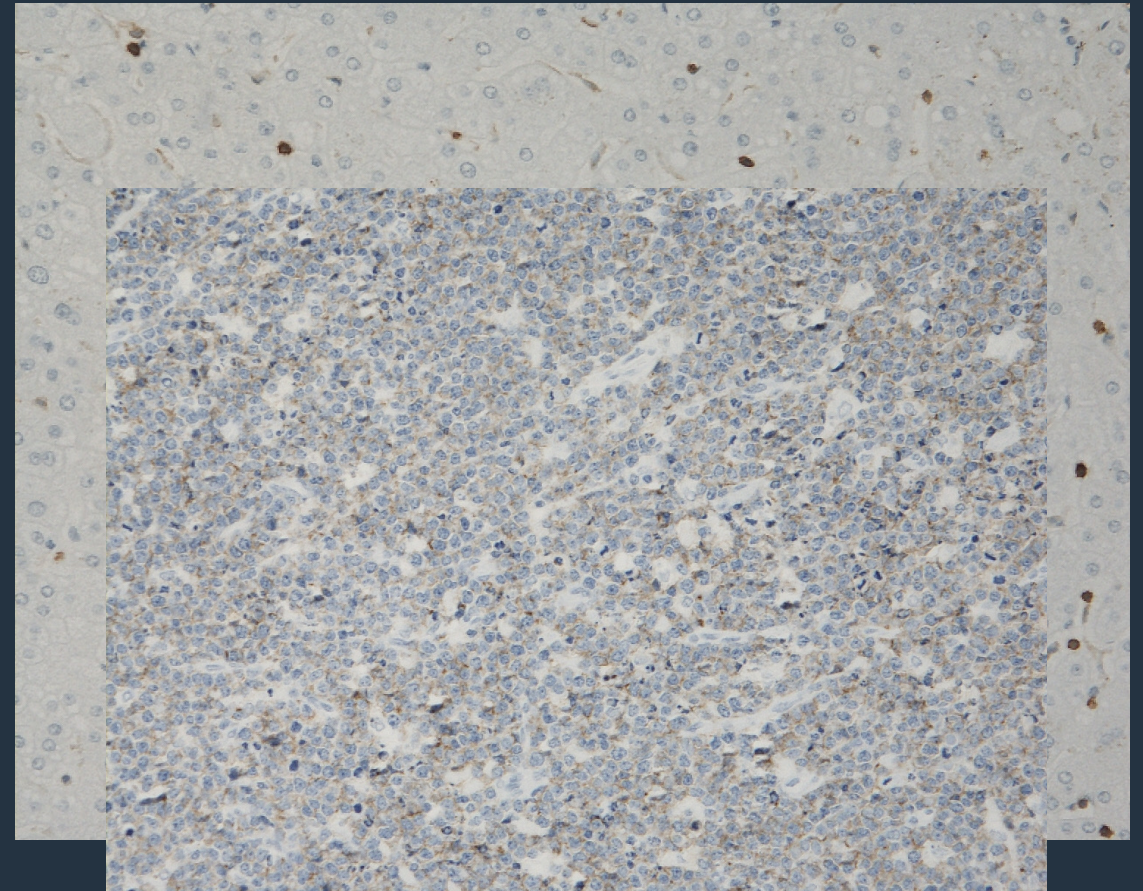
RP2/18 Ventana RTU



All lymphocytes (B- and T- cells) and histocytes must display a strong distinct membranous staining reaction. Squamous epithelial cells should be negative.

.... And Liver!

CD45, RP2/18 Ventana RTU



The Kupffer cells should show a weak to moderate staining reaction whereas hepatocytes must be negative.

CK-PAN

CK-PAN performance in NordiQC assessments

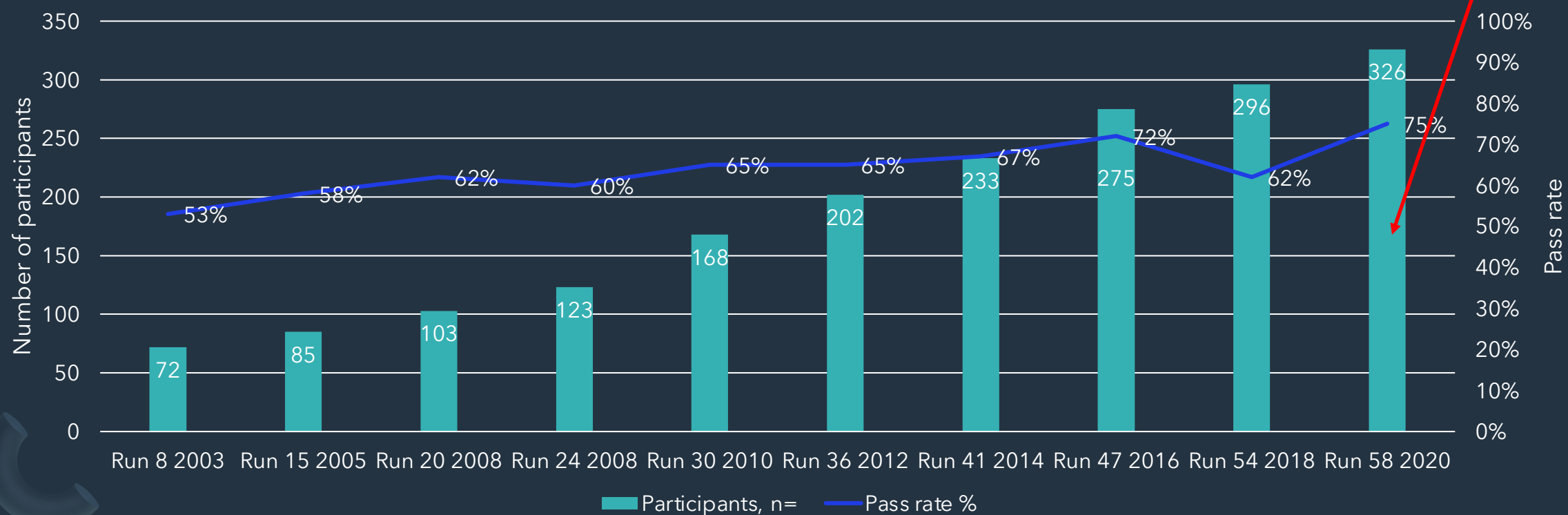
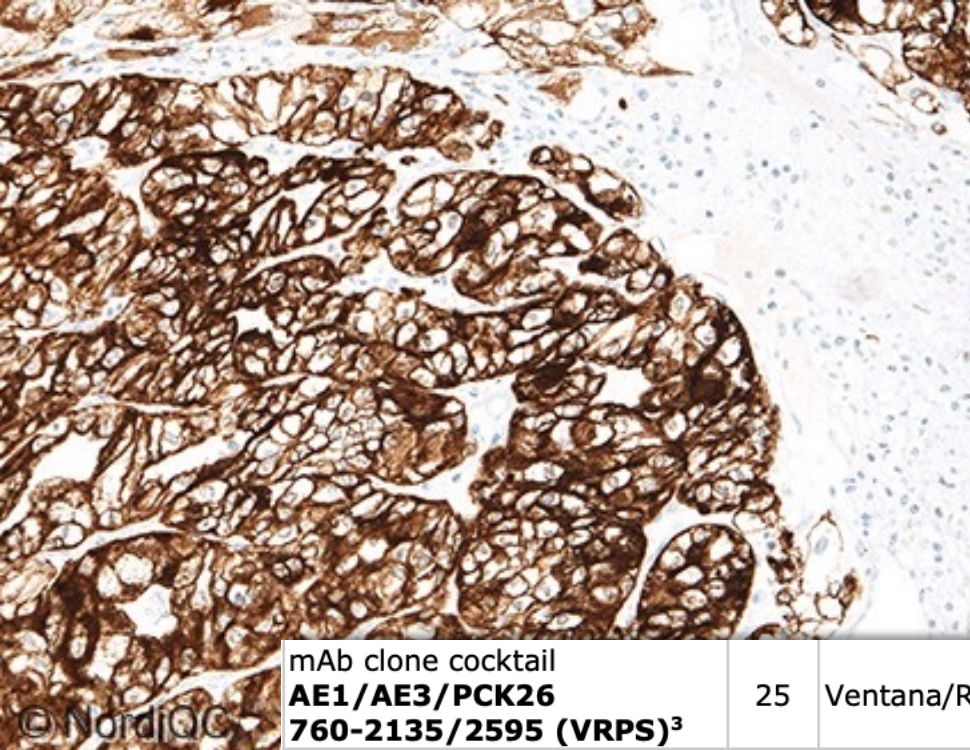


Table 2. Proportion of optimal results for CK-PAN using the mAb clone cocktail AE1/AE3 as concentrate on the four main IHC systems*

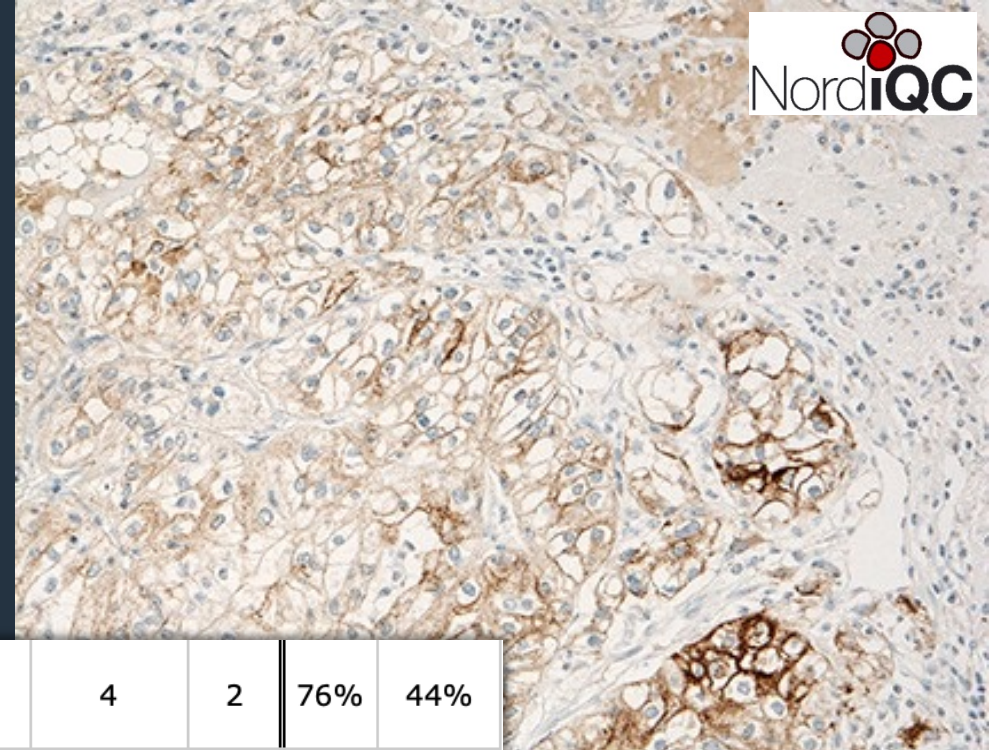
Concentrated antibodies		Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark XT / Ultra		Leica Bond III / Max		Optimal	Good	Borderline	Poor	Suff. ¹	OR. ²
		TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0						
mAb clone AE1/AE3		5/9** (56%)	-	6/6 100%	-	36/62 (58%)	-	0/12 (0%)	0/3	12	-	-	1	92%	92%
mAb clone BS5		0/2	-	1/1	-	2/3	-	3/6	1/1	10	2	2	-	86%	71%
										27	1	2	1	90%	87%
										17	1	-	-	100%	94%
										11	8	4	2	76%	44%
										29	19	10	11	70%	42%
760-2135/2595 (LMPS) ⁴															
mAb clone cocktail AE1/AE3 PA0909										-	1	1	-	-	-
mAb clone cocktail AE1/AE3 PA0094										1	3	1	-	80%	20%
mAb clone cocktail AE1/AE3 PA0012										-	3	-	-	-	-
Total										168	75	47	36	-	
Proportion										52%	23%	15%	11%	75%	

* 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.



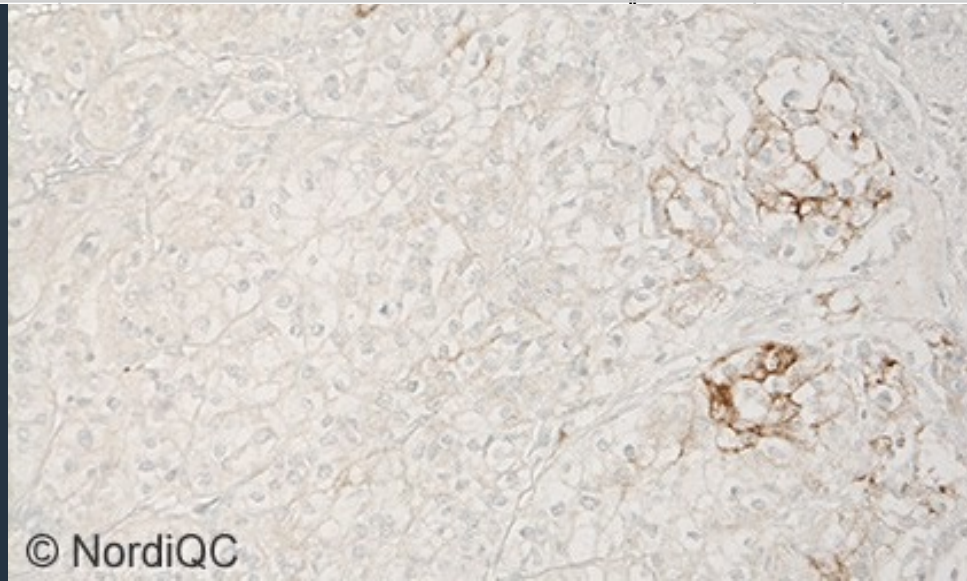
HIER+P3
OptiView



mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (VRPS) ³	25	Ventana/Roche	11	8	4	2	76%	44%
mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (LMPS) ⁴	69	Ventana/Roche	29	19	10	11	70%	42%

HIER
OptiView

Photos of Clear cell renal
cell carcinoma



P1
OptiView

mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (VRPS)³	25	Ventana/Roche	11	8	4	2	76%	44%
mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (LMPS)⁴	69	Ventana/Roche	29	19	10	11	70%	42%

Table 4. Pass rates for antibody cocktails combined with epitope retrieval methods in nine NordiQC runs

Pass rate for compiled data from run 15, 20, 24, 30, 36, 41, 47, 54 & 58								
	Total		HIER		Proteolysis		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb AE1/AE3	1145	836 (73%)	1075	826 (77%)	49	6 (12%)	9	3 (33%)
mAb AE1/AE3/5D3	48	42 (88%)	47	42 (89%)	1	0	0	0
mAb AE1/AE3/PCK26	361	219 (61%)	48	22 (46%)	48	3 (6%)	258	192 (74%)
mAb MNF116	111	31 (28%)	53	9 (17%)	48	22 (46%)	9	2 (22%)



Table 2. Recommended staining protocol for Anti-Pan Keratin (AE1/AE3/PCK26) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1 36 minutes, 95°C
Antibody (Primary)	4 minutes, 37°C	8 minutes, 37°C	8 minutes, 36°C
*ultraBlock step using VENTANA Antibody Diluent with Casein	4 minutes		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

*Use of VENTANA Antibody Diluent with Casein at the ultraBlock step is recommended to reduce staining on smooth muscle.

Table 2. Recommended staining protocol for Anti-Pan Keratin (AE1/AE3/PCK26) antibody with *ultraView* Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1 36 minutes, 95°C
Enzyme (Protease)	Protease 3, 4 minutes		
Antibody (Primary)	4 minutes, 37°C	8 minutes, 37°C	8 minutes, 36°C
ultraBlock step using VENTANA Antibody Diluent with Casein ^b	4 minutes		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

Control - Esophagus

All squamous epithelial cells throughout all the cell layers must show a strong distinct cytoplasmic staining reaction due to expression of HMW-CK types 5 and 14. Smooth muscle cells in vessels and in muscularis mucosa in esophagus will typically show a weak to moderate patchy cytoplasmic staining.

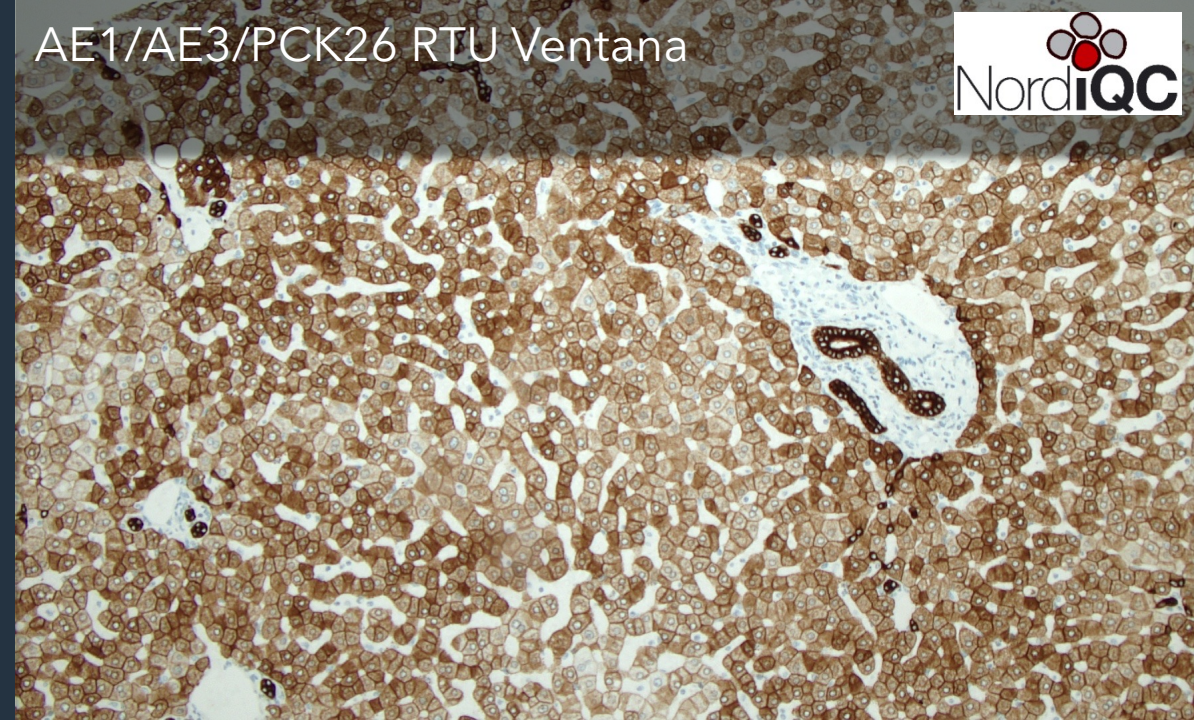
P3+HIER

AE1/AE3/PCK26 RTU Ventana

Stand alone P1

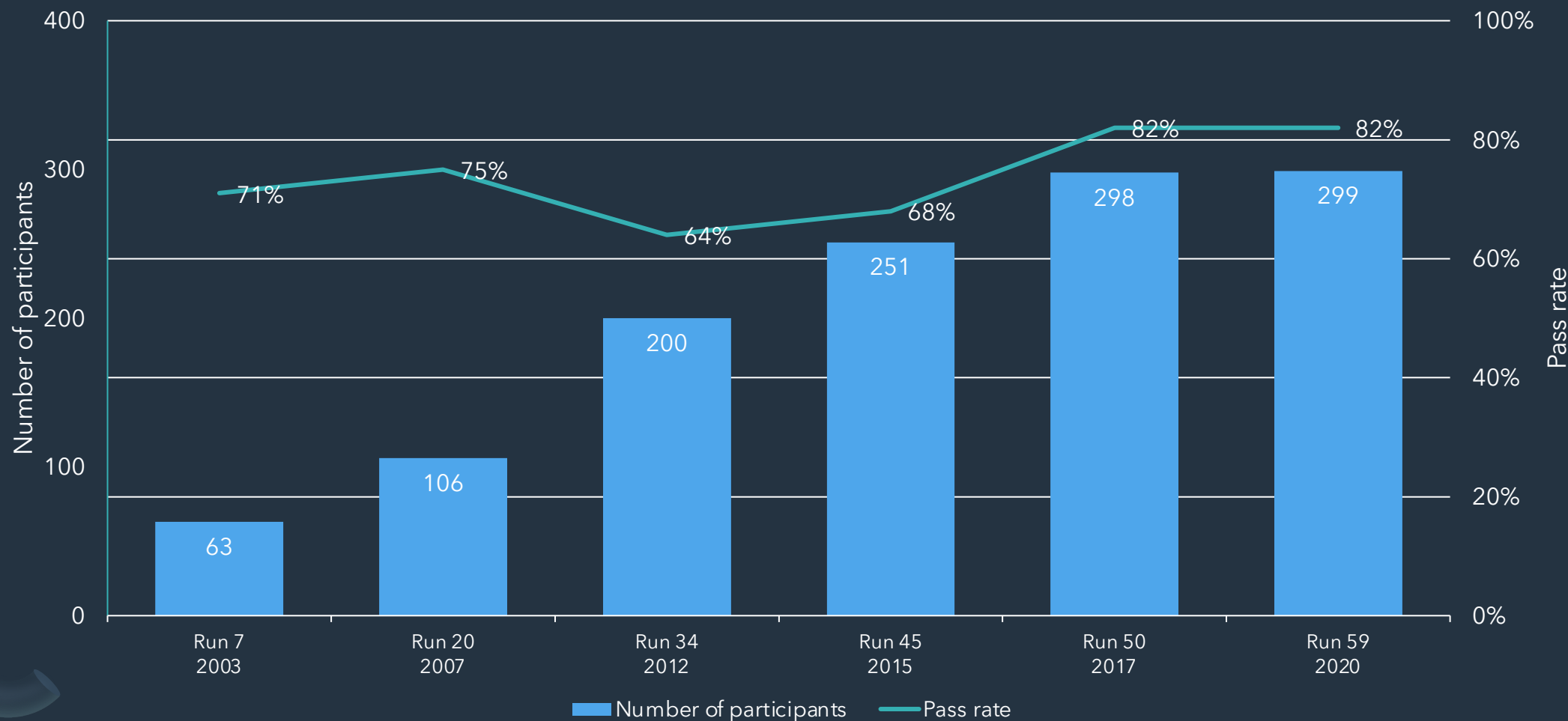
And Liver

It is crucial that the vast majority of the hepatocytes (expression only a limited amount of the primary LMW CK types 8 and 18) show an at least moderate, distinct cytoplasmic and membranous staining reaction. No staining should be seen in stromal cells in the liver.



S100

S100 performance in NordiQC assessments



TRY TO KEEP UP



Back in 2003 the main problem among the non-sufficient protocols was omission of HIER or use of proteolytic pretreatment, and guess what – it still is!!

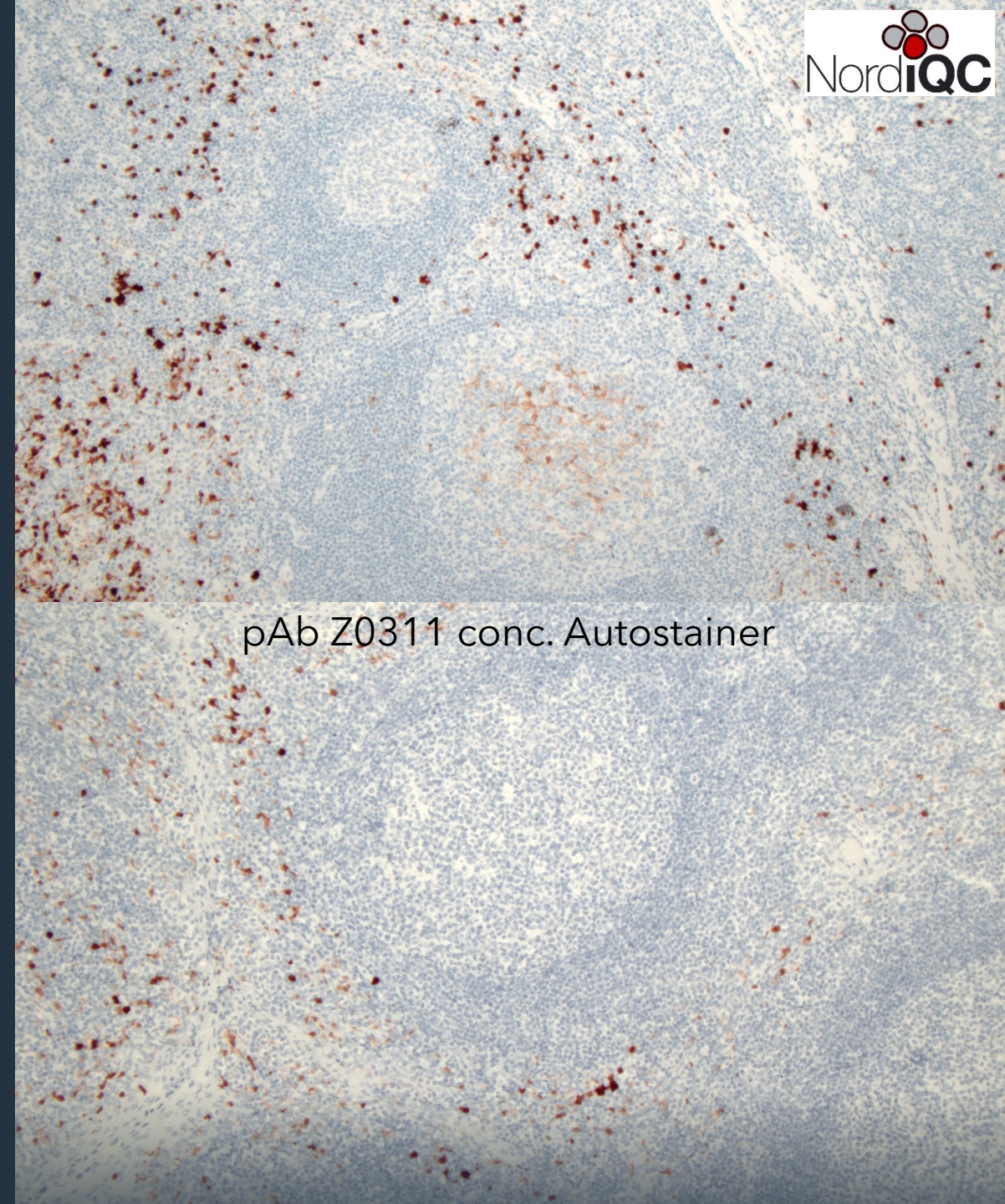
Table 5. **Pass rates for S100 antibody combined with epitope retrieval methods in the last three NordiQC runs**

Pass rate for compiled data from run 45, 50 & 59										
	Total		HIER		Proteolysis		HIER + proteolysis		No pretreatment	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb 4C4.9	137	80 (58%)	110	71 (65%)	4	0	2	1	21	8 (38%)
pAb NCL-L- S100p	30	18 (60%)	21	14 (67%)	6	2 (33%)	0	0	3	2
pAb Z0311	494	417 (84%)	444	386 (87%)	26	15 (58%)	3	2	21	14 (67%)
pAb 760- 2523	97	68 (70%)	82	62 (76%)	2	1	0	0	13	5 (39%)
Total	758	583 (77%)	657	533 (81%)	38	18 (47%)	5	3	58	29 (50%)

Controls

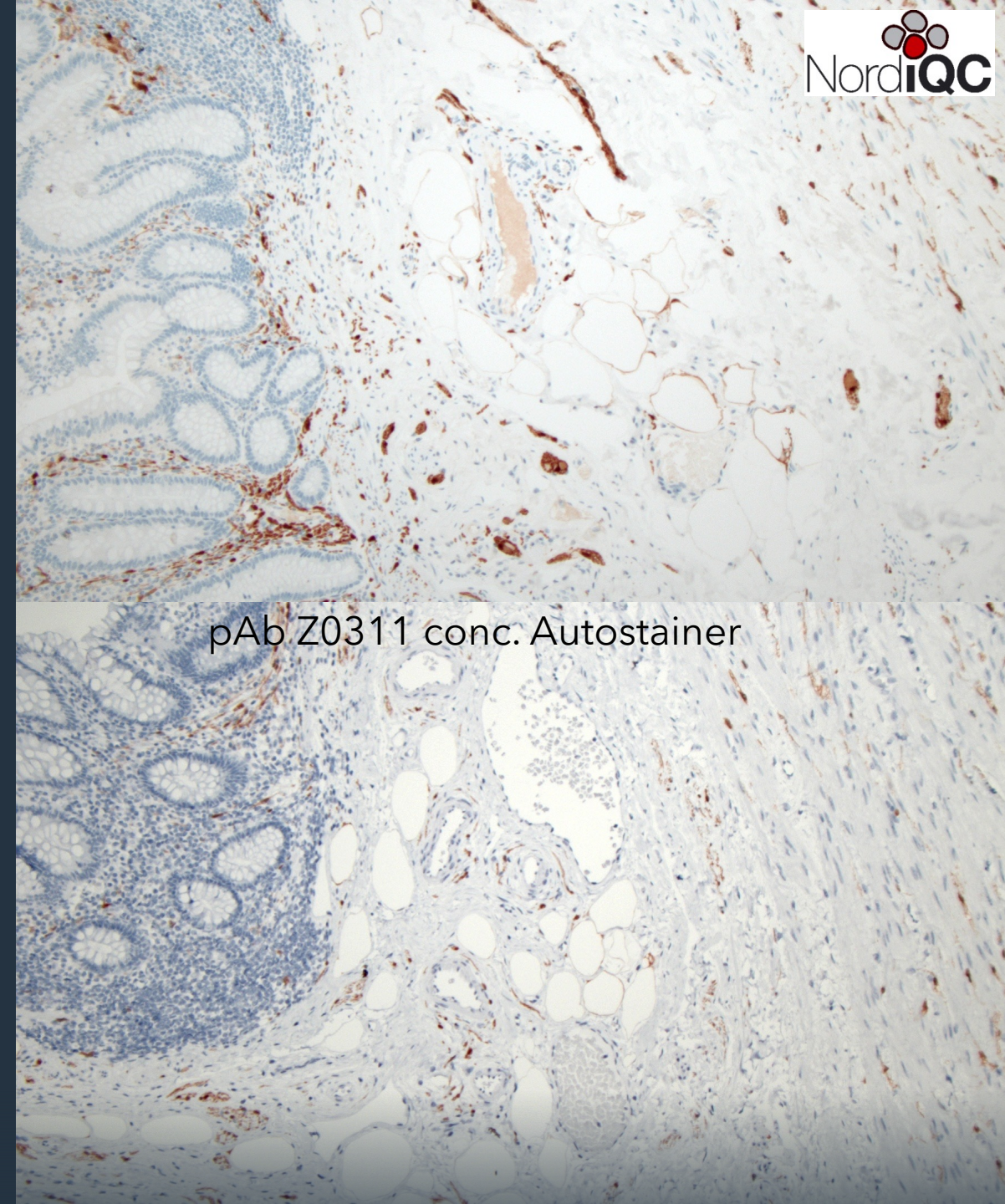
Only Z0311

In the tonsil, interfollicular dendritic cells and Langerhans cells of the squamous epithelium, must display a moderate to strong staining intensity whereas the follicular dendritic cell meshwork of the germinal centres should show an at least weak to moderate nuclear and cytoplasmic staining reaction.



Appendix

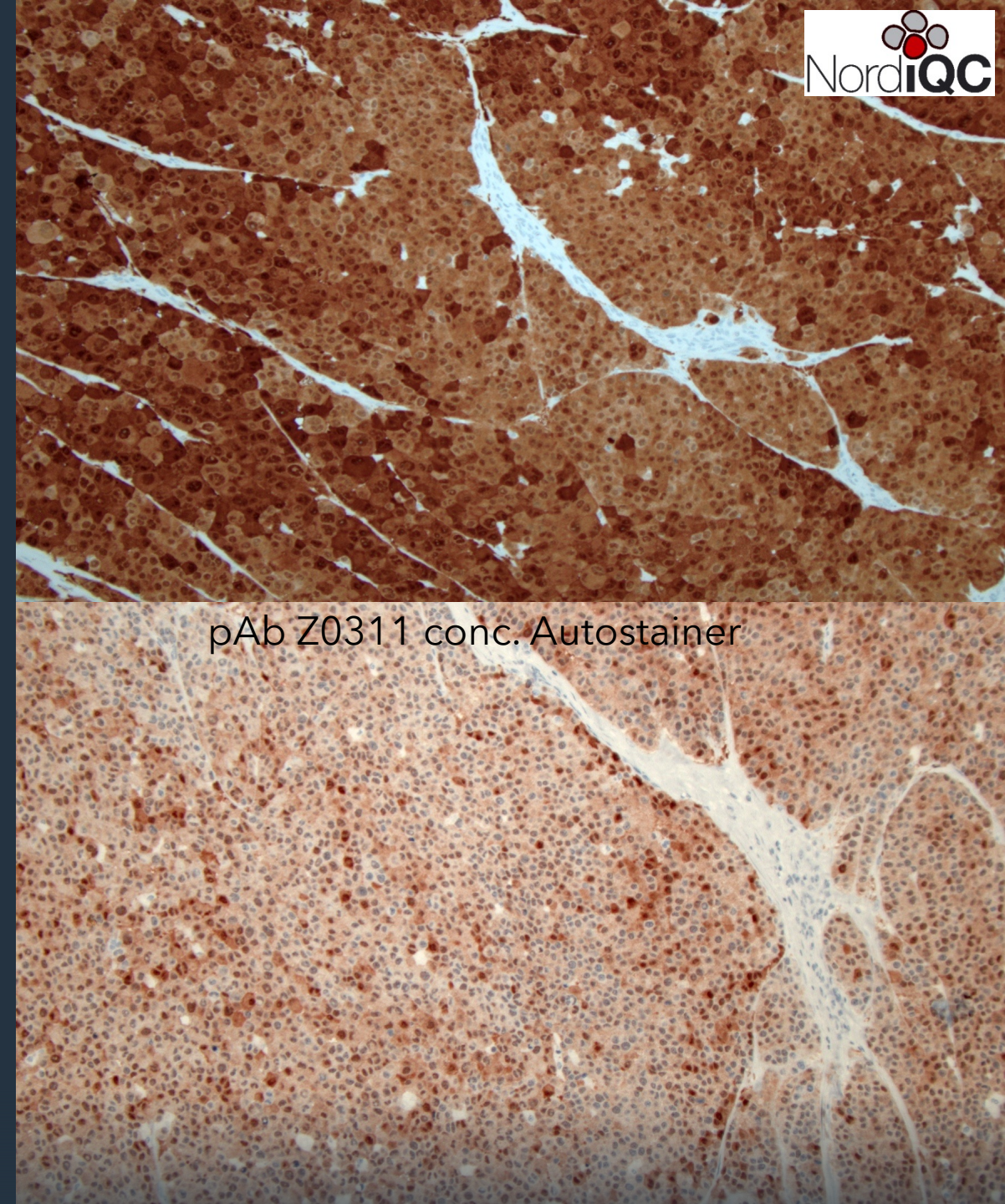
Virtually all adipocytes and Schwann cells of peripheral nerves, must show an as strong as possible nuclear and cytoplasmic staining reaction without any staining reaction of the smooth muscle or epithelial cells.



pAb Z0311 conc. Autostainer

In addition

All neoplastic cells should show a strong nuclear and cytoplasmic staining reaction in the malignant melanoma



pAb Z0311 conc. Autostainer

The clone Z0311 which was used by 57% both as concentrate and RTU is now terminated from vendor as a concentrate.



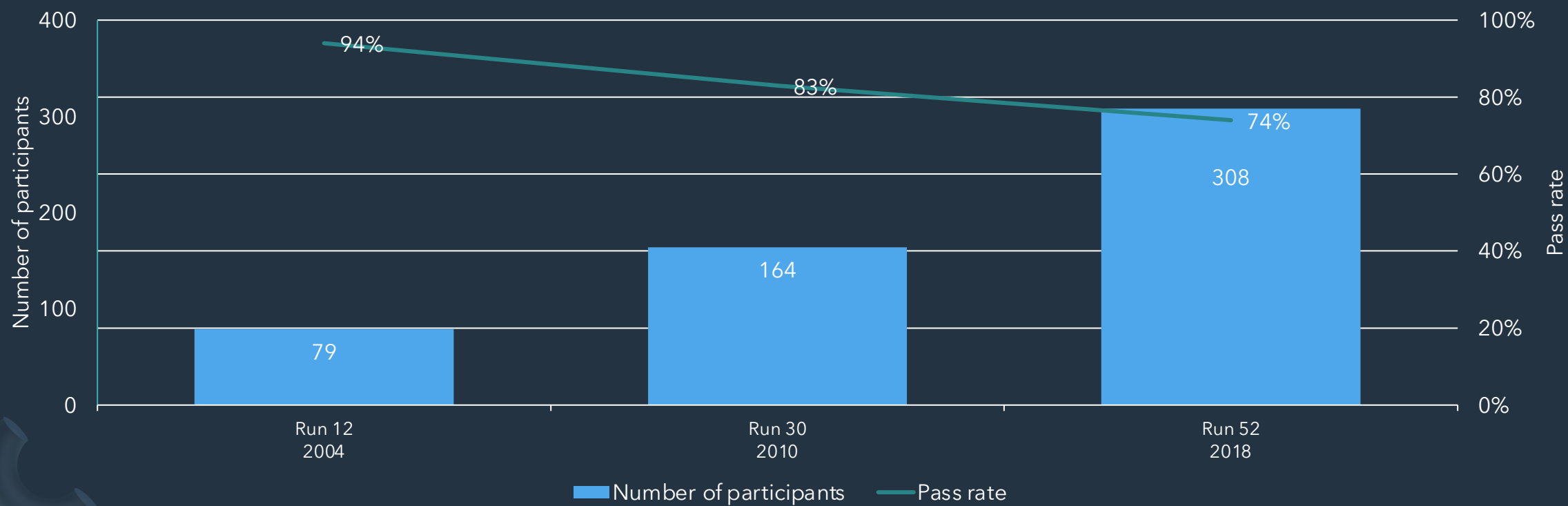
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone 4C4.9	1	Thermoscientific						
	1	Immunologic						
	2	Cell Marque						
	1	Diagnostic BioSystems						
	1	DCS	2	5	2	2	63%	18%
	2	BioCare Medical						
	2	Zytomed Systems						
	1	Zeta Corporation						
pAb Z0311 ⁵	100	Aqilent/Dako	55	27	15	3	82%	55%
pAb NCL-L-S100p	8	Leica/Novocastra	1	4	2	1	62%	13%
Ready-To-Use antibodies							Suff. ¹	OR. ²
mAb clone 4C4.9 790-2914 (VRPS) ³	4	Roche/Ventana	-	4	-	-	-	-
mAb clone 4C4.9 790-2914 (LMPS) ⁴	33	Roche/Ventana	9	15	8	1	73%	27%
pAb 760-2523 (VRPS) ³	11	Roche/Ventana	3	7	1	-	91%	27%
pAb 760-2523 (LMPS) ⁴	32	Roche/Ventana	8	15	9	-	72%	25%
pAb IS/IR504 (VRPS) ³	6	Aqilent/Dako	4	2	-	-	100%	67%
pAb IS/IR504 (LMPS) ⁴	19	Aqilent/Dako	14	4	1	-	95%	74%
pAb GA504 (VRPS) ³	29	Aqilent/Dako	28	1	-	-	100%	97%
pAb GA504 (LMPS) ⁴	17	Aqilent/Dako	13	3	1	-	94%	77%
pAb PA0900 (VRPS) ³	3	Leica/Novocastra	-	-	3	-	-	-
pAb PA0900 (LMPS) ⁴	10	Leica/Novocastra	1	6	3	-	70%	10%
Total	299		142	102	48	7	-	
Proportion			48%	34%	16%	2%	82%	

SOX10

Total	250		167	63	16	4	-	
Proportion			67%	25%	6%	2%	92%	

Vimentin

Vimentin performance in NordiQC assessments



Tonsil is out

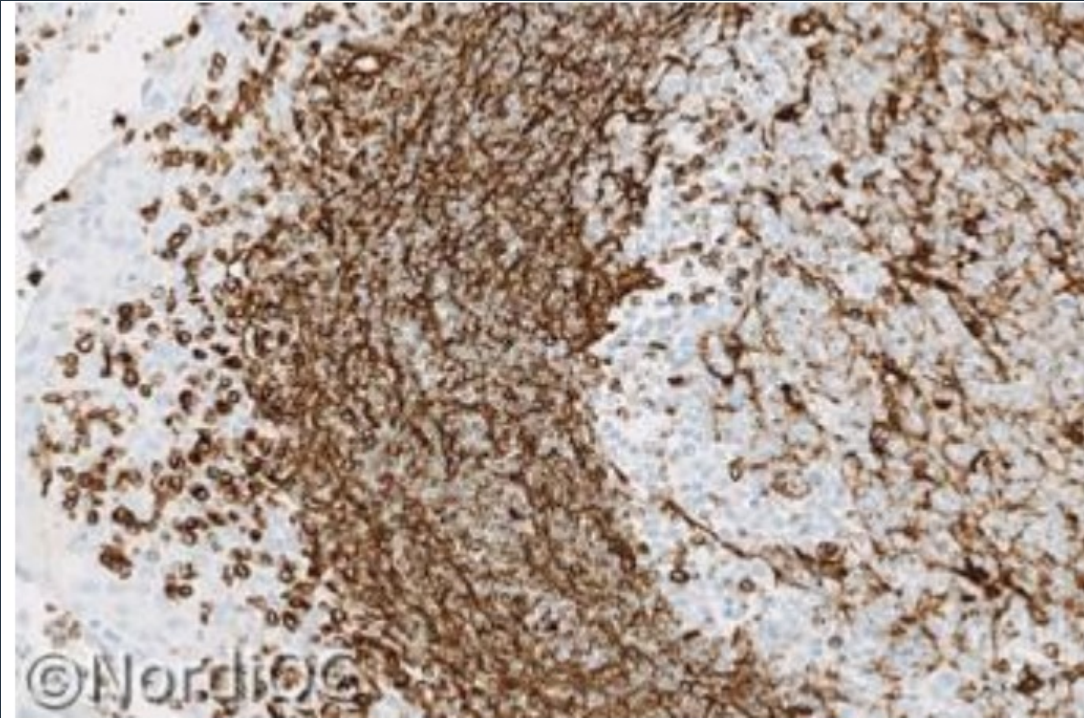
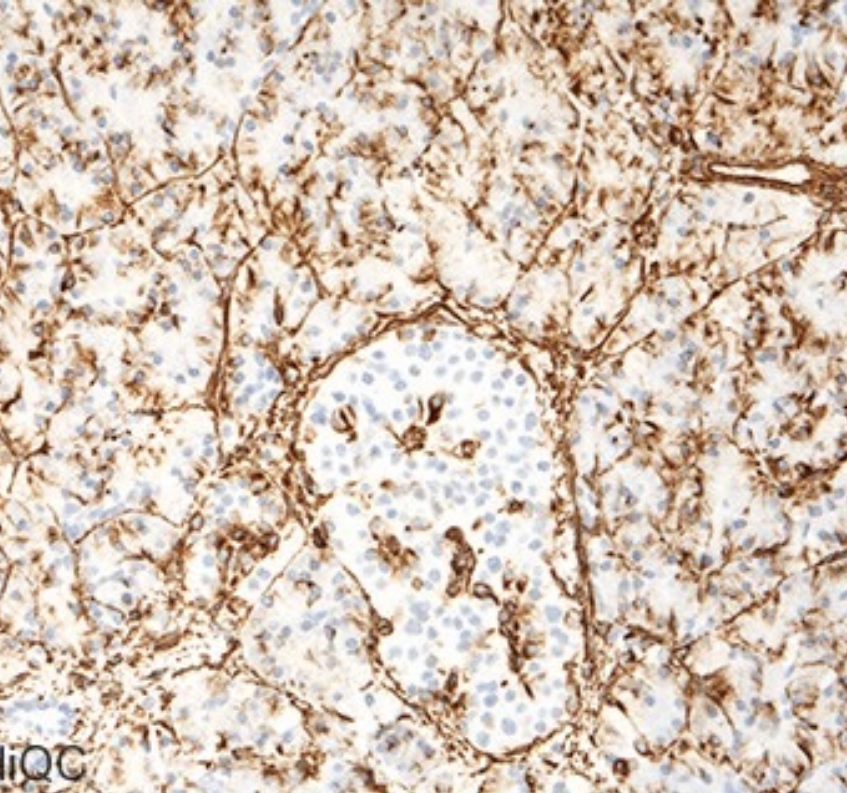


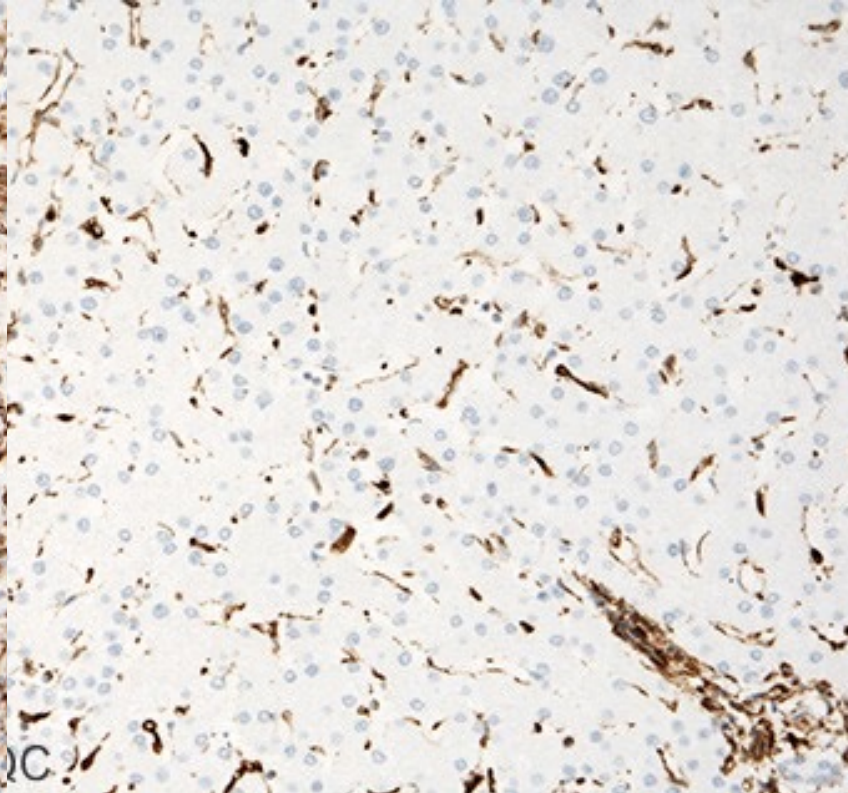
Fig. 1a
Optimal VIM staining of the tonsil using the mAb clone V9 carefully calibrated after HIER. The intraepithelial lymphocytes, the mantle zone B-cells and the germinal centre macrophages show a strong and distinct staining. No staining is seen in the squamous epithelial cells.



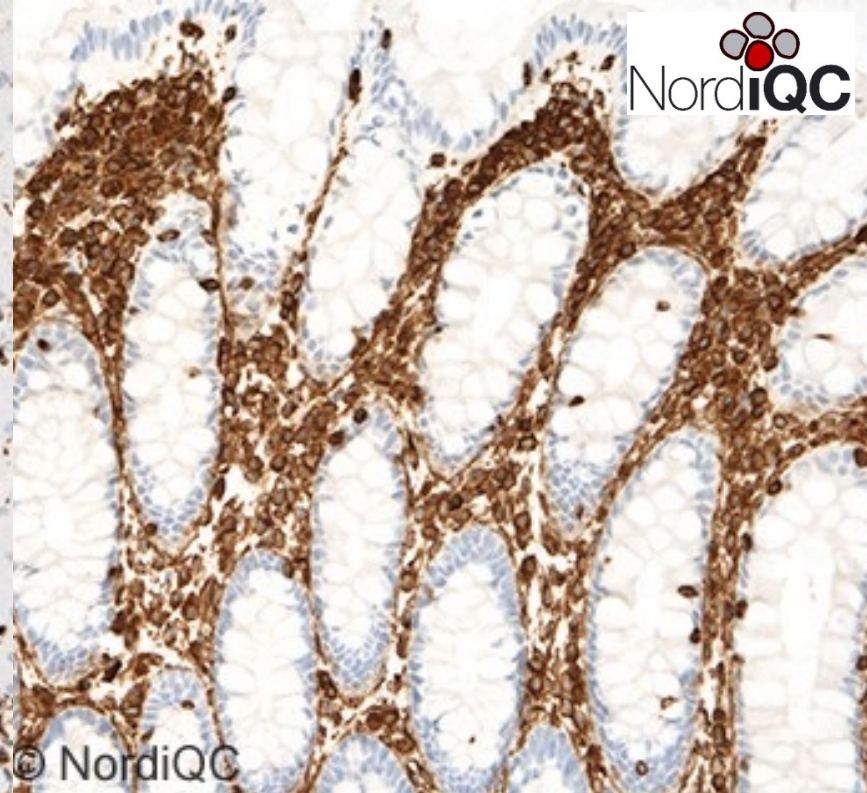
According to the new guidelines provided by the International Ad Hoc Expert Committee (Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18.)



Pancreas: Epithelial cells of exocrine acini must show a weak but distinct cytoplasmic staining reaction.



Liver: Virtually all Kupffer cells must show an at least moderate and distinct cytoplasmic staining reaction, while endothelial cells of the sinusoids must display an at least weak staining reaction



Colon: Endothelial cells of large vessels and stromal cells (e.g. fibroblasts and lymphocytes) must show a strong and distinct cytoplasmic staining reaction, while intraepithelial T-cells must at least display a moderate staining intensity.

Why go with V8 when you can try V9

Modified table 1

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone V9 IR630	31	Agilent/Dako	27	1	3	0	90%	87%
mAb clone V9 GA630	29	Agilent/Dako	23	2	4	0	86%	79%
mAb clone V9 790-2917	100	Roche/Ventana	21	51	19	9	72%	21%
mAb clone V9 PA0640	7	Leica/Novocastra	5	2	0	0	100%	71%
Total	308		133	96	49	30	-	
Proportion			43%	31%	16%	10%	74%	

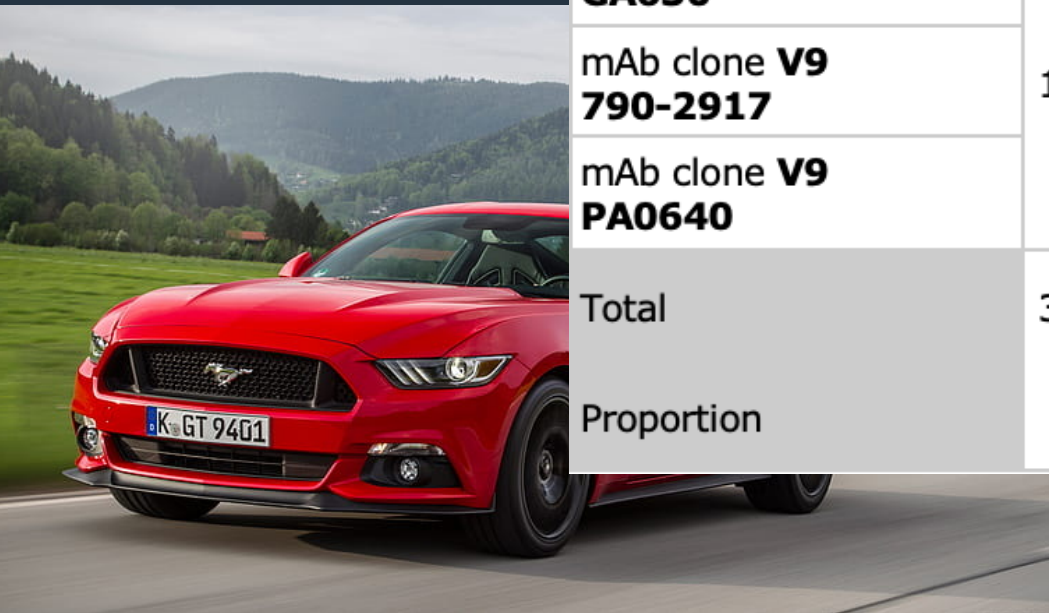


Table 4. **Proportion of sufficient and optimal results for VIM for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Leica BOND MAX/III mAb V9 PA0640	3/3	2/3	4/4	3/4
Dako AS mAb V9 IR630	92% (11/12)	92% (11/12)	88% (15/17)	82% (14/17)
Dako Omnis mAb V9 GA630	100% (16/16)	100% (16/16)	64% (7/11)	45% (5/11)
VMS Ultra/XT/GX mAb V9 790-2917	1/1	0/1	72% (71/99)	21% (21/99)

* 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.

“Recommendations from the vendor during run 52 2018: HIER in CC1 for 64 min., 16 min. incubation time in primary Ab and used the biotin-based iView as the detection system (...)” The information provided in the spec sheet of the RTU product was outdated and needed to be revised.

100% of the insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 79/79 of the insufficient results.

NQC: HIER CC1 32-64 min
+ AB 16-32 min
= 78% pass-rate

Updated recommendations 2022

and staining protocol for CONFIRM anti-Vimentin (V9) antibody with detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 24 minutes	CC1, 24 minutes	ULTRA CC1 24 minutes, 100 °C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	16 minutes, 37 °C	16 minutes, 37 °C	16 minutes, 36 °C
OptiView HQ Linker	8 minutes (default)		
OptiView HRP Multimer	8 minutes (default)		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

Overview

Marker	Last run	Pass rate/optimal	No. of labs
CD45	Run 59 2020	94% / 79%	296
PAN-CK	Run 58 2020	75% / 52%	326
S100	Run 59 2020	82% / 48%	299
Vimentin	Run 52 2018	74% / 43%	308

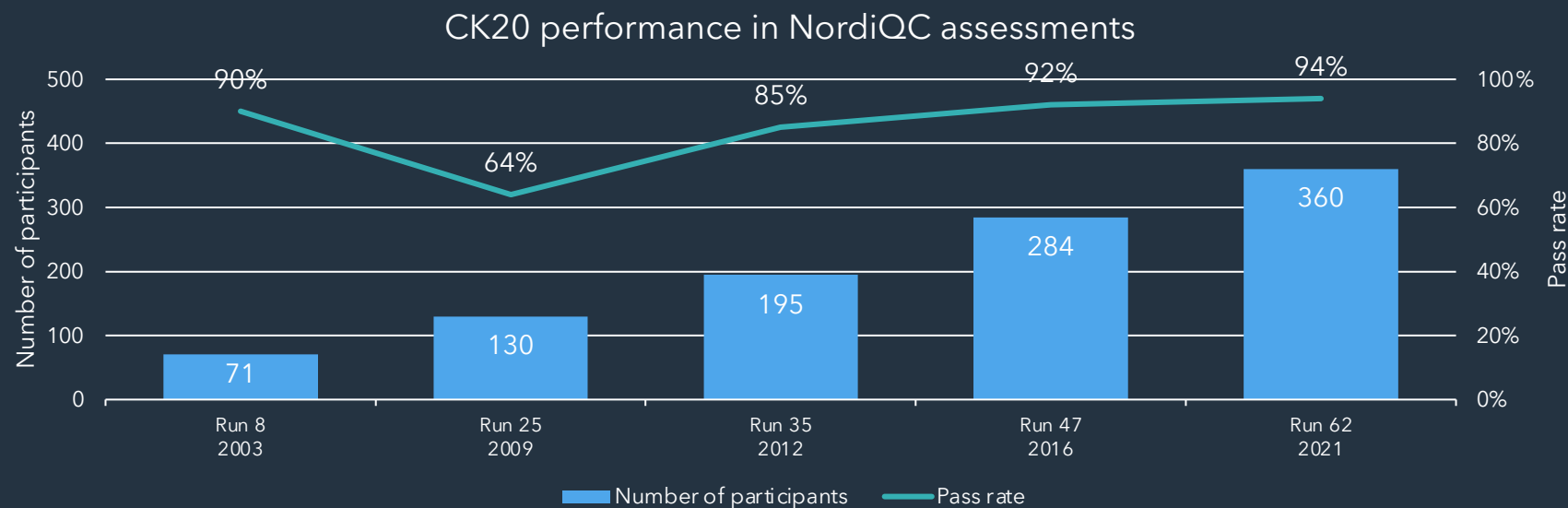
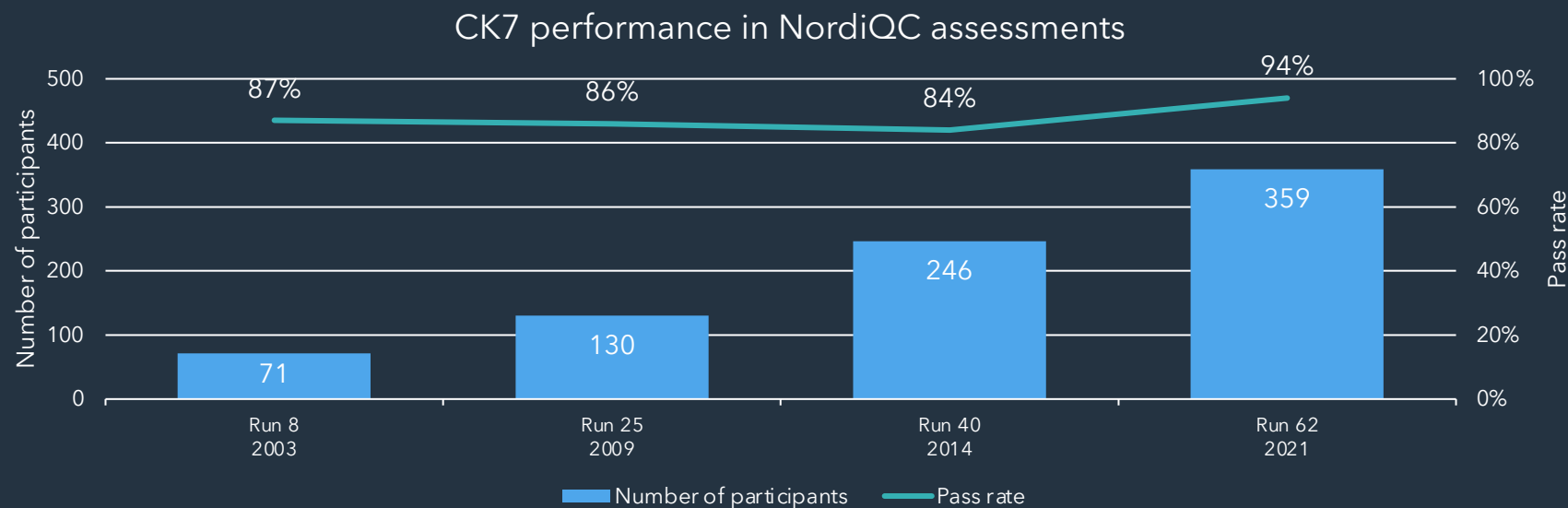
What else do you have?



Markers	Control	Last run	Pass rate / Optimal	No. of labs
CK20	Appendix	62 2021	94% / 74%	360
CK7	Pancreas, appendix	62 2021	94% / 74%	359
SATB2	Appendix, testis, tonsil	64 2022	75% / 42%	173
CDX2	Pancreas, tonsil	61 2021	91% / 79%	325
AMACR	Kidney	65 2022	93% / 74%	334
MLA	Skin, low level MLA tumors	60 2020	88% / 26%	312
SOX10	Skin, appendix	60 2020	92% / 67%	250
Uroplakin	Urethra, tonsil	59 2020*	45% / 21%	66
Pax8	Kidney, fallopian tube	64 2022	52% / 27%	337
GATA3	Tonsil, uterine cervix	63 2021	68% / 41%	320
Desmin	Appendix, placenta	64 2022	69% / 41%	370
BRAF	Positive and negative tumors	62 2021*	72% / 35%	135
CD56	Tonsil	64 2022	72% / 47%	363
CD31	Liver, tonsil	62 2021	79% / 56%	342

*First NordiQC run

When the concept works!



CK7 and CK20 – you GO!

Table 1. Antibodies and assessment marks for CK7, Run 62

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone OV-TL 12/30, IR619³	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone OV-TL 12/30, IR619⁴	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone OV-TL 12/30, GA619³	32	Dako/Agilent	31	1	0	0	100%	97%
mAb clone OV-TL 12/30, GA619⁴	30	Dako/Agilent	27	2	1	0	97%	90%
mAb clone RN7, PA0942³	6	Leica Biosystems	1	5	0	0	100%	45%
mAb clone RN7, PA0942/PA0138⁴	11	Leica Biosystems	5	6	0	0	100%	69%
rmAb clone SP52, 790-4462³	16	Ventana/Roche	11	5	0	1	97%	85%
rmAb clone SP52, 790-4462⁴	101	Ventana/Roche	86	12	2	1	-	-
Total	359		265	71	20	3	94%	-
Proportion			74%	20%	5%	1%		

Table 1. Antibodies and assessment marks for CK20, run 62

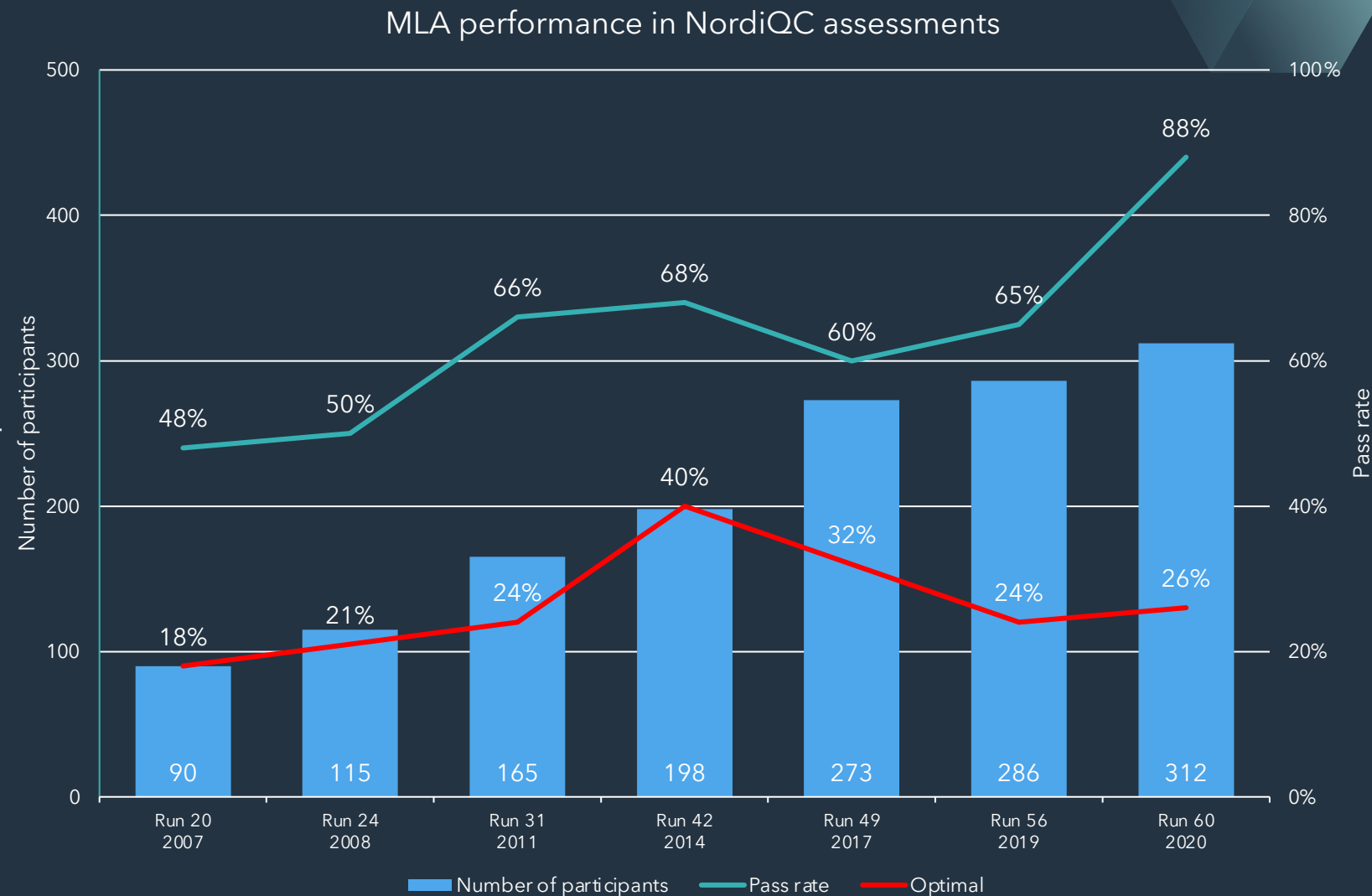
Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone SP33 790-4431³	19	Ventana/Roche	16	3	-	-	100%	84%
rmAb clone SP33 790-4431⁴	89	Ventana/Roche	89	14	2	-	98%	85%
mAb clone Ks20.8 IR/IS777³	18	Dako/Agilent	14	4	-	-	100%	78%
mAb clone Ks20.8 IR/IS777⁴	16	Dako/Agilent	12	3	1	-	94%	75%
mAb clone Ks20.8 GA777³	33	Dako/Agilent	31	2	-	-	100%	94%
mAb clone Ks20.8 GA777⁴	27	Dako/Agilent	19	7	1	-	96%	70%
mAb clone Ks20.8 PA0022³	5	Leica Biosystems	4	1	-	-	100%	80%
mAb clone Ks20.8 PA0022⁴	10	Leica Biosystems	7	3	-	-	100%	70%
Total	360		266	72	21	1	94%	-
Proportion			74%	20%	6%	-		

Markers	Control	Last run	Pass rate / Optimal	No. of labs
CK20	Appendix	62 2021	94% / 74%	360
CK7	Pancreas, appendix	62 2021	94% / 74%	359
SATB2	Appendix, testis, tonsil	64 2022	75% / 42%	173
CDX2	Pancreas, tonsil	61 2021	91% / 79%	325
AMACR	Kidney	65 2022	93% / 74%	334
MLA	Skin, low level MLA tumors	60 2020	88% / 26%	312
SOX10	Skin, appendix	60 2020	92% / 67%	250
Uroplakin	Urethra, tonsil	59 2020*	45% / 21%	66
Pax8	Kidney, fallopian tube	64 2022	52% / 27%	337
GATA3	Tonsil, uterine cervix	63 2021	68% / 41%	320
Desmin	Appendix, placenta	64 2022	69% / 41%	370
BRAF	Positive and negative tumors	62 2021*	72% / 35%	135
CD56	Tonsil	64 2012	72% / 47%	363
CD31	Liver, tonsil	62 2021	79% / 56%	342

*First NordiQC run

Melan A

Run 56 2019 the pass rate was a low 65%. In Run 60 it has increased to 88%. The amount of optimal however was low in both runs 24% and 26% respectively.



Aim and purpose

In previous NordiQC MLA assessments, laboratories using the mAb clone A103 have been assessed on their ability to detect both the specific MLA and the unknown cross-reacting protein in steroid hormone producing cells and corresponding tumours, whereas laboratories using other clones have been assessed on their ability to detect MLA only.



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

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT/GX mAb A103 790-2990	0/3	0/3	85% (80/94)	6% (6/94)
Dako AS mAb A103 IR633/IS633	100% (14/14)	7% (1/14)	92% (11/12)	17% (2/12)
Leica Bond III/MAX mAb A103 PA0233/PA0044	100% (7/7)	29% (2/7)	100% (10/10)	60% (6/10)

87% of the insufficient protocols were to weak or false negative staining reaction in structures expected to be positive - they were all A103.

Concentrated antibodies		n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	
clone A103	mAb clone EP43	57	Dako/Agilent						
		19	Novocastra/Leica						
		6	Cell Marque						
		1	Abcam						
		1	Biocare						
		1	Monosan						
Ready-To-Use antibodies	mAb clone A103 790-2990 ³	1	Biogenex	21	57	9	0	90%	24%
		1	Zeta Corporation						
		9	Nordic Biotite						
		9	Epitomics						
		1	Cell Marque						
				18	1	0	0	100%	95%
mAb clone A103 790-2990 ⁴	mAb clone A103 IR633/IS633 ³	3	Ventana/Roche						
		94	Ventana/Roche	0	0	3	0	-	-
		14	Dako/Agilent	6	74	11	3	85%	6%
		56	Dako/Agilent	1	13	0	0	100%	7%
		7	Leica Biosystems	12	36	7	1	85%	21%
		10	Leica Biosystems	2	5	0	0	100%	29%
mAb clone A103 PA0233/PA0044 ⁴	mAb clone A103 PA0233/PA0044 ⁴	6		4	0	0	0	100%	60%

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

Concentrated antibodies	Dako Autostainer		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	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone A103	0/5** (0%)	0/1	6/9 (66%)	-	5/34 (15%)	-	-	7/17 (41%)	0/1
rmAb clone EP43	-	-	5/5 (100%)	-	5/6 (83%)	6/6 (100%)	-	1/1	-



Immunostainer	
Type:	Ventana Benchmark Ultra
Primary antibody	
Clone:	A103
Producer:	Ventana Roche
Product no. / lot no.:	790-2990 / f27660
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	32 min. / 37°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Ventana Ultra CC1
Heating time at max. temp.:	64 min.
Maximum heating temp.:	98°C
Visualization system	
Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	37°C

Immunostainer	
Type:	Leica BOND III
Primary antibody	
Clone:	A103
Producer:	Leica/Novocastra
Product no. / lot no.:	PA0233/PA0044 / 66904
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	15 min. / 20°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Leica Bond Epitope Retrieval Solution 2
Heating time at max. temp.:	20 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	20°C



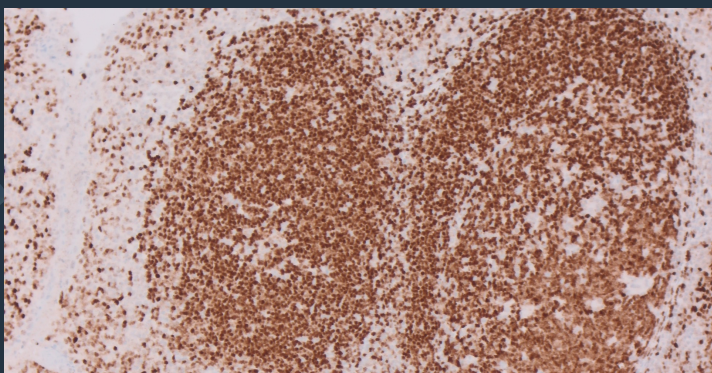
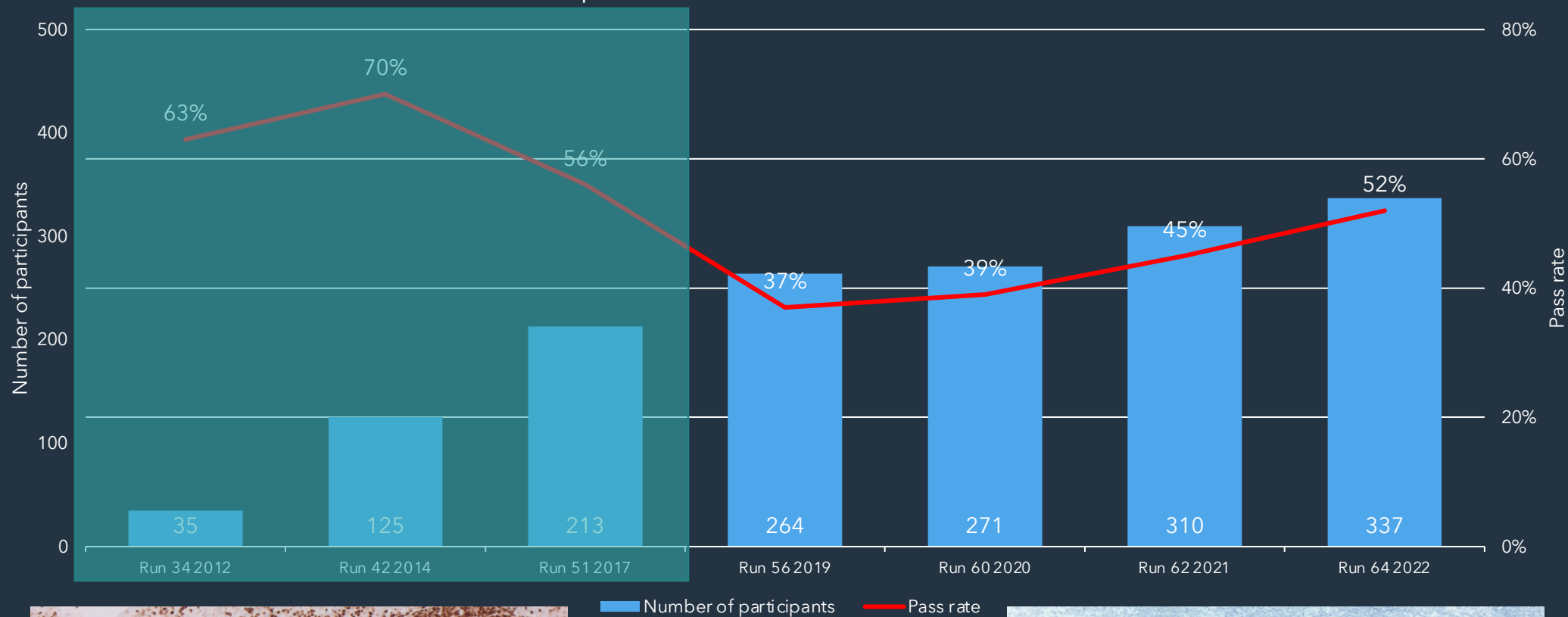
Immunostainer	
Type:	Dako Omnis
Primary antibody	
Clone:	A103
Producer:	Dako
Product no. / lot no.:	IR633/IS633 / 20079125
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	20 min. / 32°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Dako Omnis Target Retrieval Solution, 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

Immunostainer	
Type:	Dako Autostainer Link 48 +
Primary antibody	
Clone:	A103
Producer:	Dako
Product no. / lot no.:	IR633/IS633 / 20067423
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	20 min. / 22°C
Epitope retrieval, HIER	
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	99°C
Visualization system	
Producer:	Dako
Product / no:	EnVision FLEX / K8000/SM802
Linker:	None
Incubation time polymer:	20 min.
Incubation temperature:	22°C



PAX8

PAX8 performance in NordiQC assessments



Pushing in the right direction



Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone BC12*	6	Biocare	2	4	4	-	60%	20%
mAb clone MRQ-50	4	Zytomed Systems	-	17	7	1	68%	-
rmAb clone EP298⁵*	25	Cell Marque	-	1	-	3	-	-
rmAb clone EP331*	3	Cell Marque	-	8	6	1	53%	-
rmAb clone EP331*	1	Epitomics	-	1	-	3	-	-
rmAb clone SP348*	9	Cell Marque	-	8	6	1	53%	-
rmAb clone SP348*	6	Epitomics	-	8	6	1	53%	-
rmAb clone SP348*	105	Abcam	81	24	4	2	95%	73%
rmAb clone SP348*	3	Gennova	81	24	4	2	95%	73%
rmAb clone SP348*	3	Spring Bioscience	81	24	4	2	95%	73%
rmAb clone ZR-1*	3	Zeta Corporation	2	1	1	1	60%	40%
rmAb clone ZR-1*	1	Gene Tech	2	1	1	1	60%	40%
rmAb clone ZR-1*	1	ImmunoForce	2	1	1	1	60%	40%
pAb, 10336-1-AP	13	Proteintech	-	3	6	4	23%	-
Conc total	199		88	63	34	14	76%	44%
Ready-To-Use antibodies							Suff. ¹	OR. ²
rmAb clone, EP331* 760-6077(VRPS)³	5	Ventana/Cell Marque	-	-	5	-	-	-
rmAb clone, EP331* 760-6077(LMPS)⁴	15	Ventana/Cell Marque	-	-	11	4	-	-
mAb clone MRQ-50 , 760-4618 (VRPS)³	3	Ventana/Roche	-	-	-	3	-	-
mAb clone MRQ-50 , 760-4618 (LMPS)⁴	55	Ventana/Roche	-	4	33	18	7%	-
RTU total	138		3	20	76	39	17%	2%
Total	337		91	83	110	53	-	
Proportion			27%	25%	33%	15%	52%	

Not an easy antibody

Table 3. Proportion of optimal results for PAX8 for the most commonly used antibodies as concentrate on the four main IHC systems*

Concentrated antibodies	Dako/Agilent Autostainer		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	CC1 pH 8.5 + P3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
rmAb SP348	4/8 (50%)	-	29/31 (94%)	0/1	45/63 (71%)	3/5 (60%)	0/2	0/1	-
rmAb ZR-1	1/1	-	-	-	0/1		-	0/2	-
mAb BC12	-	-	-	-	-	-	-	2/6 (33%)	-

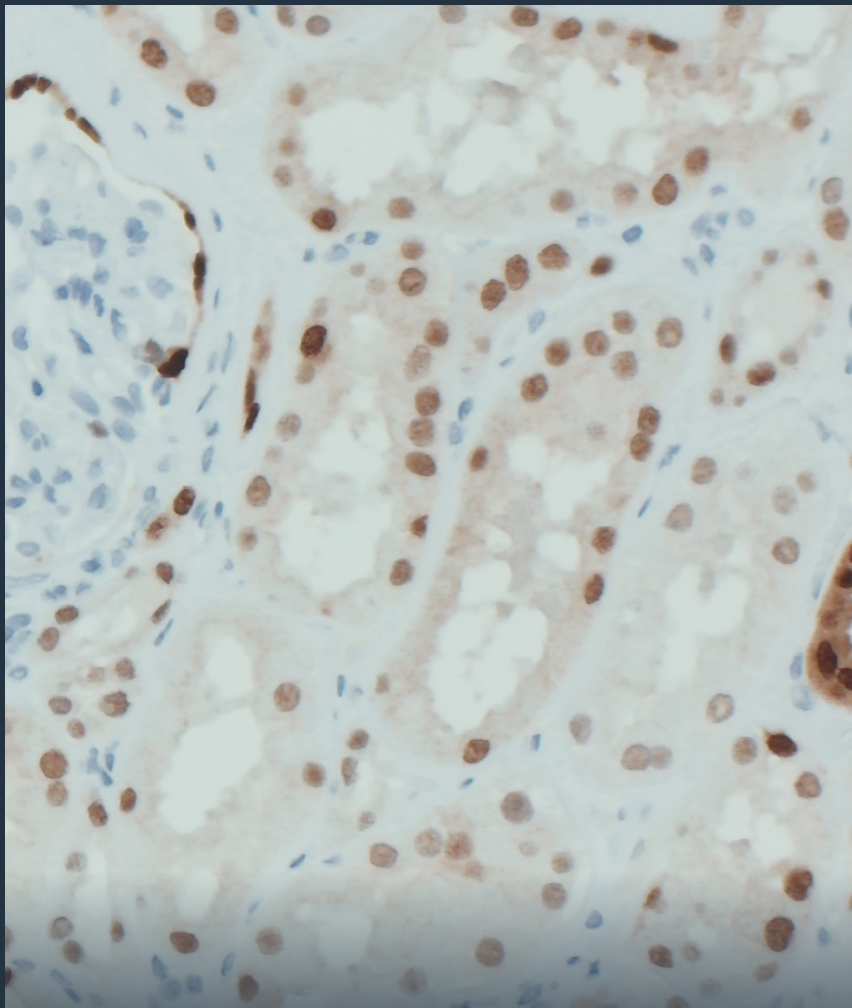


Table 5. Overview of the assessment marks for mAb clone MRQ-50 on the four main IHC instruments in runs 62 and 64 (cumulated data for both RTU and concentrate).

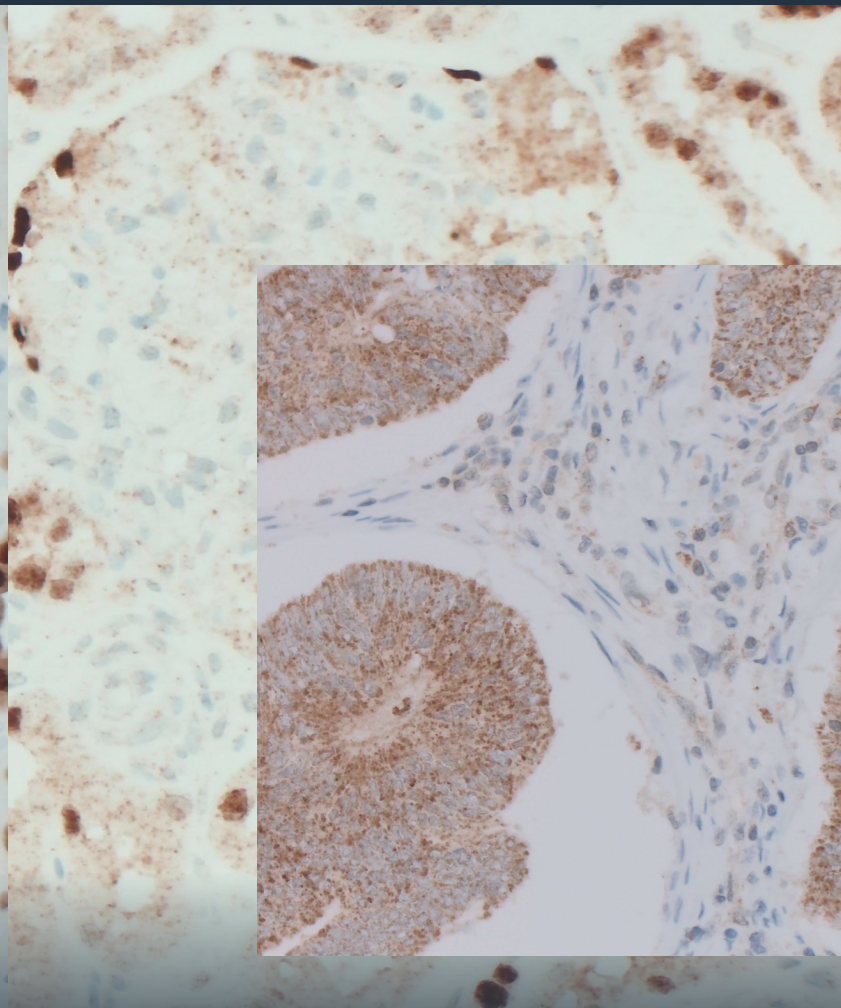
MRQ-50 score	Dako/Agilent Autostainer	Dako/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max
Optimal	-	-	-	-
Good	22	-	12	31
Borderline	4	11	110	3
Poor	-	3	59	-
Total	26	14	181	34
Sufficient %	85%	0%	7%	91%

Kidney

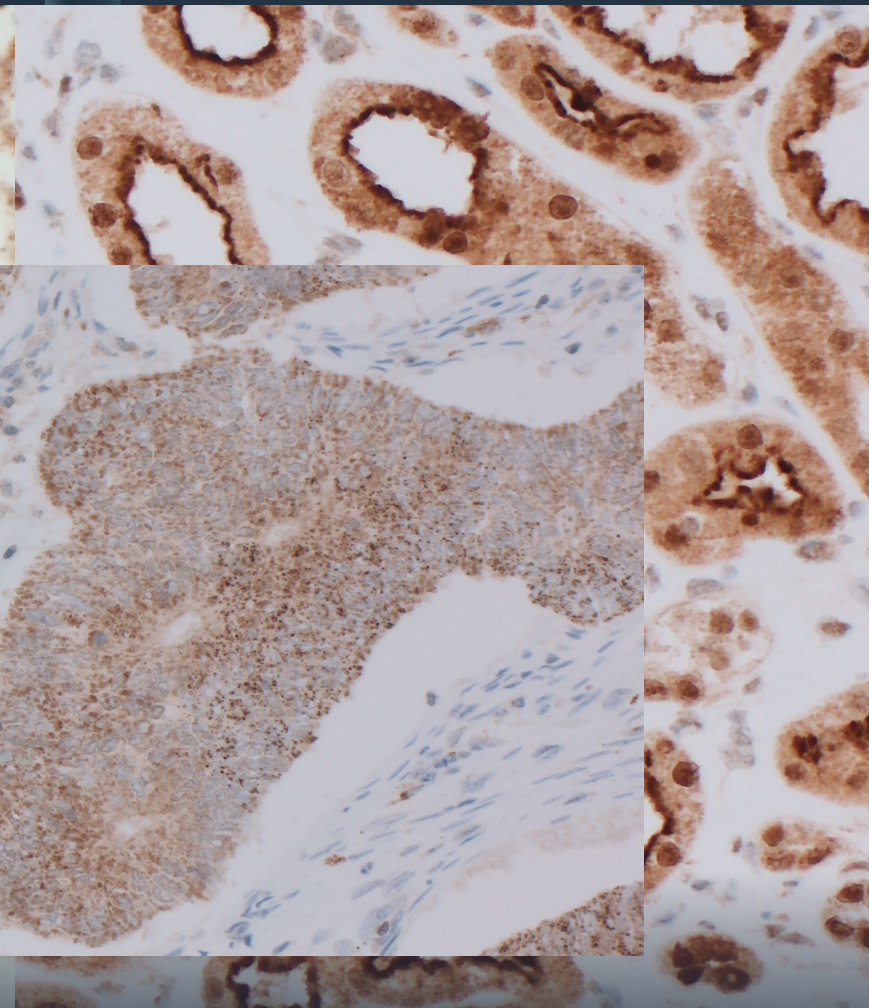
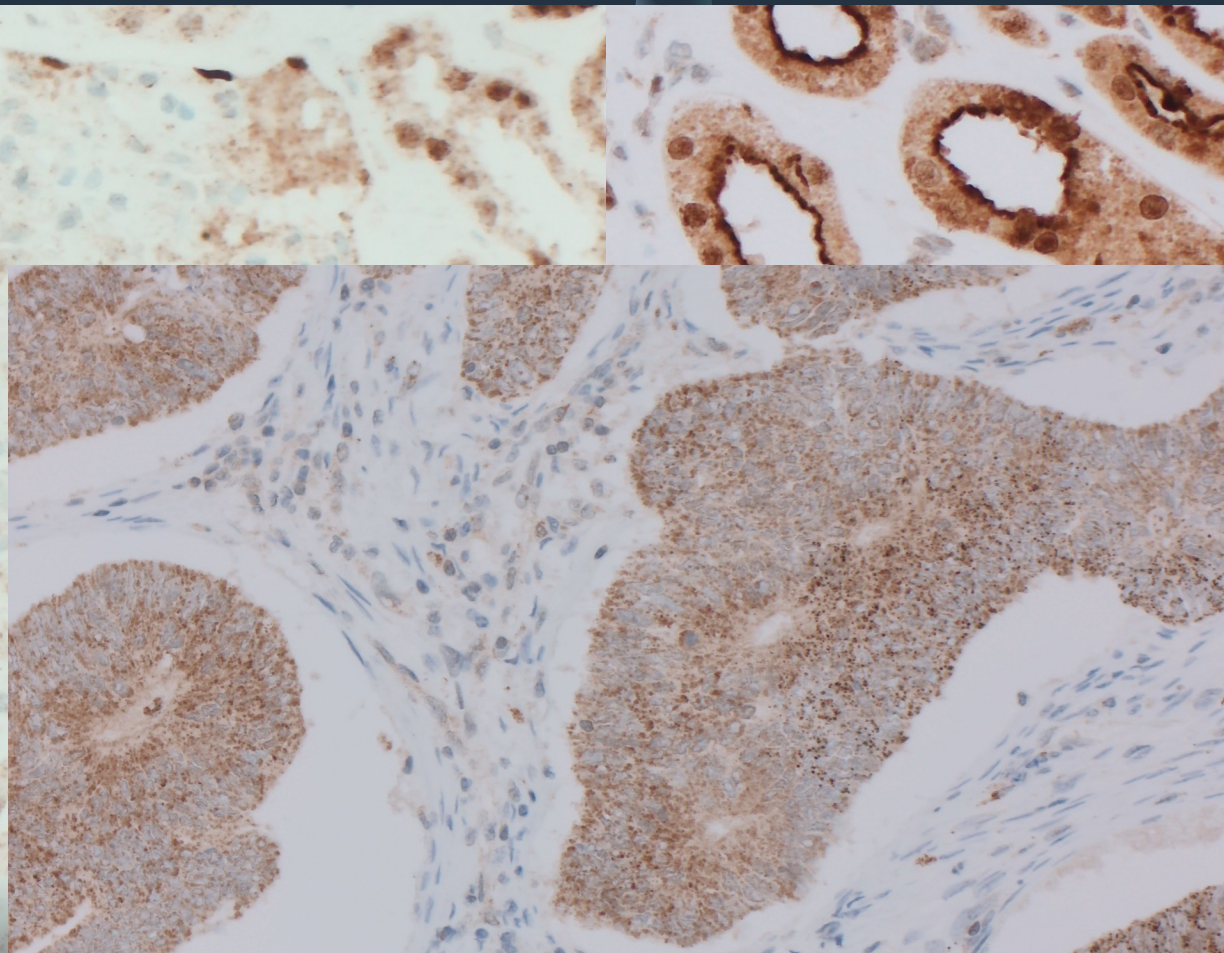
SP348 - Ventana



MRQ-50 - Ventana

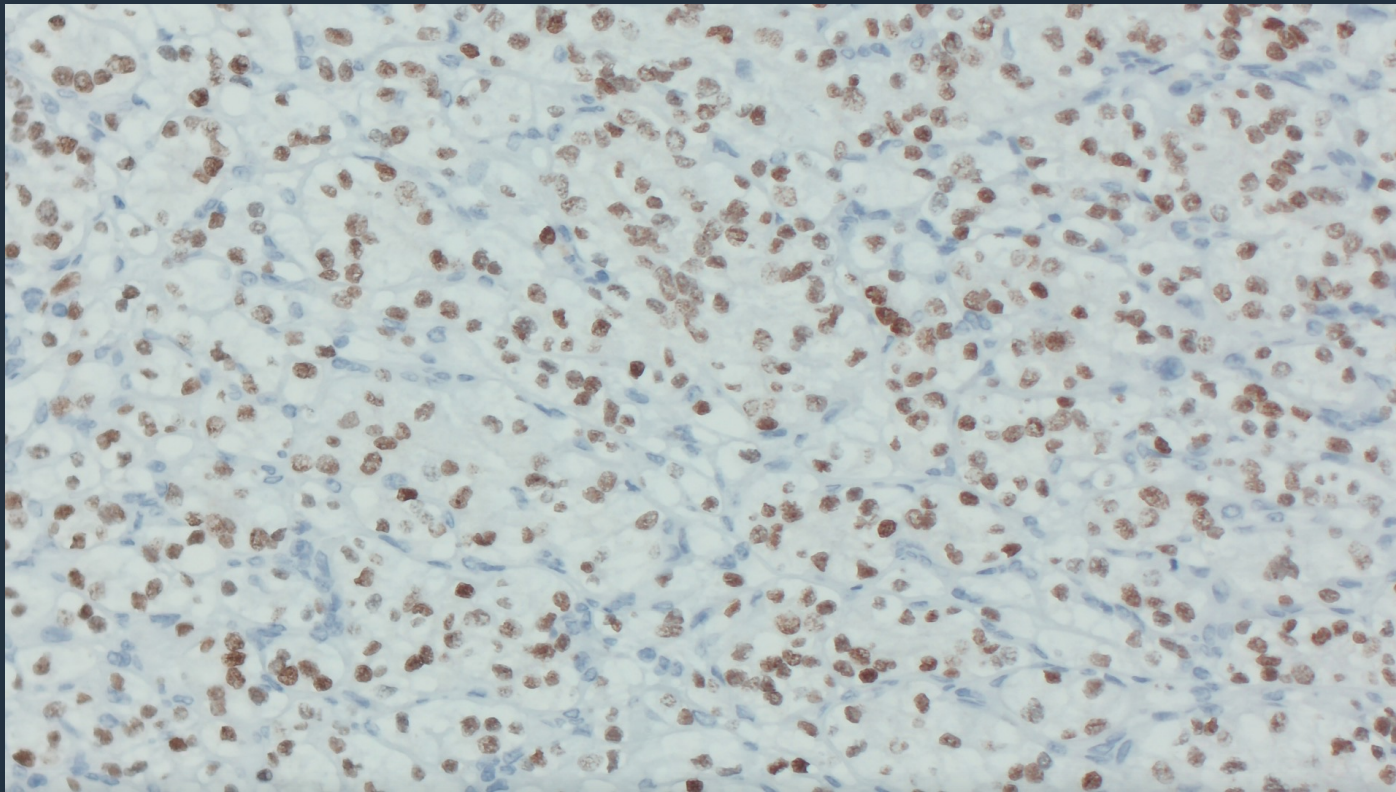


EP331 Autostainer

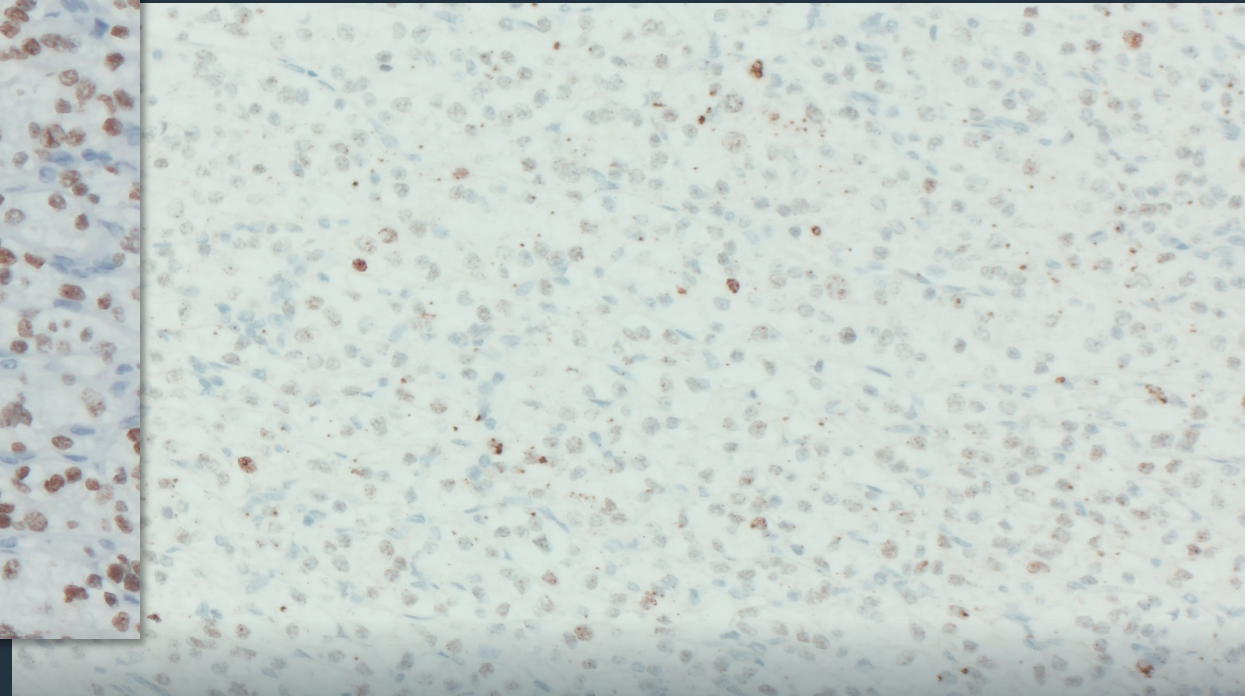


Renal clear cell carcinoma

SP348 ventana



MRQ-50 ventana





Immunostainer	
Type:	Ventana Benchmark Ultra
Primary antibody	
Clone:	SP348
Producer:	Abcam
Product no. / lot no.:	ab227707 / GR3298900-1
Diluent:	Antibody Diluent
Dilution factor:	1:100
Incubation time / temperature:	32 min. / 36°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Ventana Ultra CC1
Heating time at max. temp.:	64 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	36°C



Immunostainer	
Type:	Leica BOND III
Primary antibody	
Clone:	ZR1
Producer:	Zeta Corportion
Product no. / lot no.:	Z2202 / Z220RT
Diluent:	Bond Antibody Diluent
Dilution factor:	1:25
Incubation time / temperature:	45 min. / 20°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Leica Bond Epitope Retrieval Solution 2
Heating time at max. temp.:	30 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	20°C



Immunostainer	
Type:	Dako Omnis
Primary antibody	
Clone:	SP348
Producer:	Abcam
Product no. / lot no.:	ab227707 / GR33234272
Diluent:	Renoir Red Diluent
Dilution factor:	1:200
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:	Rabbit LINKER
Incubation time linker:	10 min.
Incubation time polymer:	20 min.
Incubation temperature:	32°C

Immunostainer	
Type:	Dako Autostainer Link 48 +
Primary antibody	
Clone:	SP348
Producer:	Gennova
Product no. / lot no.:	AP10761CM / .
Diluent:	Antibody Diluent
Dilution factor:	1:100
Incubation time / temperature:	30 min. / 23°C
Epitope retrieval, HIER	
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	97°C
Visualization system	
Producer:	Dako
Product / no:	EnVision FLEX+ / K8002/SM802
Linker:	Linker, Rabbit
Incubation time linker:	10 min.
Incubation time polymer:	20 min.
Incubation temperature:	23°C



GATA3

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone L50-823	88	Cell Marque	31	40	33	24	56%	25%
	24	Biocare						
	4	BD Pharmingen						
	3	Zytomed Systems						
	3	Gennova						
	2	Bio-SB						
	2	Immunologic						
	1	Anacrom						
	1	DBS						
rmAb clone EP368	5	Cell Marque	4	-	1	1	67%	67%
	1	Quartett						
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 L50-823 760-4897³	56	Ventana/Roche	36	12	8	-	86%	64%
mAb clone L50-823 760-4897⁴	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	Master Diagnostica	1	2	1	-	-	-
	1	Vitro SA						
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%	

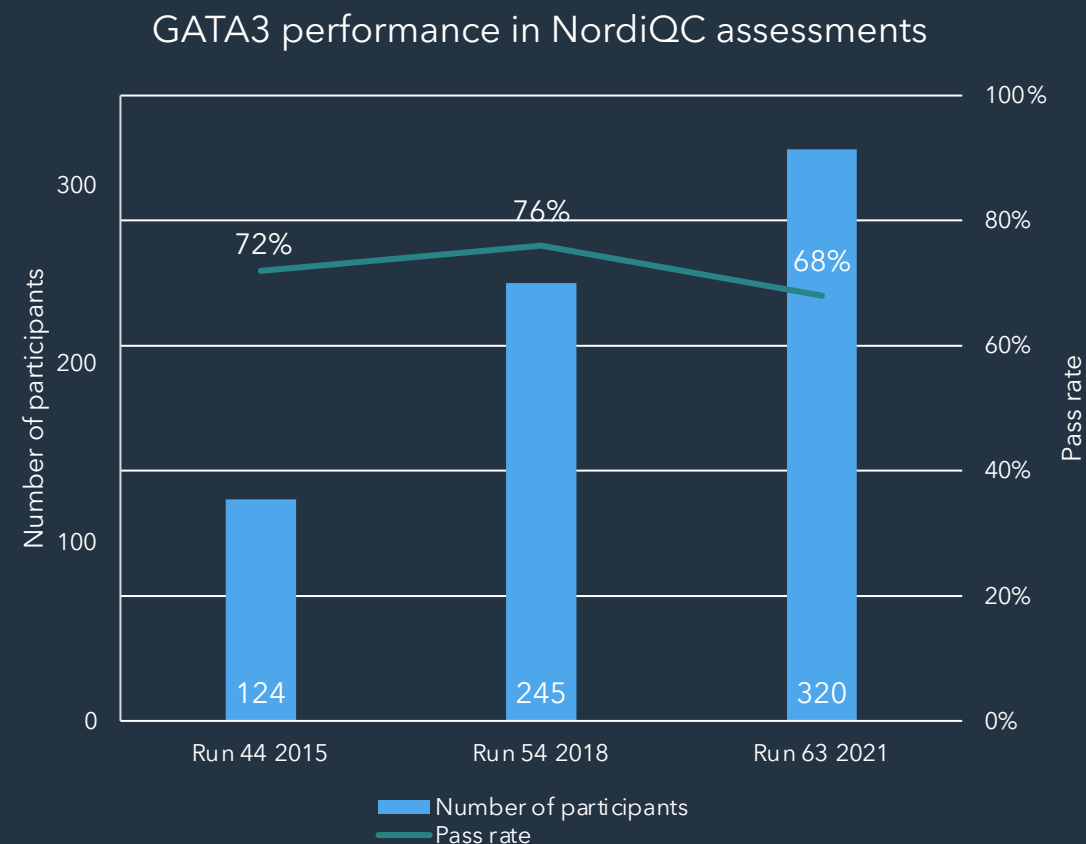


Table 4. Summarization of the proportion of sufficient and optimal marks using either 2- or 3-layer detection systems.**

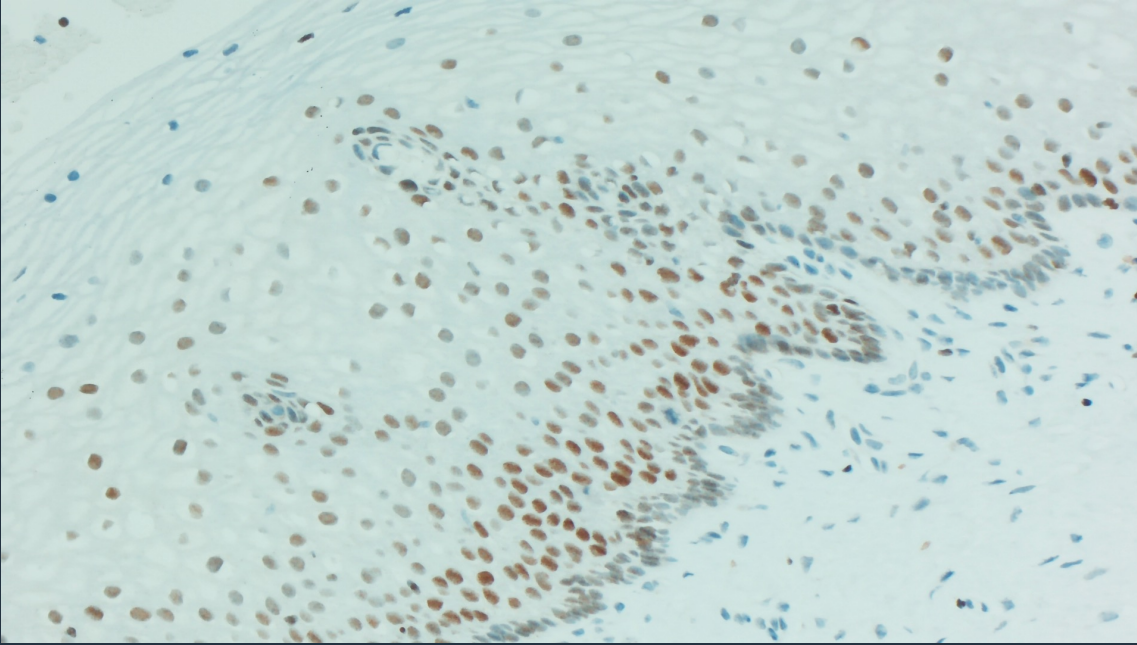
		2-layer detection system		3-layer detection system	
Antibodies	n	Sufficient	Optimal	Sufficient	Optimal
mAb conc L50-823 Cell Marque	88	36% (4/11)	9% (1/11)	74% (57/77)	36% (28/77)
mAb conc L50-823 Biocare Medical	24	(0/1)	(0/1)	26% (6/23)	9% (2/23)
mAb clone RTU L50-823 760-4897* Ventana/Roche	107	53% (18/34)	6% (2/34)	99% (84/85)	88% (75/85)
mAb clone RTU L50-823 390M-17,18,10 Cell Marque	42	27% (4/15)	13% (2/15)	96% (26/27)	52% (14/27)
mAb clone RTU L50-823 PM 405AA Biocare Medical	12	(0/2)	(0/2)	80% (8/10)	50% (5/10)

*Only protocols performed on the intended IHC stainer device are included.

** regardless of the protocol settings applied e.g., HIER time and/or incubation time in the primary Ab (≥10 protocols assessed).

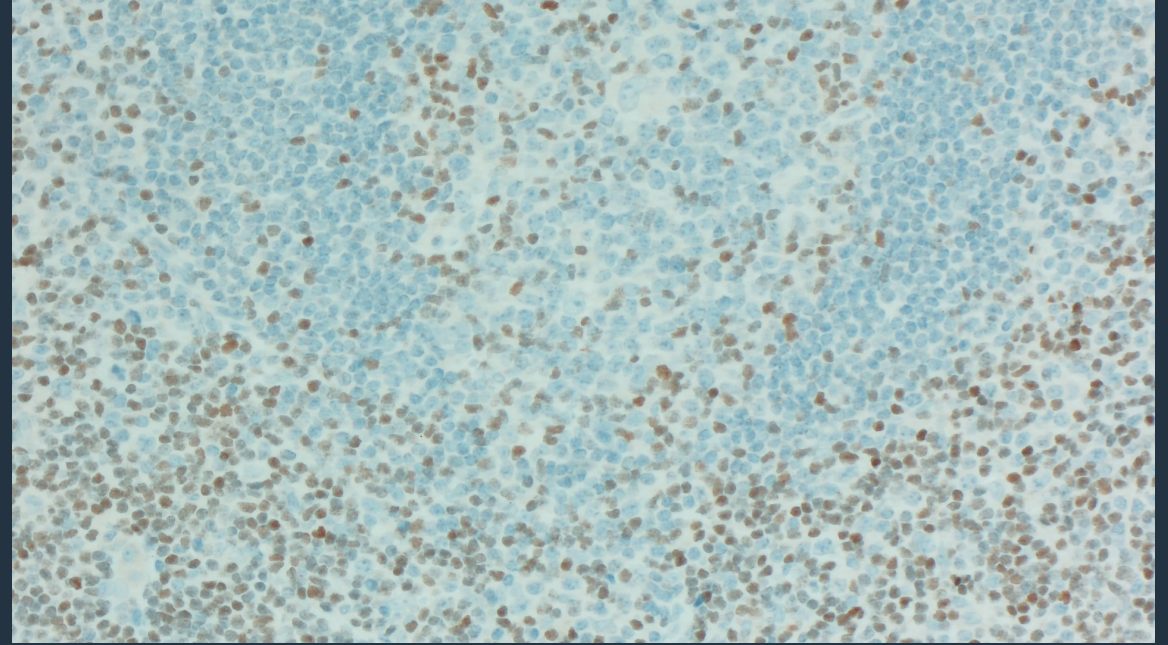
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Uterine Cervix

The squamous epithelial cells in the basal and intermediate layer of the surface epithelium display a weak to moderate, but distinct nuclear staining reaction, whereas the nuclei of superficial layers and stroma cells are negative.

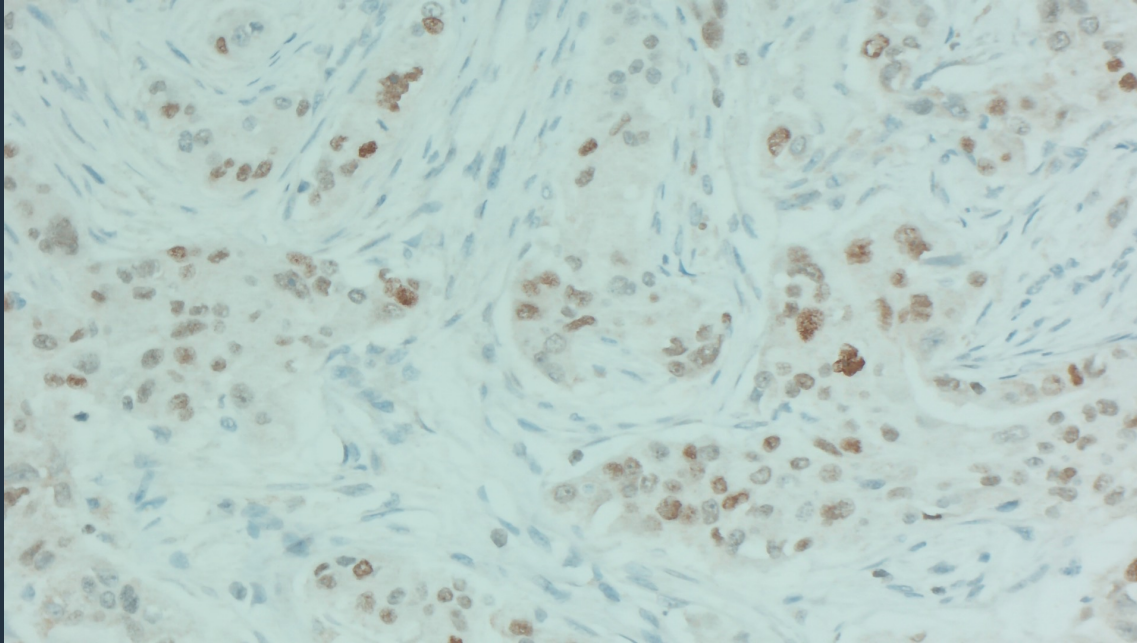


Tonsil

The vast majority of T helper cells (Th2) display a moderate but distinct nuclear staining reaction, whereas the B-cells are negative.

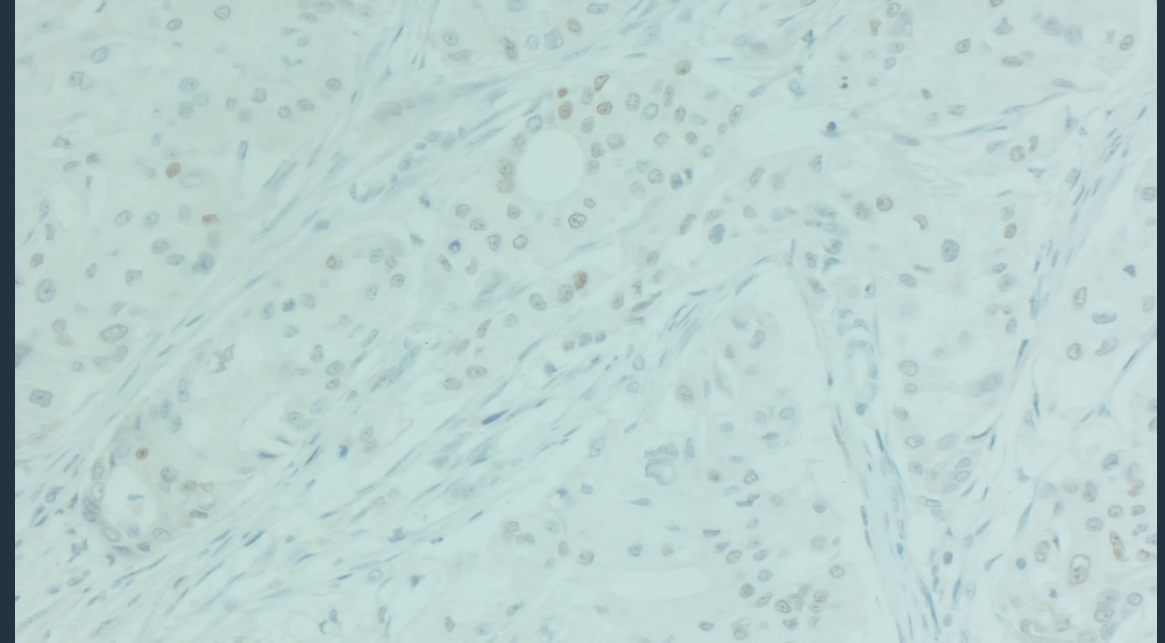


Trippel negative breast tumor with low level GATA3



Optimal

RTU system 760-4897 (Ventana/Roche), based on the mAb clone L50-823, applying vendor recommended protocol settings and **OptiView** as detection system. A weak to strong nuclear staining reaction of virtually all neoplastic cells are seen.



Insufficient

RTU system 760-4897 (Ventana/Roche), based on the mAb clone L50-823, applying vendor recommended protocol settings and **UltraView** as detection system. The vast majority of neoplastic cells are false negative and only few are weakly positive



Type:	Ventana Benchmark Ultra
Primary antibody	
Clone:	L50-823
Producer:	Cell Marque
Product no. / lot no.:	390M-16 / 0000052937
Diluent:	Antibody Diluent
Dilution factor:	1:100
Incubation time / temperature:	48 min. / 36°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Ventana CC1
Heating time at max. temp.:	48 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	36°C

Type:	Leica BOND III
Primary antibody	
Clone:	L50-823
Producer:	Cell Marque
Product no. / lot no.:	390M-16 / 0000091031
Diluent:	Bond Antibody Diluent
Dilution factor:	1:250
Incubation time / temperature:	15 min. / 21°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Leica Bond Epitope Retrieval Solution 2
Heating time at max. temp.:	20 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	21°C

Type:	Dako Omnis
Primary antibody	
Clone:	L50-823
Producer:	Cell Marque
Product no. / lot no.:	390M-16 / 1602105L
Diluent:	Antibody Diluent
Dilution factor:	1:100
Incubation time / temperature:	20 min. / 21°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:	21°C

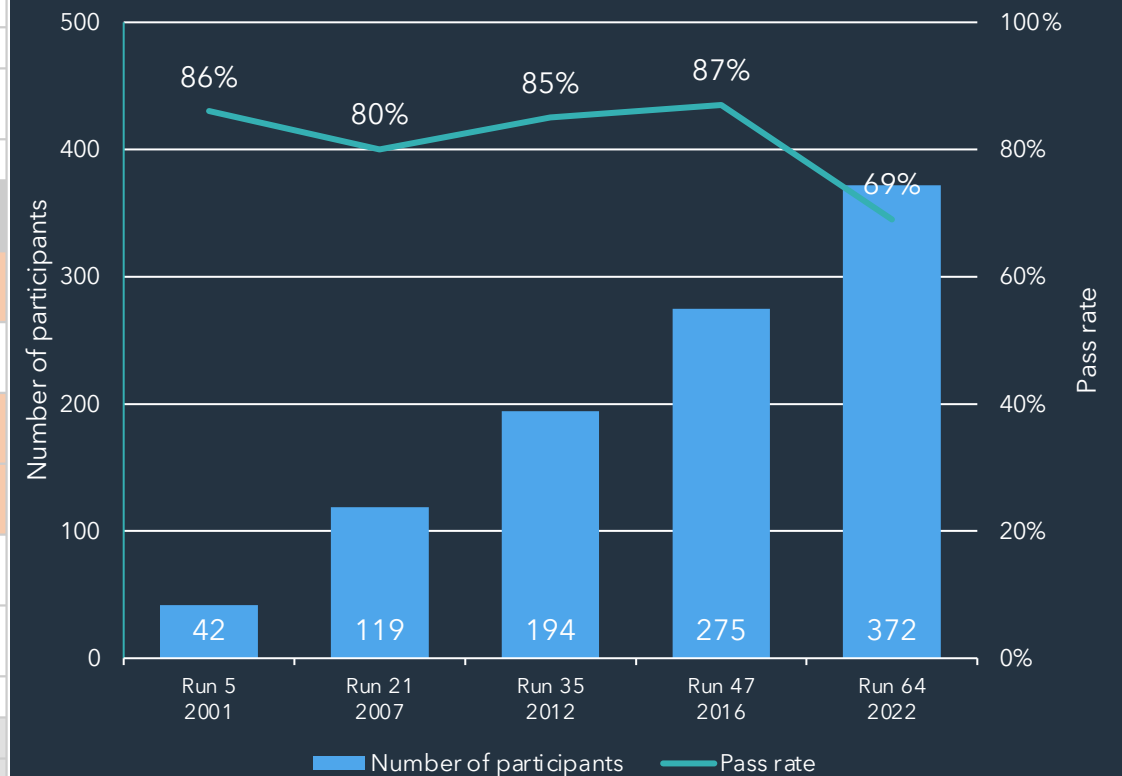
Type:	Dako Autostainer Link 48 +
Primary antibody	
Clone:	L50-823
Producer:	Cell Marque
Product no. / lot no.:	390M-16 / 0000010688
Diluent:	Antibody Diluent
Dilution factor:	1:250
Incubation time / temperature:	20 min. / 23°C
Epitope retrieval, HIER	
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	97°C
Visualization system	
Producer:	Dako
Product / no:	EnVision FLEX+ / K8002/SM802
Linker:	Linker, Mouse
Incubation time linker:	15 min.
Incubation time polymer:	20 min.
Incubation temperature:	23°C



Desmin

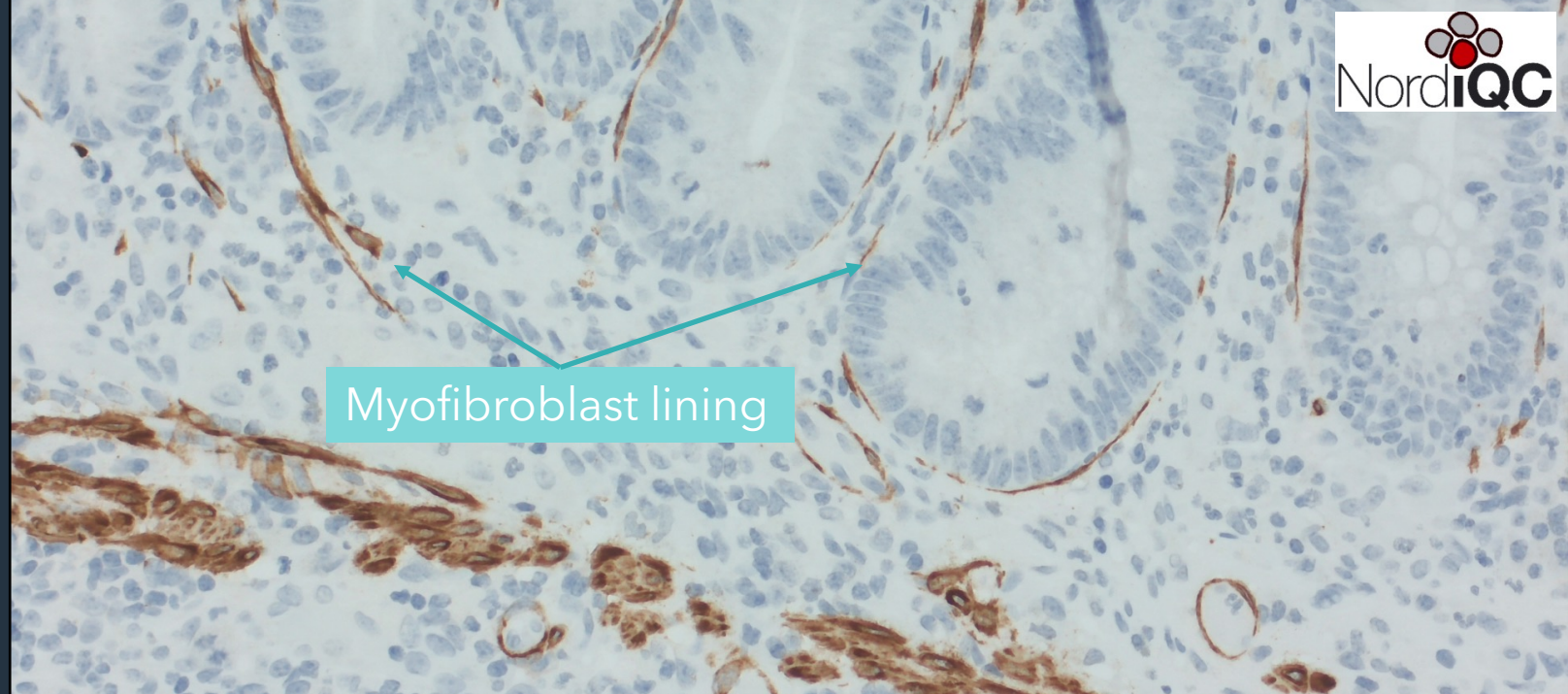
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone D33	79 5 2 2 2 1 1 1	Dako/Agilent Cell Marque Epredia Monosan Zytomed BioGenex Biolyx Biotech Diagnostic Biosystems	28	45	17	3	78%	30%
mAb clone DE-R-11	15	Leica Biosystems	9	4	2	-	87%	60%
mAb clone BS21	8 1	Nordic Biosite Optibodies	8	1	-	-	100%	89%
Conc total	122		47	52	20	3	81%	39%
Ready-To-Use antibodies							Suff. ¹	OR. ²
mAb clone DE-R-11³ 760-2513	1	Ventana/Roche	-	-	1	-	-	-
mAb clone DE-R-11⁴ 760-2513	139	Ventana/Roche	75	23	32	9	71%	54%
mAb clone D33 IR/IS606³	21	Dako/Agilent	3	9	8	1	57%	14%
mAb clone D33 IR/IS606⁴	50	Dako/Agilent	2	9	18	21	22%	4%
mAb clone DE-R-11 PA0032³	12	Leica Biosystems	11	1	-	-	100%	92%
mAb clone DE-R-11 PA0032⁴	9	Leica Biosystems	7	2	-	-	100%	88%
RTU total	248		105	52	59	32	63%	42%
Total	370		152	104	79	35	256	
Proportion			41%	28%	21%	10%	69%	

Desmin performance in NordiQC assessments



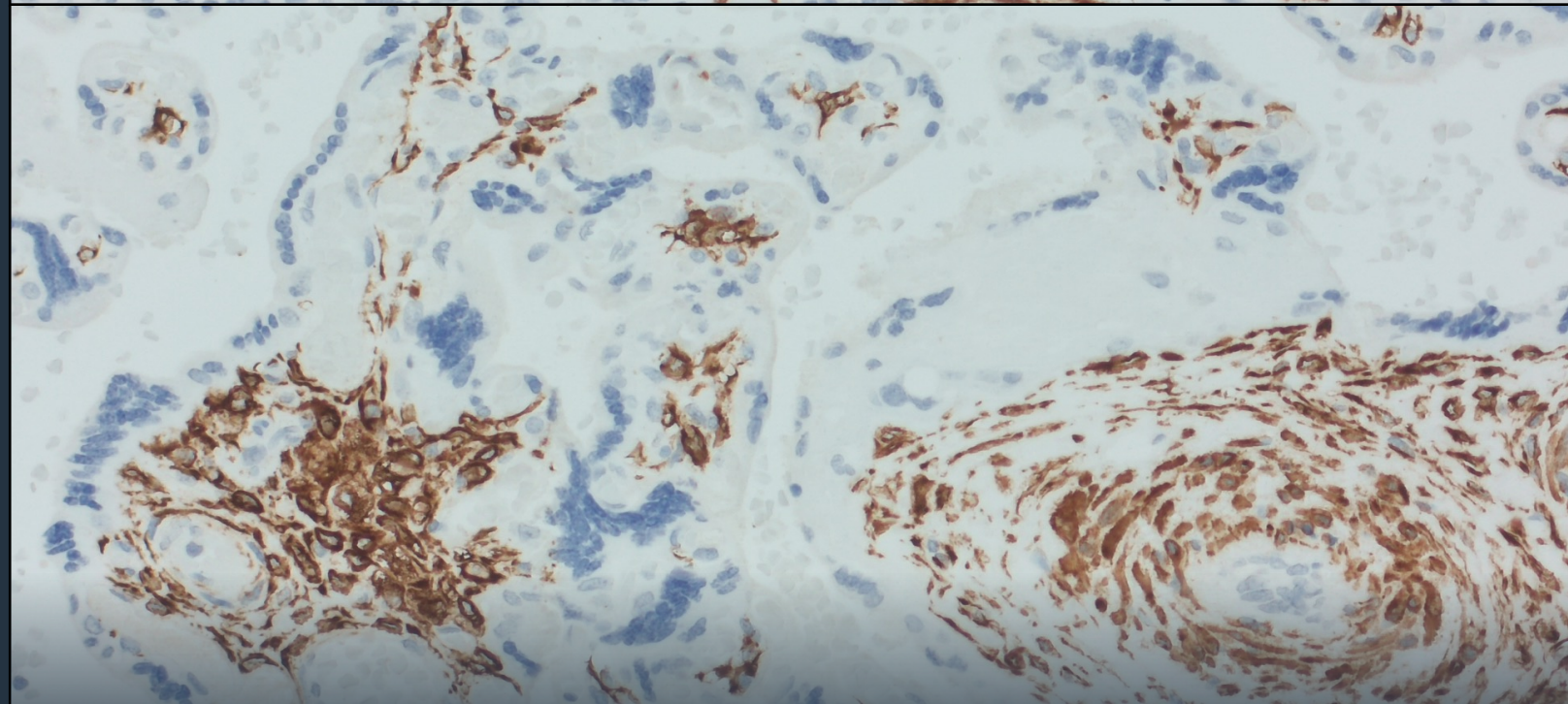
Appendix

The smooth muscle cells of lamina muscularis propria and myofibroblasts lining the epithelial cells show a moderate to strong staining reaction. No background staining is seen.



Placenta

The vast majority of smooth muscle in vessels in the stromal compartment of villi show a moderate to strong cytoplasmic staining reaction. No reaction of the cytotrophoblastic and syncytiotrophoblastic cells in the placenta was seen.



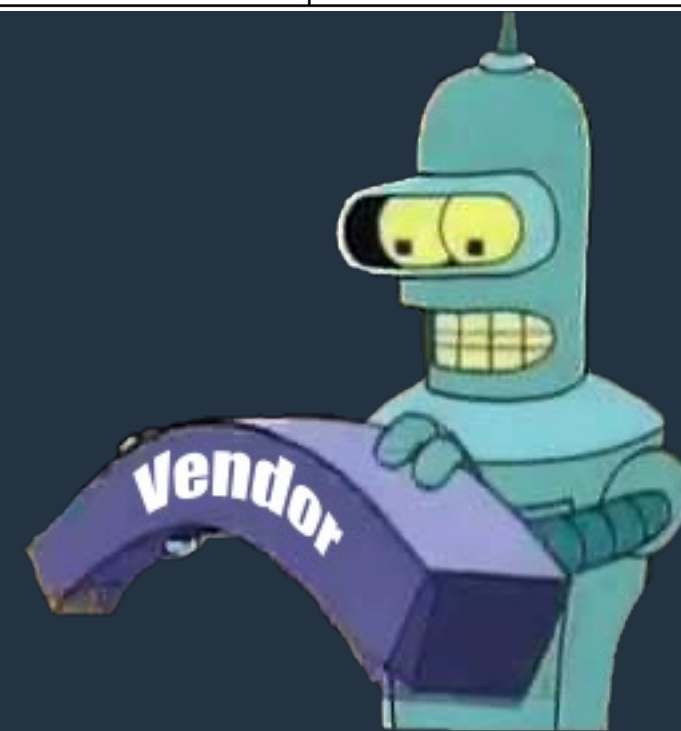
RTU product IR/IS606 D33 on Omnis using Flex+ protocol

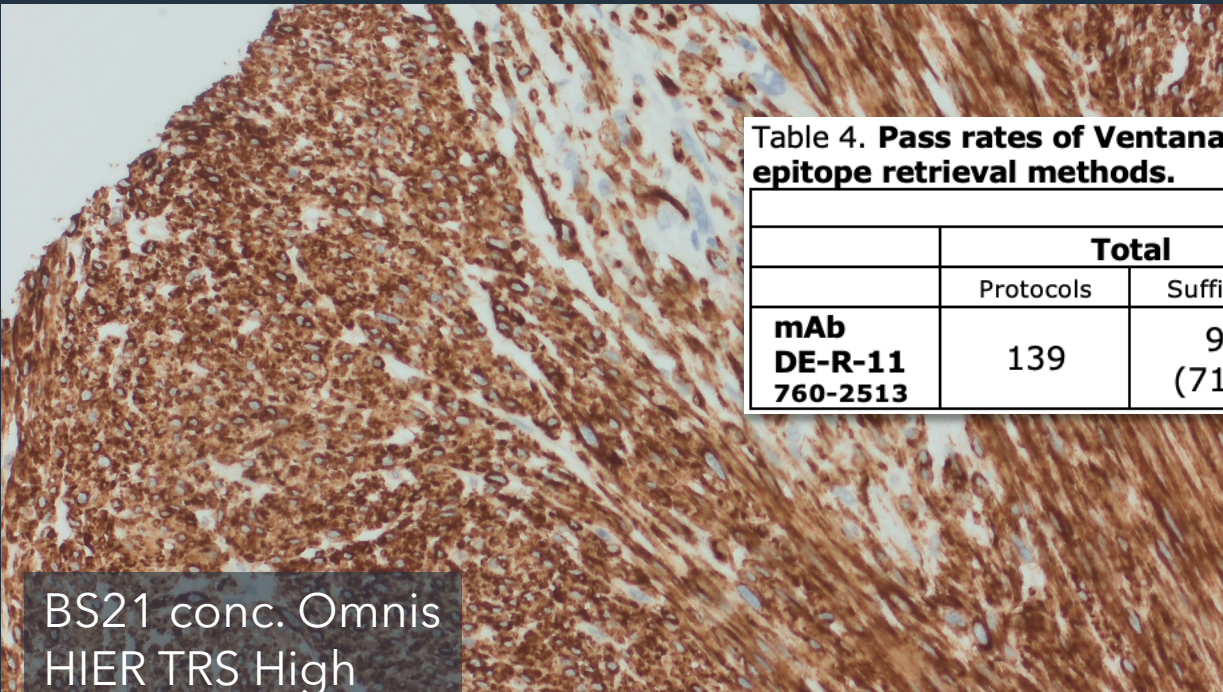
Table 3. **Proportion of sufficient and optimal results for Desmin for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb DE-R-11 760-2513	-	-	70% (97/138)	53% (74/138)
Dako AS mAb D33 IR/IS606	57% (12/21)	14% (3/21)	100% (6/6)	17% (1/6)
Leica Bond III/MAX mAb DE-R-11 PA0032	100% (12/12)	92% (11/12)	100% (5/5)	100% (5/5)

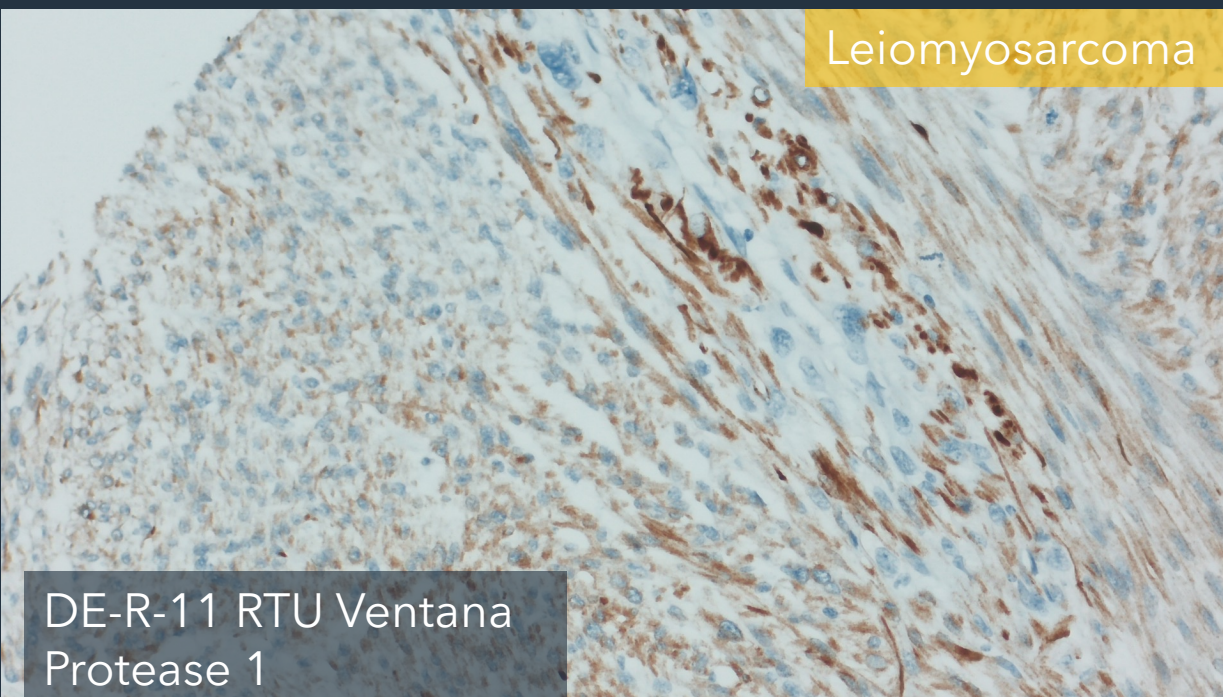
Table 2. **Proportion of optimal results for Desmin for the most commonly used antibodies as concentrate on the four main IHC systems***

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	ER2 pH 9.0	ER1 pH 6.0
mAb clone D33	4/9** (44%)	0/1	0/14	-	9/43 (21%)	-	13/16 (81%)	1/2
mAb clone DE-R-11	-	-	3/5 (60%)	-	2/6 (33%)	-	4/4 (100%)	-
mAb clone BS21	-	-	7/7 (100%)	-	-	-	0/1	-





BS21 conc. Omnis
HIER TRS High



Leiomyosarcoma

DE-R-11 RTU Ventana
Protease 1

Table 4. **Pass rates of Ventana/Roche RTU DE-R-11 antibody on the Benchmark platform for different epitope retrieval methods.**

Pass rate								
	Total		HIER		Proteolysis		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb DE-R-11 760-2513	139	98 (71%)	74	73 (99%)	49	11 (22%)	16	13 (81%)

Table 1. Recommended Staining Protocols for CONFIRM anti-Desmin (DE-R-11)

Procedure Type	Platform or Method	
	NexES IHC	BenchMark Series
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None required	None required
Enzyme (Protease)	Protease 1, 8 minutes	Protease 1, 8 minutes
Antibody (Primary)	Approximately 32 minutes, 37° C	Approximately 16 minutes, 37° C
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin II, 2 to 4 minutes	Hematoxylin II, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes



Type:	Ventana Benchmark Ultra
Primary antibody	
Clone:	DE-R-11
Producer:	Ventana
Product no. / lot no.:	760-2513 / H04767
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	24 min. / 36°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Ventana Ultra CC1
Heating time at max. temp.:	24 min.
Maximum heating temp.:	100°C
Epitope retrieval, proteolysis	
Enzyme:	Protease 3
Enzyme producer / no:	Ventana / 760-2020
Incubation time / temp:	4 min. / 36°C
Visualization system	
Producer:	Ventana
Product / no:	OptiView DAB IHC Detection Kit / 760-700
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	36°C

Type:	Leica BOND III
Primary antibody	
Clone:	DE-R-11
Producer:	Leica
Product no. / lot no.:	PA0032 / 69490
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	15 min. / 20°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Leica Bond Epitope Retrieval Solution 2
Heating time at max. temp.:	20 min.
Maximum heating temp.:	100°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	20°C

Type:	Dako Omnis
Primary antibody	
Clone:	BS21
Producer:	Nordic Biosite
Product no. / lot no.:	BSH-7082 / BSH-11u
Diluent:	Antibody Diluent
Dilution factor:	1:50
Incubation time / temperature:	30 min. / 21°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:	21°C

Type:	Dako Autostainer Link 48 +
Primary antibody	
Clone:	D33
Producer:	Dako
Product no. / lot no.:	IR606 / IS606 / 20082709
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	20 min. / 21°C
Epitope retrieval, HIER	
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	99°C
Visualization system	
Producer:	Dako
Product / no:	EnVision FLEX / K8000/SM802
Linker:	None
Incubation time polymer:	20 min.
Incubation temperature:	21°C



