NordiQC data: Antibody selection, protocols and controls

The generel module

Tanya Julio
Histotechnologist
Pathology department
Aarhus University Hospital, DK
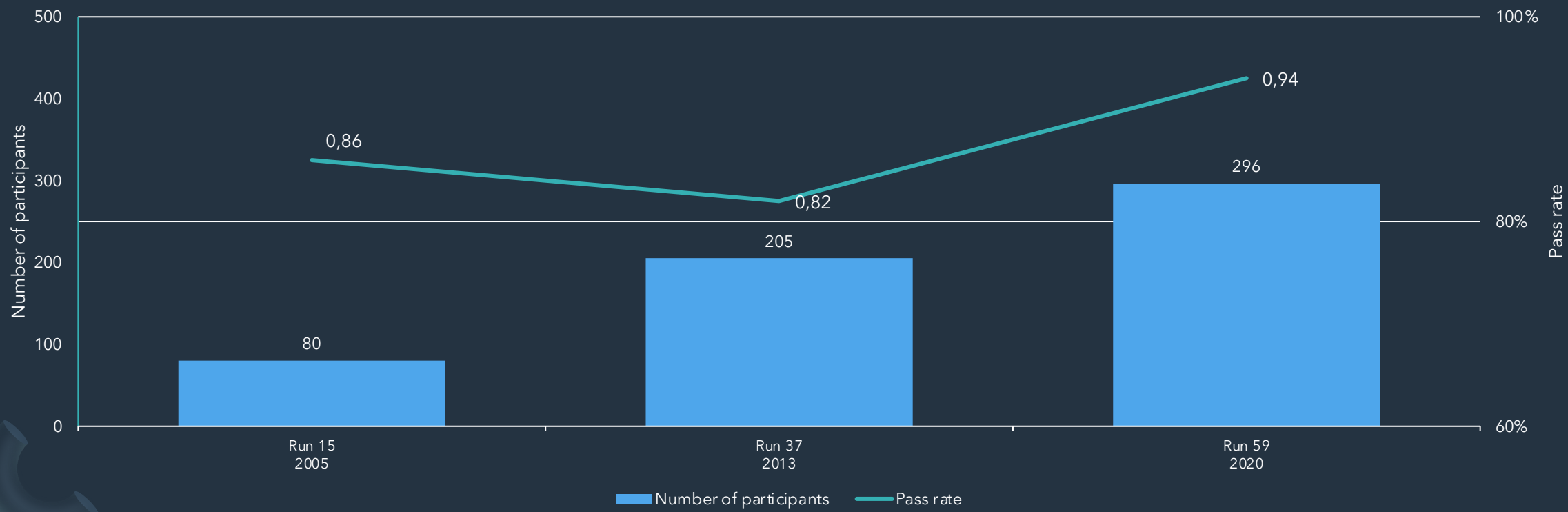
Primary panel for the unknown primary tumour

	CD45	Pan-CK	S100	Vimentin
Haematolymphoid neoplasms	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial neoplasms	-	+/(-)	-/+	-/+
mesothelial neoplasms	-	+	-	+
mesenchymal and neuronal neoplasms	-	-/(+)	-/+	+
non-neuronal neuroephithelial 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

WHAT ARE YOU SAYING?

YOUR LANGUAGE J... LIKE GIBBERIS...
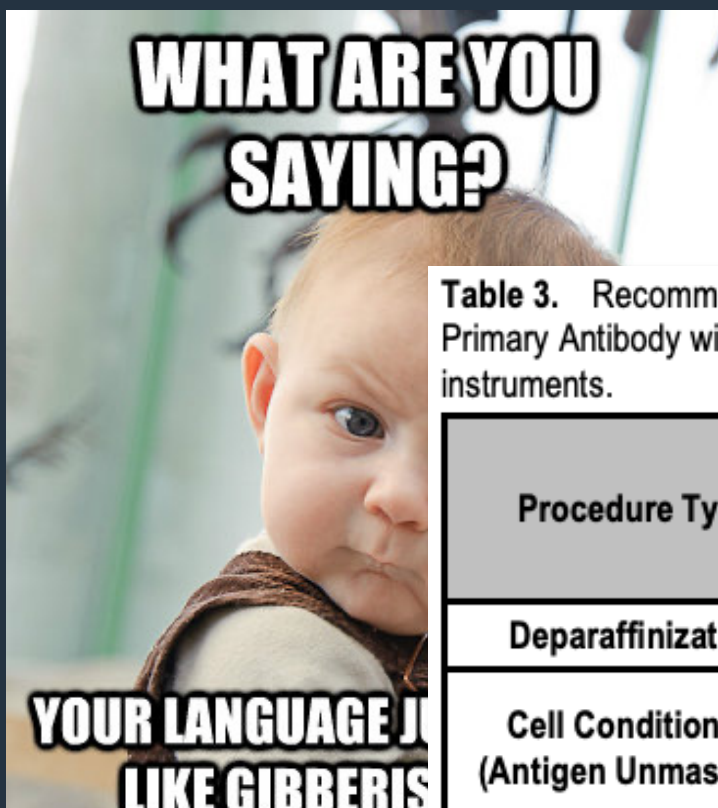
Table 1. Recommended Staining Protocols for CONFIRM anti-CD45, LCA (RP2/18)

Procedure Type	Platform or Method	
	NexES IHC	**BenchMark Series**
Deparaffinization	Off Line	Selected
		None required
		None required
		Approximately 16 minutes, 37° C
		Optional
		Optional
		Hematoxylin II, 2 to 4 minutes
		Bluing, 2 to 4 minutes

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
Cell Conditioning (Antigen Unmasking)	CC1, 16 minutes	CC1, 24 minutes	ULTRA CC1, 24 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	4 minutes, 37°C	4 minutes, 37°C	4 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

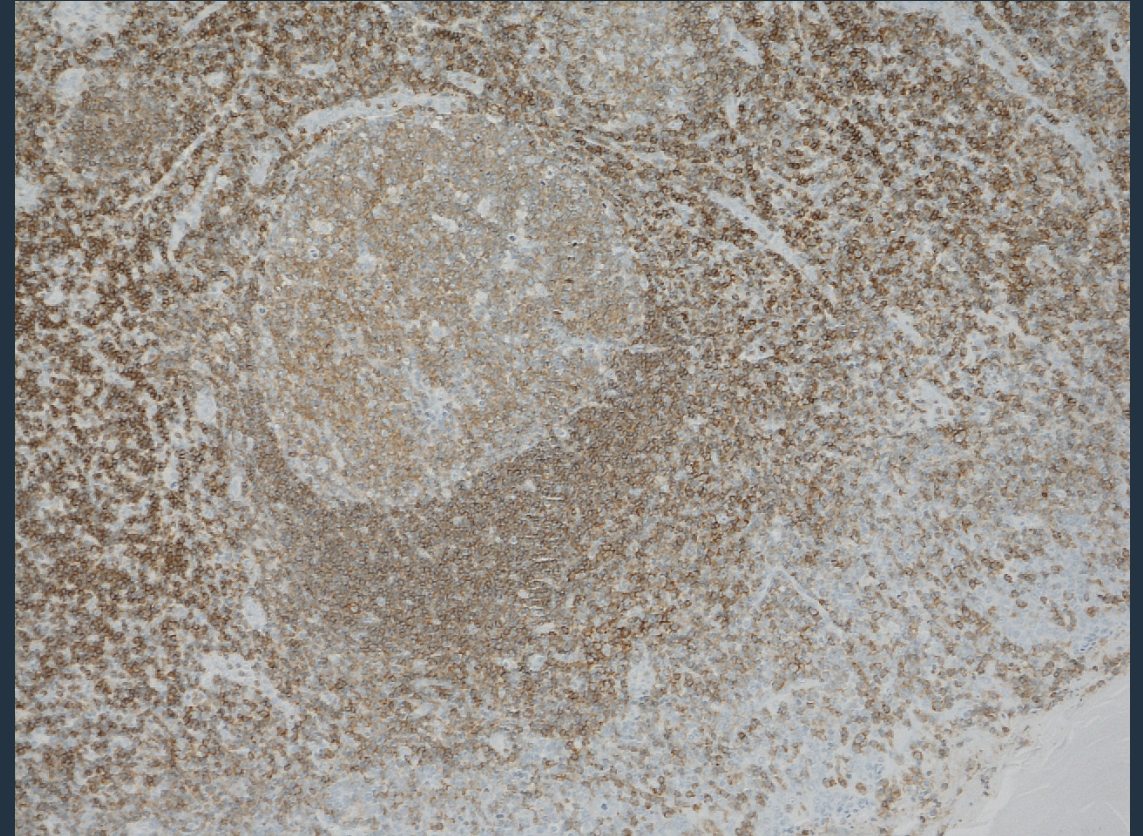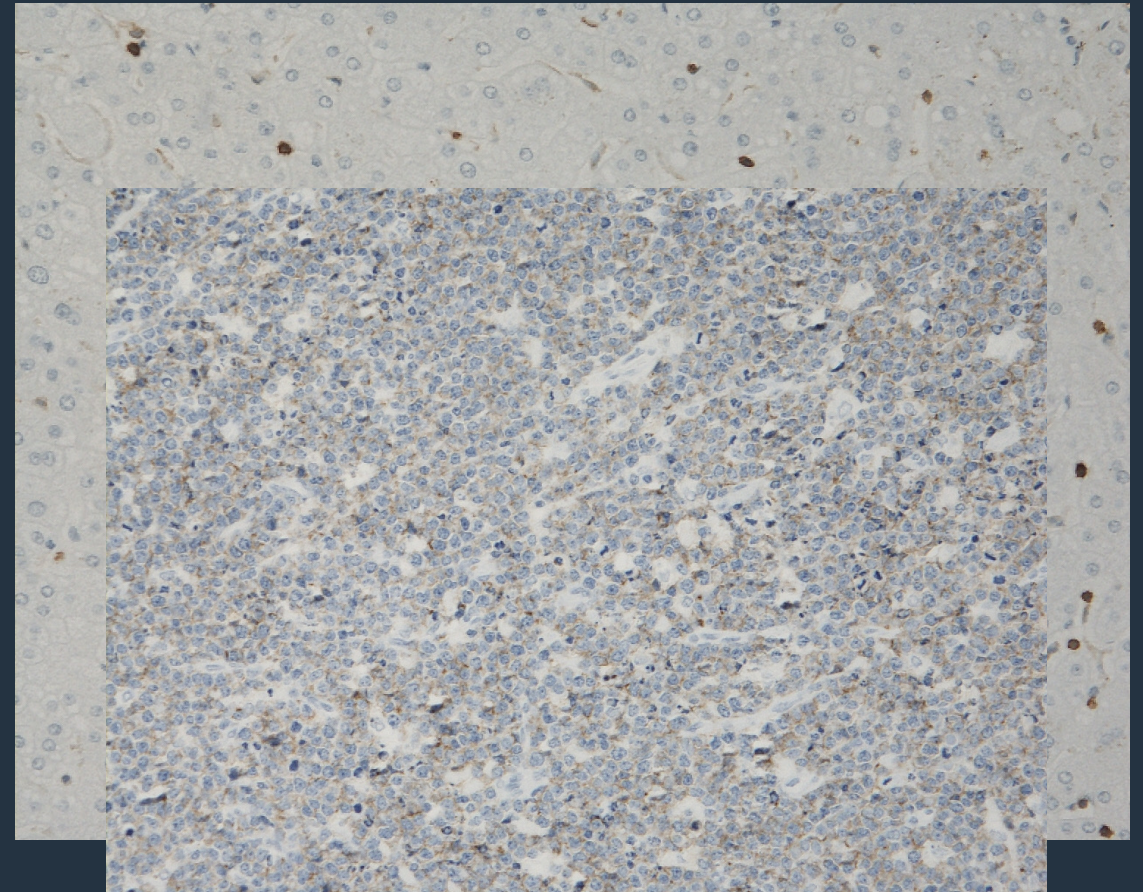|		
mAb clone **RP2/18** 760-2505 (VRPS)[3]	3	Ventana/Roc...
mAb clone **RP2/18** 760-2505 (LMPS)[4]	45	Ventana/Roc...

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

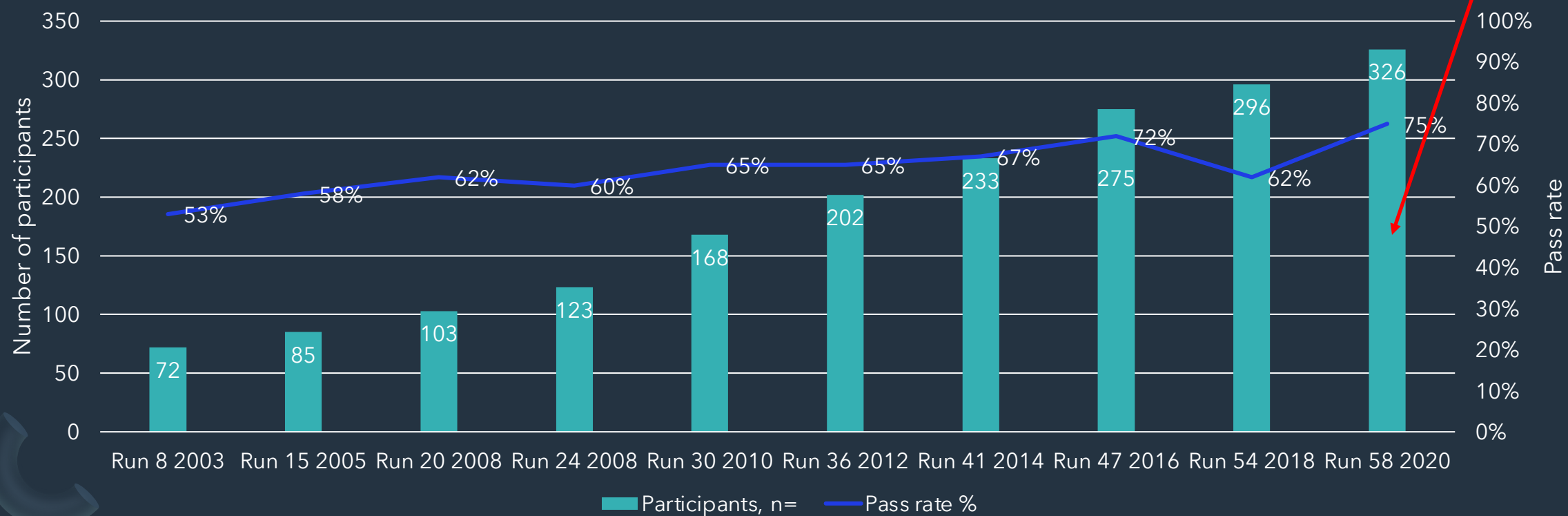CK-PAN performance in NordiQC assesments

52% optimal

Table (Ready-To-Use antibodies):

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
mAb clone cocktail AE1/AE3 IR053 (VRPS)[3]	13	Dako/Agilent	12	-	-	1	92%	92%
mAb clone cocktail			10	2	2	-	86%	71%
			27	1	2	1	90%	87%
			17	1	-	-	100%	94%
			11	8	4	2	76%	44%
AE1/AE3/PCK26 760-2135/2595 (LMPS)[4]		Ventana/Roche	29	19	10	11	70%	42%
mAb clone cocktail AE1/AE3 PA0909	2	Leica/Novocastra	-	1	1	-	-	-
mAb clone cocktail AE1/AE3 PA0094	5	Leica/Novocastra	1	3	1	-	80%	20%
mAb clone cocktail AE1/AE3 PA0012	3	Leica/Novocastra	-	3	-	-	-	-
Total	326		168	75	47	36	-	
Proportion			52%	23%	15%	11%	75%	

Table 2. **Proportion of optimal results for CK-PAN using the mAb clone cocktail AE1/AE3 as concentrate on the four main IHC systems***
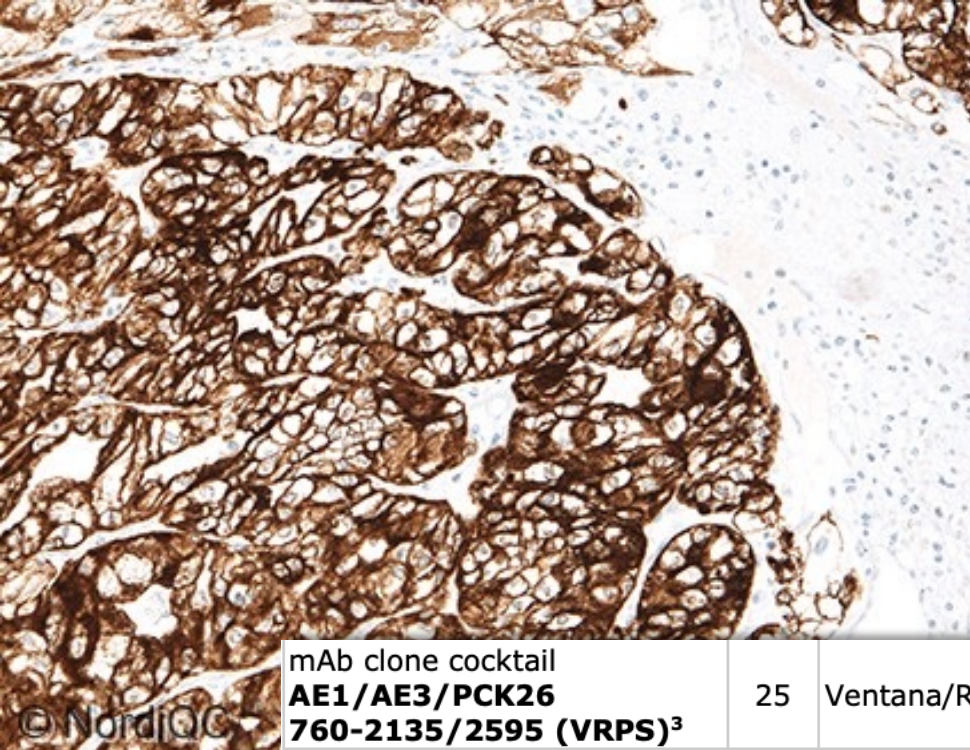
Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone AE1/AE3	5/9** (56%)	-	6/6 100%	-	36/62 (58%)	-	0/12 (0%)	0/3
mAb clone BS5	0/2	-	1/1	-	2/3	-	3/6	1/1

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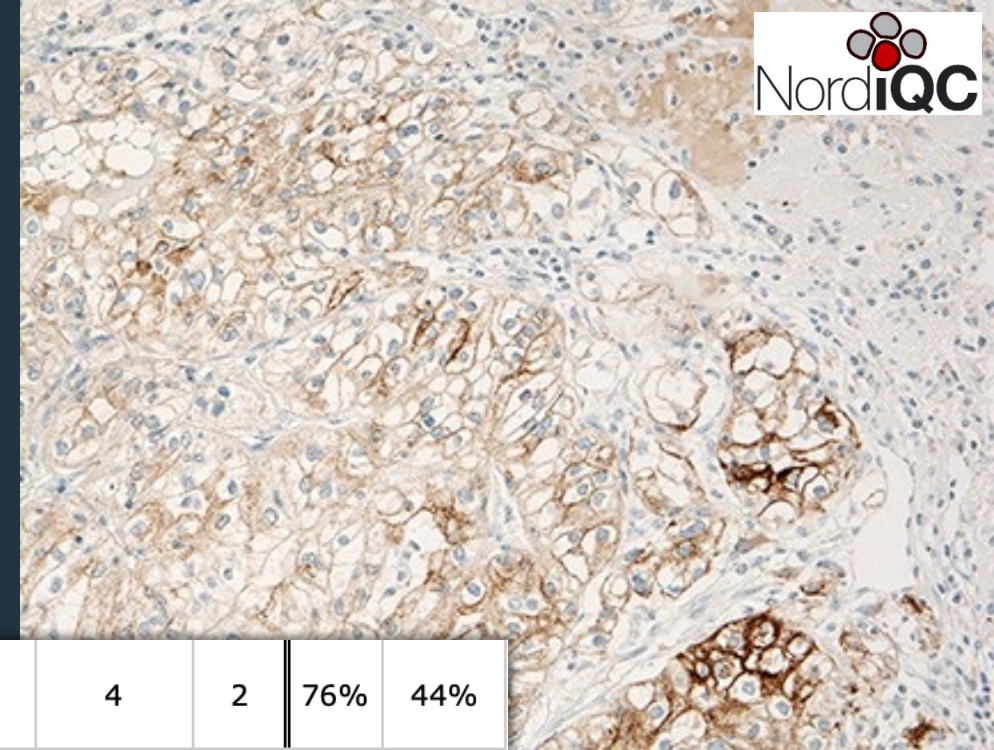** Number of optimal results/number of laboratories using this buffer.

HIER+P3
OptiView

HIER
OptiView

P1
OptiView

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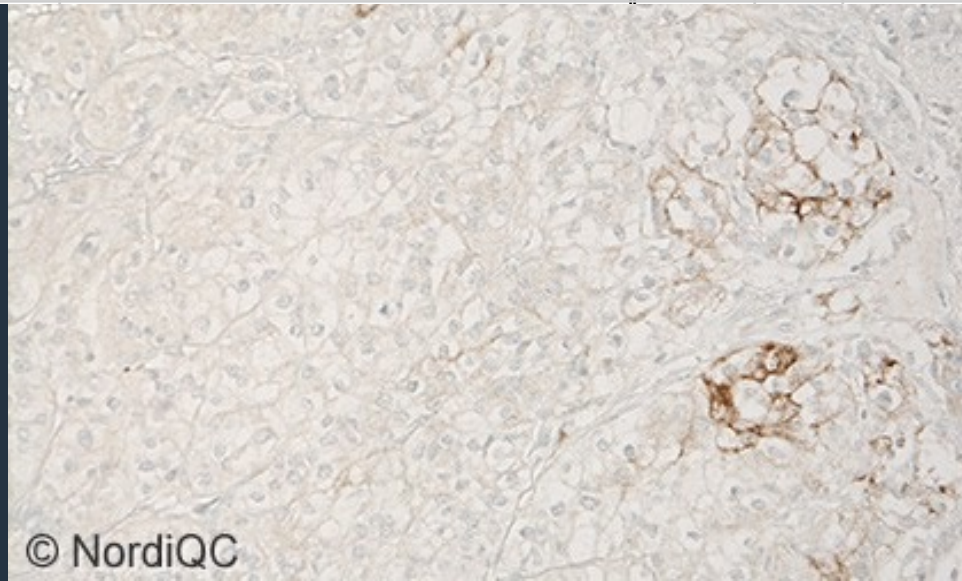
mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (VRPS)[3]	25	Ventana/Roche	11	8	4	2	76%	44%
mAb clone cocktail AE1/AE3/PCK26 760-2135/2595 (LMPS)[4]	69	Ventana/Roche	29	19	10	11	70%	42%

© NordiQC

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 *ultra*View 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 *ultra*View 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

P3+HIER

AE1/AE3/PCK26 RTU Ventana

Stand alone P1

All squarmous 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.
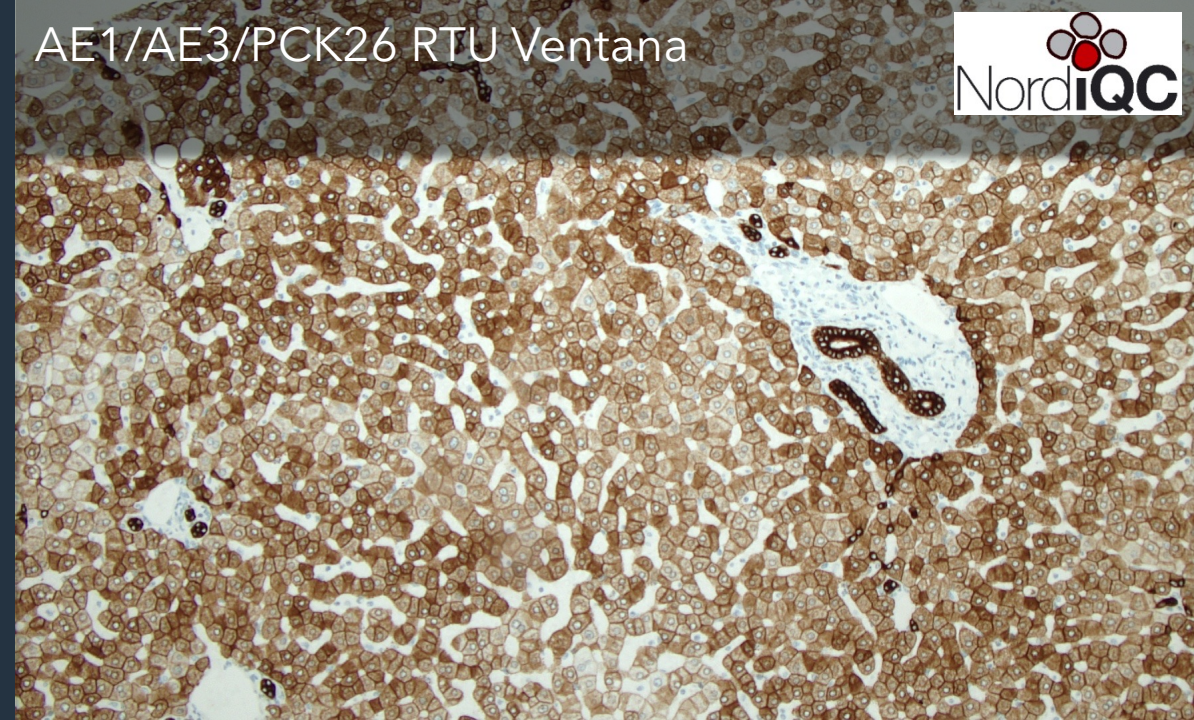
And Liver

AE1/AE3/PCK26 RTU Ventana

AE1/AE3 conc. Ventana

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
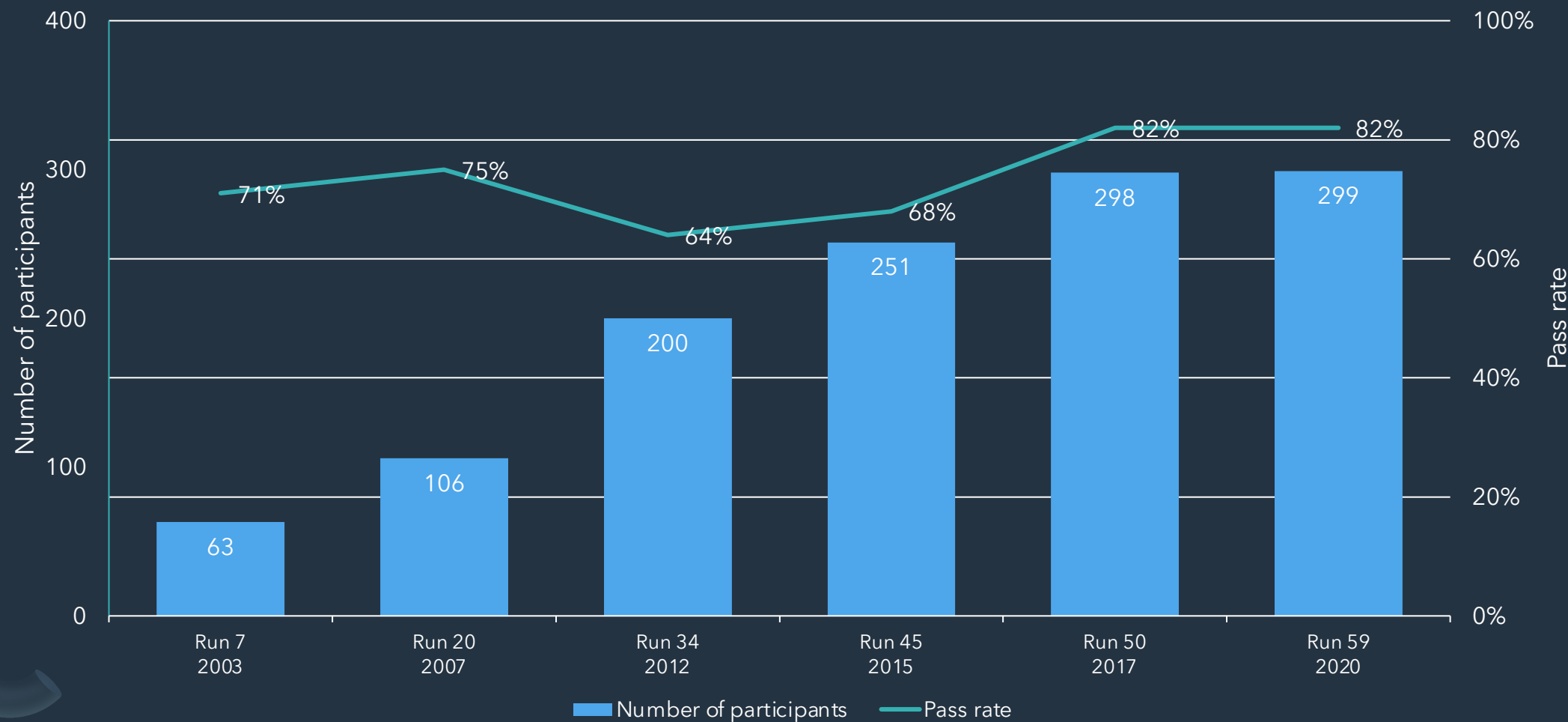
TRY TO KEEP UP

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**

	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%)

*Pass rate for compiled data from run 45, 50 & 59*

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.
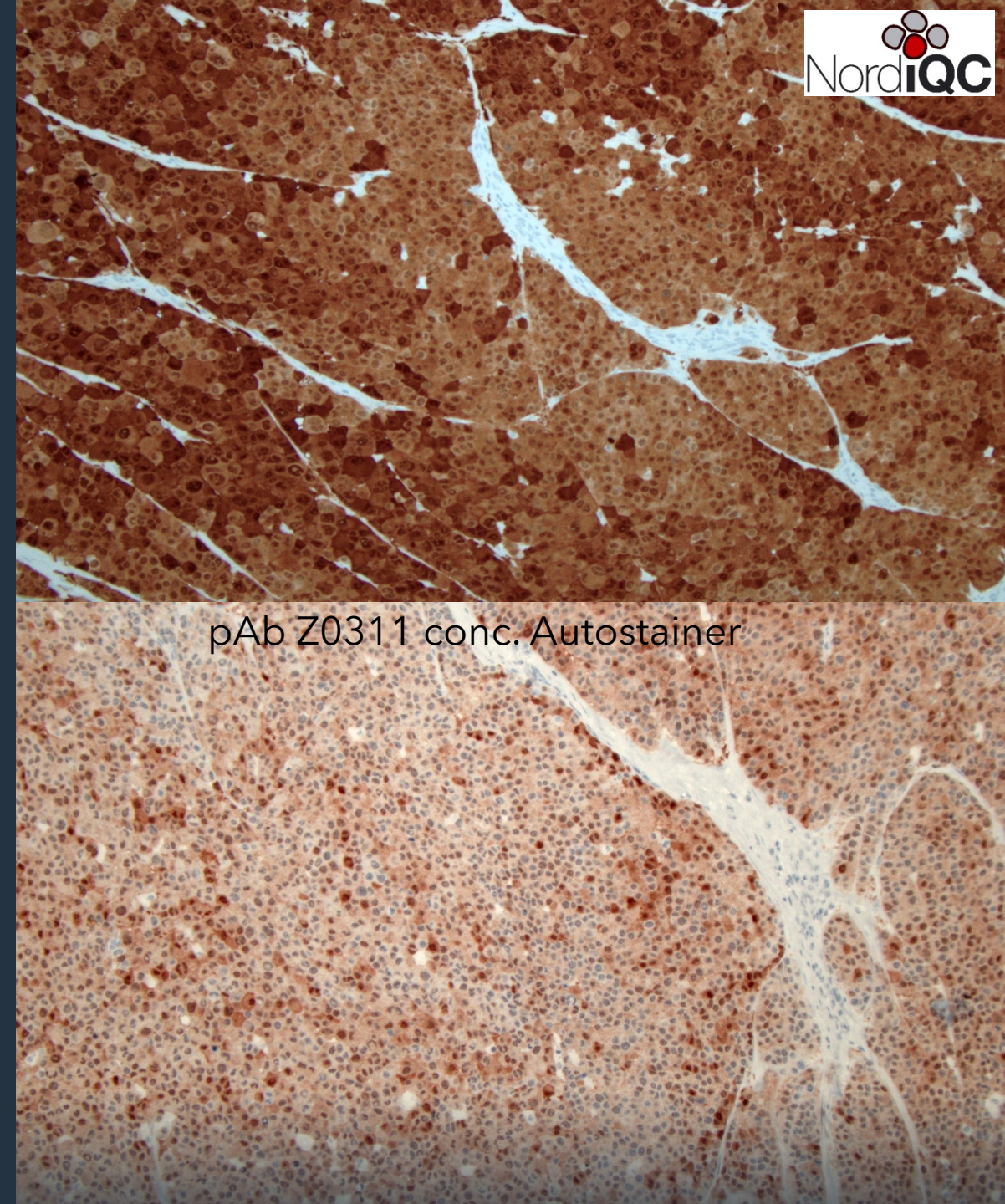
pAb Z0311 conc. Autostainer
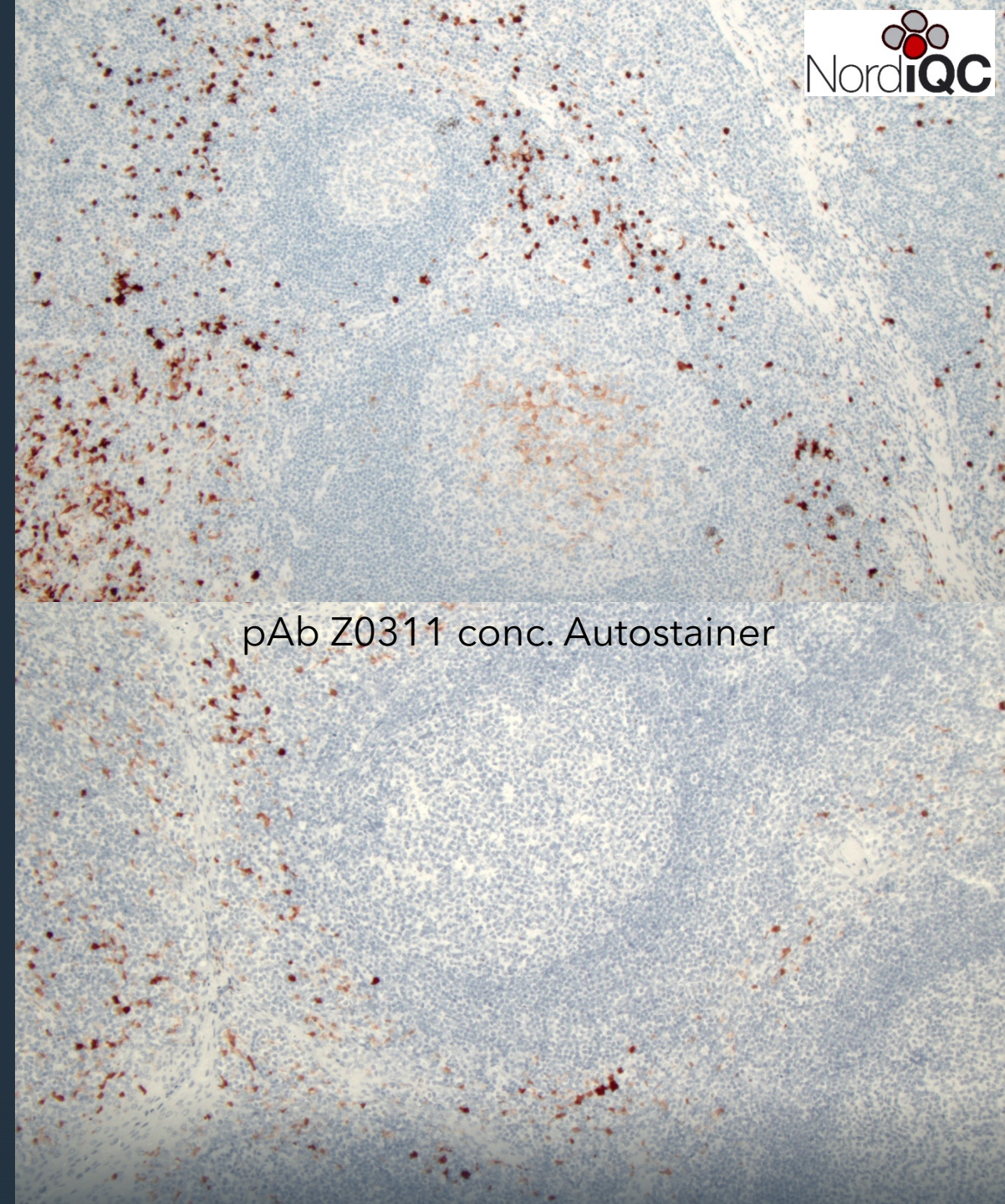
Appendix

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.
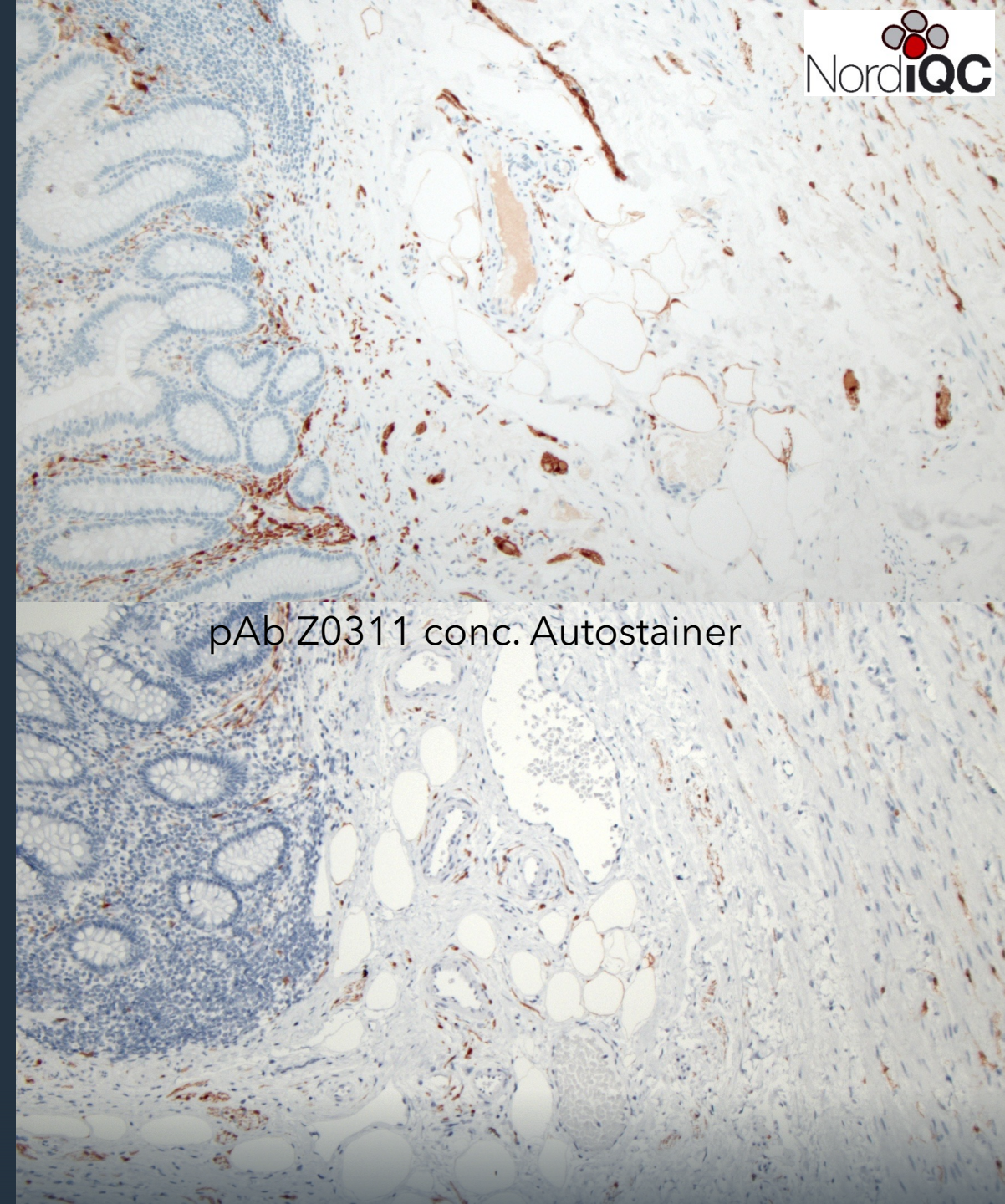
pAb Z0311 conc. Autostainer

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	2	5	2	2	63%	18%
	1	DCS						
	2	BioCare Medical						
	2	Zytomed Systems						
	1	Zeta Corporation						
pAb **Z0311**[5]	100	Agilent/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	Agilent/Dako	4	2	-	-	100%	67%
pAb **IS/IR504 (LMPS)**[4]	19	Agilent/Dako	14	4	1	-	95%	74%
pAb **GA504 (VRPS)**[3]	29	Agilent/Dako	28	1	-	-	100%	97%
pAb **GA504 (LMPS)**[4]	17	Agilent/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
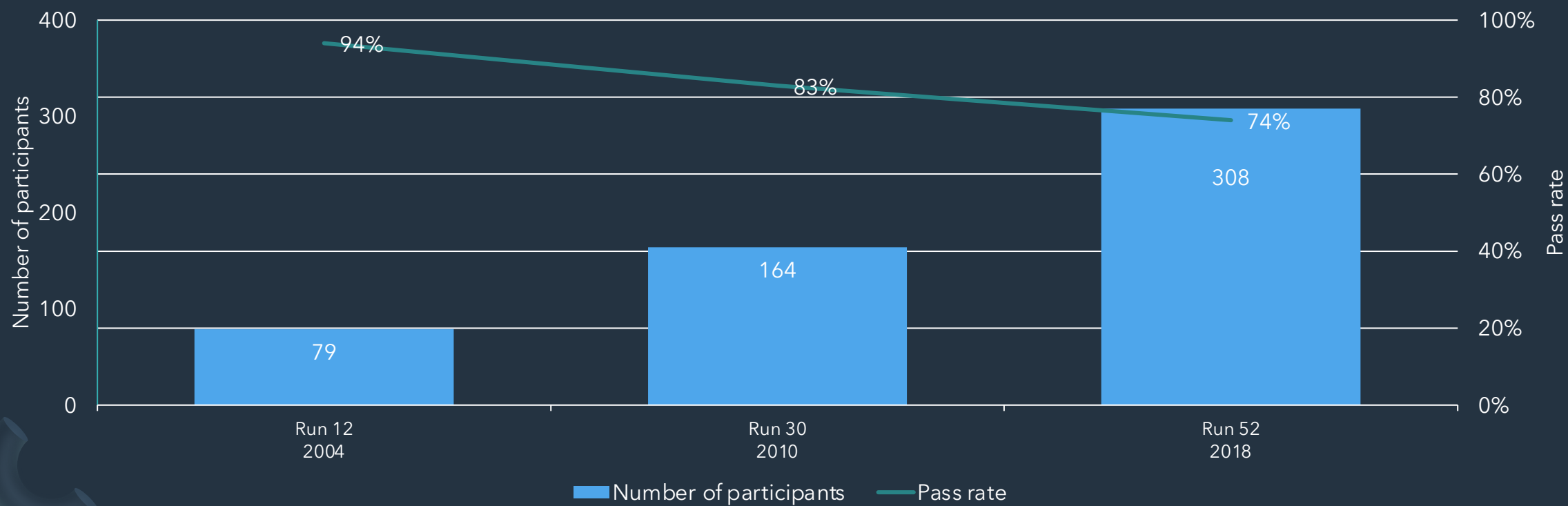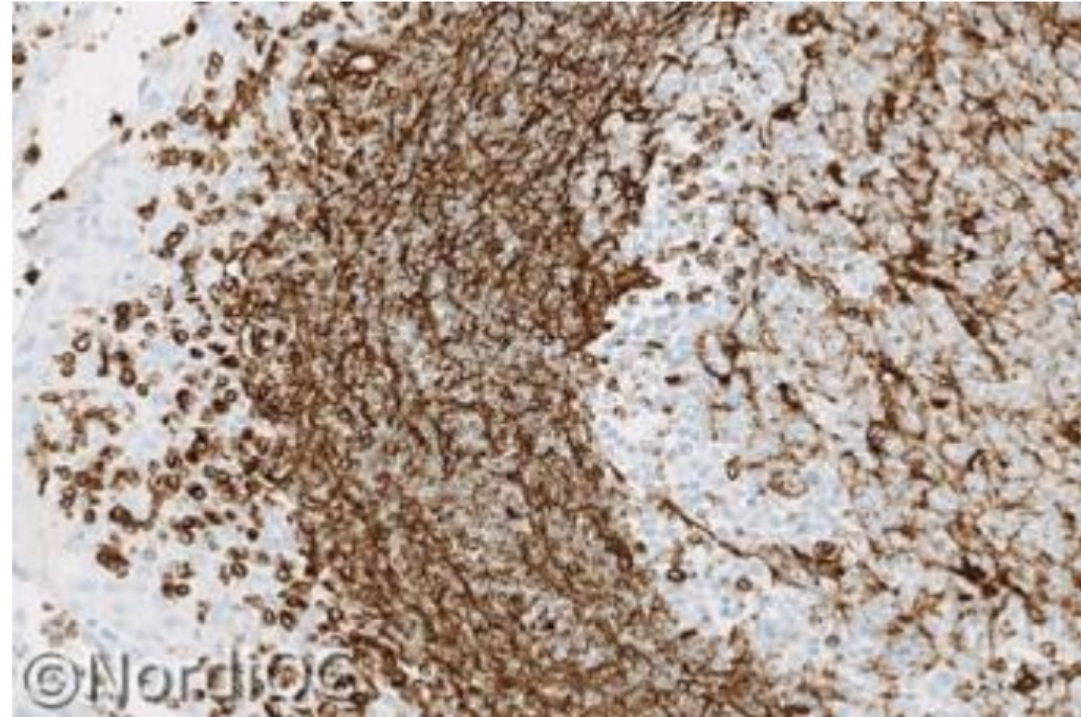
Tonsil is out

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 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.)
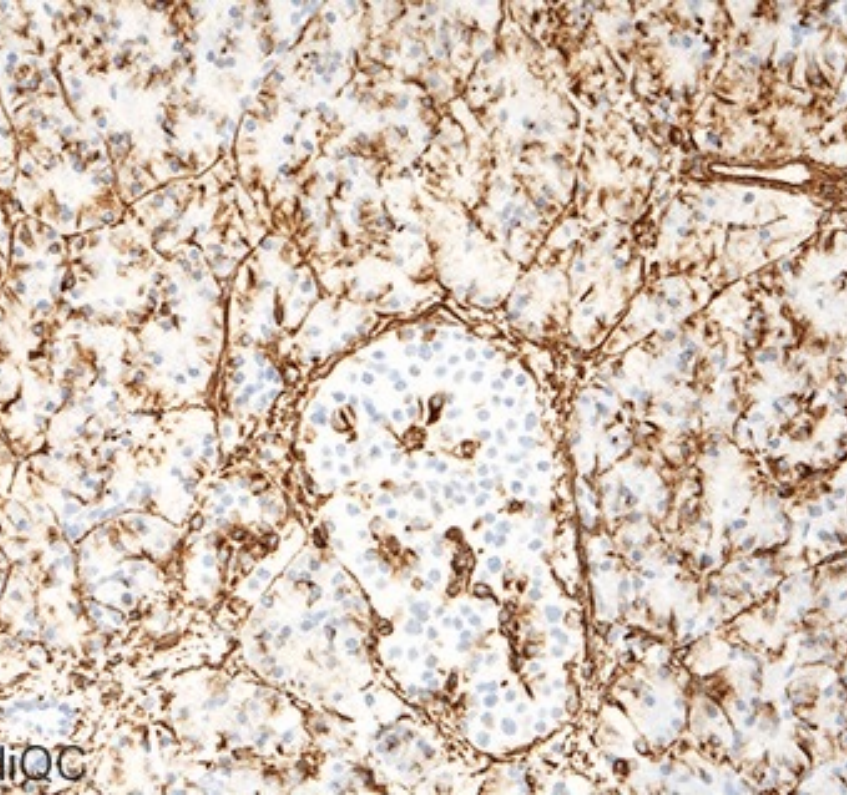
Pancreas: Epithelial cells of exocrine acini must show a weak but distinct cytoplasmic staining reaction.
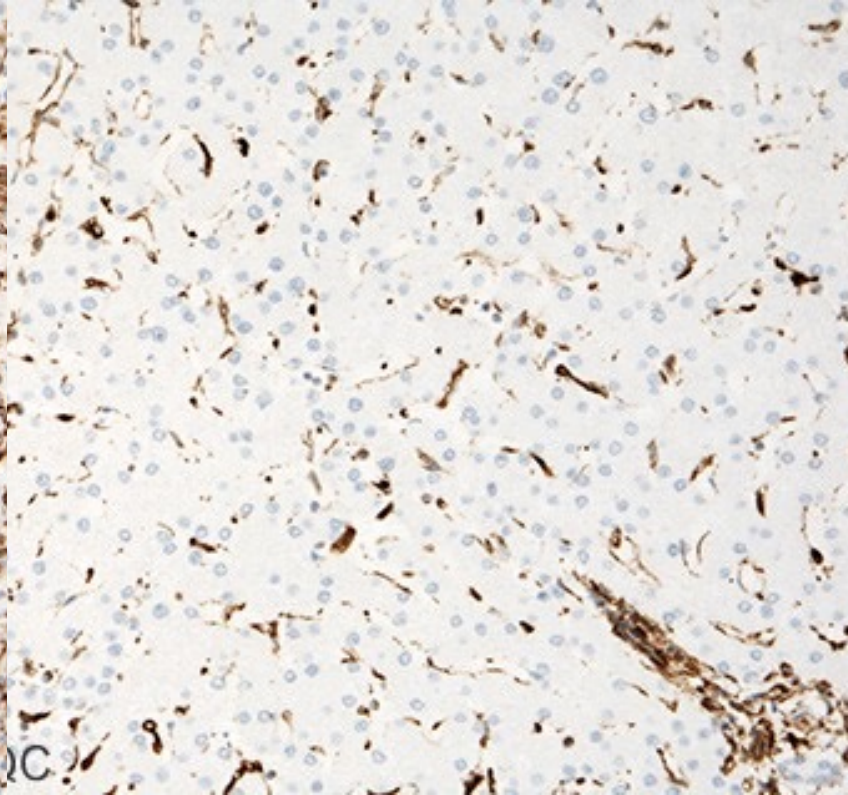
Liver: Virtually all Kupffer cells must show an at least moderate and distinct cytoplasmic staining reaction, while endothelial cells of the sinusoids must display an at least weak staining reaction
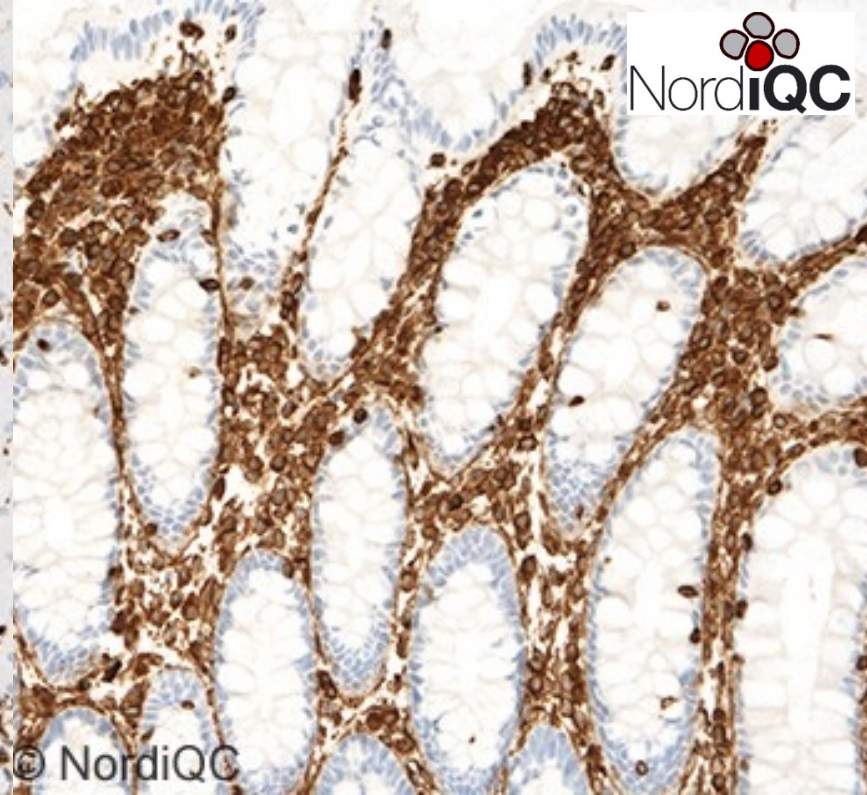
Colon: Endothelial cells of large vessels and stromal cells (e.g. fibroblasts and lymphocytes) must show a strong and distinct cytoplasmic staining reaction, while intraepithelial T-cells must at least display a moderate staining intensity.

Optimal staining with 3B4 conc. on Bond platform

Why go with V8 when you can try V9

Modified table 1

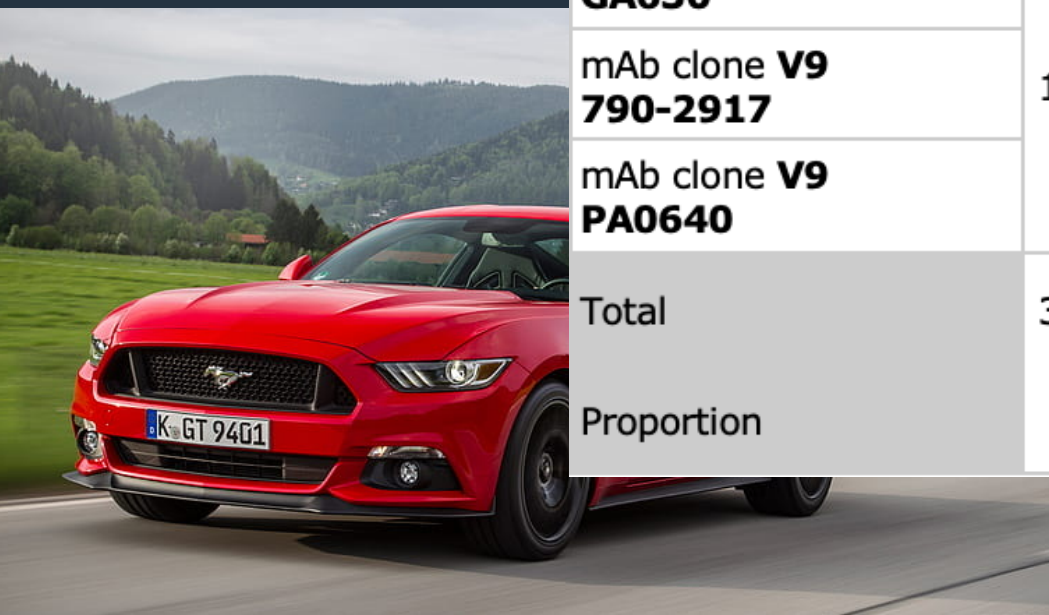| Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **V9** **IR630**	31	Agilent/Dako	27	1	3	0	90%	87%
mAb clone **V9** **GA630**	29	Agilent/Dako	23	2	4	0	86%	79%
mAb clone **V9** **790-2917**	100	Roche/Ventana	21	51	19	9	72%	21%
mAb clone **V9** **PA0640**	7	Leica/Novocastra	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.

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

Updated recommendations 2022

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

"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.

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?

Run 66-68

Markers	Control	Last run	Pass rate / Optimal	No. of labs
P53	Tonsil, appendix	67 2023	65% / 29%	372
MLH1	tonsil	67 2023	71% / 46% (90%/64%)	342
Prame	Testis, skin	68 2023	New comer 72% / 38%	222
Pax8	Fallopian tube, kidney, (tonsil)	68 2023	54% / 32% (52%/27%)	368
Uroplakin II/III	Urethra, tonsil	68 2023	49% / 29% (45%/21%)	106 (66)
MSH2	Tonsil	68 2023	91% / 62% (81%/53%)	350

*First NordiQC run

P53 – new purpose

**p53 performance in NordiQC assessments**
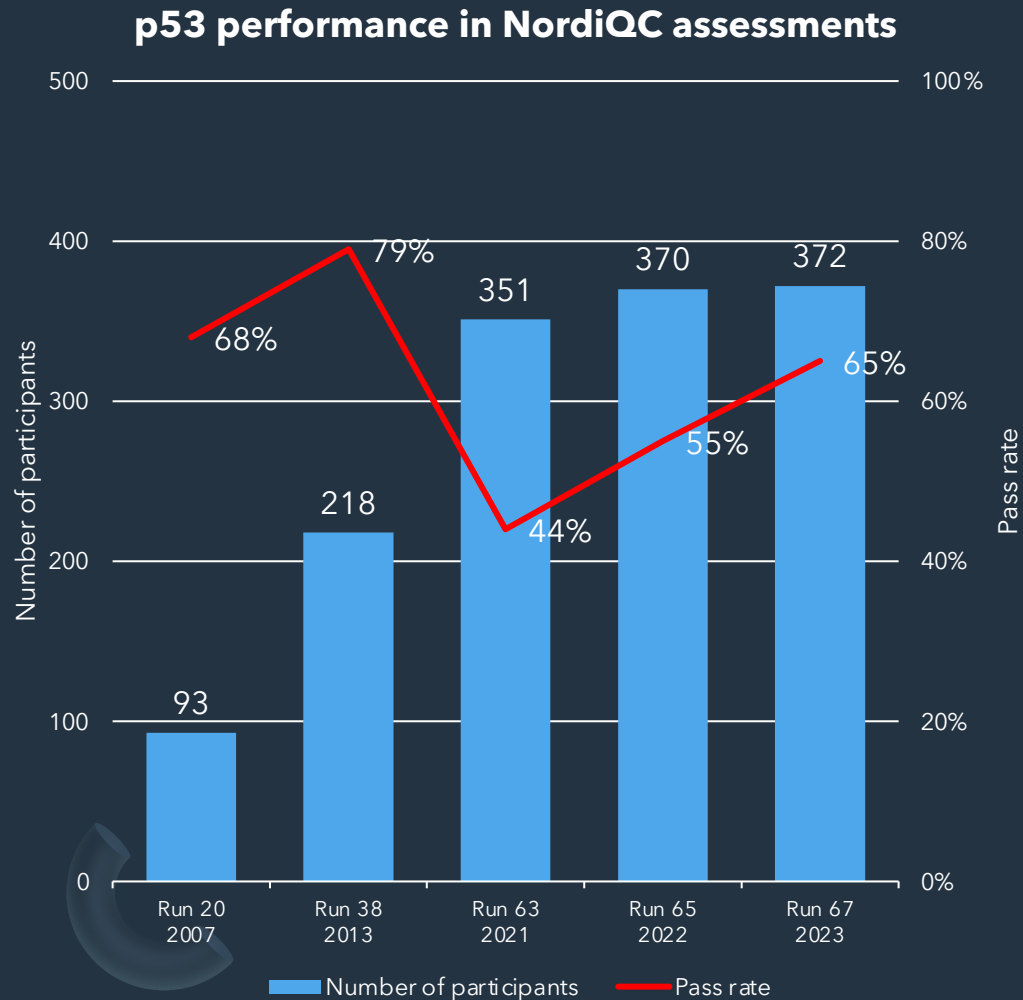
International Journal of Gynecological Pathology
38:S123–S131, Lippincott Williams & Wilkins, Baltimore
Copyright © 2018 International Society of Gynecological Pathologists. Published by Wolters Kluwer Health, Inc.
on behalf of the International Society of Gynecological Pathologists.

OPEN

Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility

Martin Köbel, M.D., Brigitte M. Ronnett, M.D., Naveena Singh, M.D., Robert A. Soslow, M.D., C. Blake Gilks, M.D., and W. Glenn McCluggage, M.D.
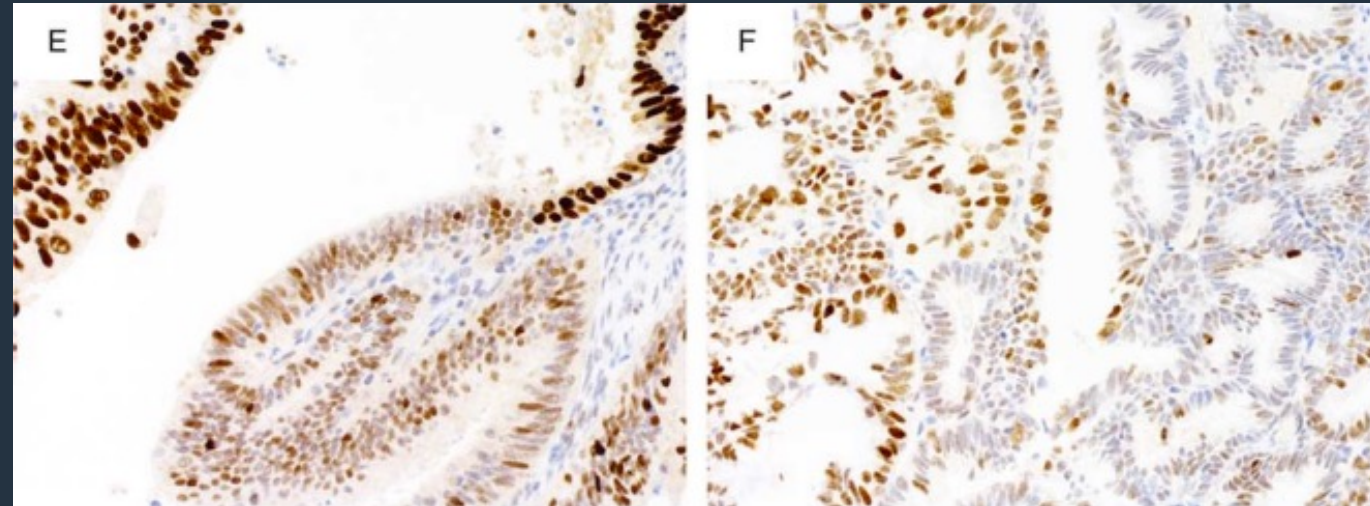
E. High wild-type   F. Low wild-type

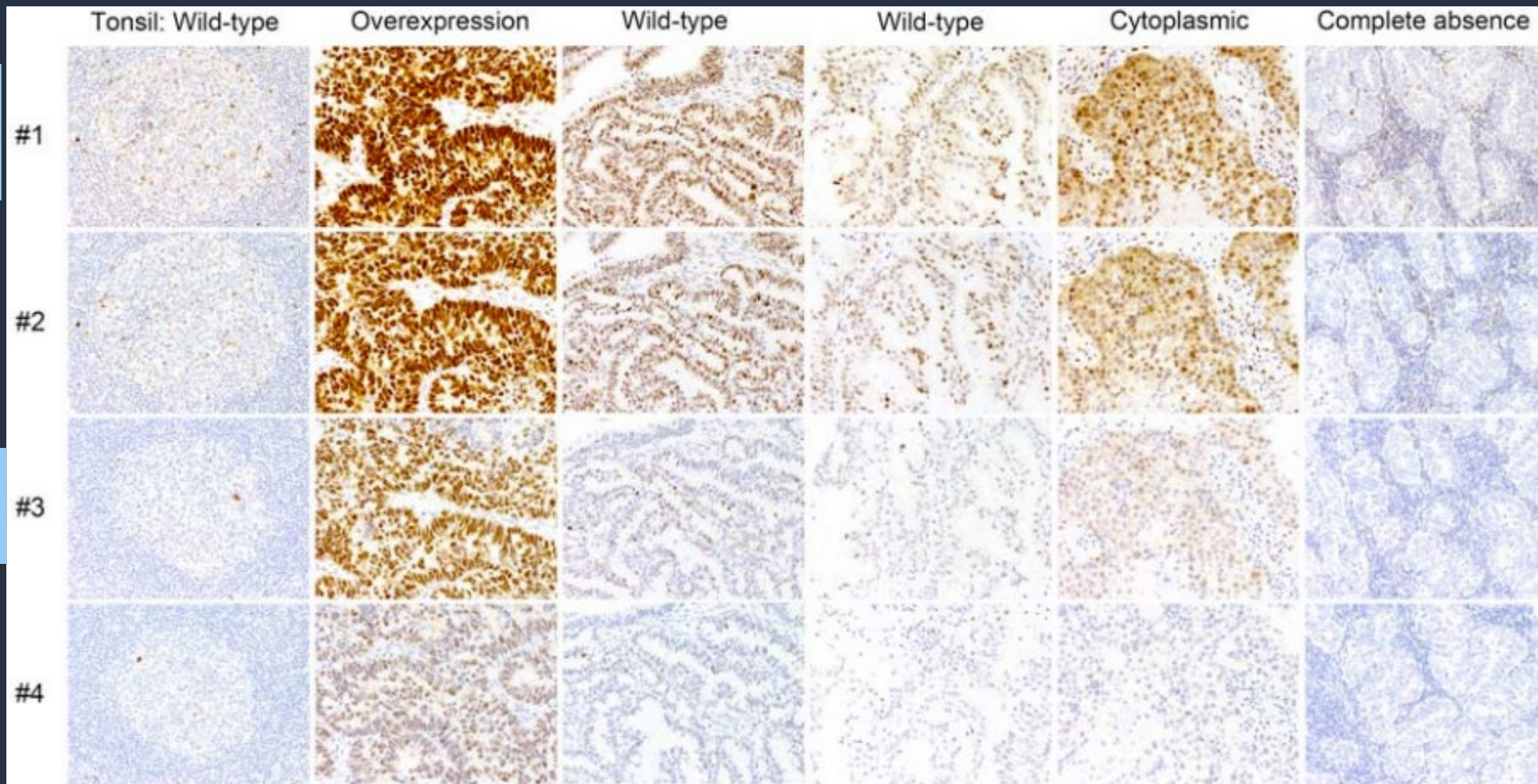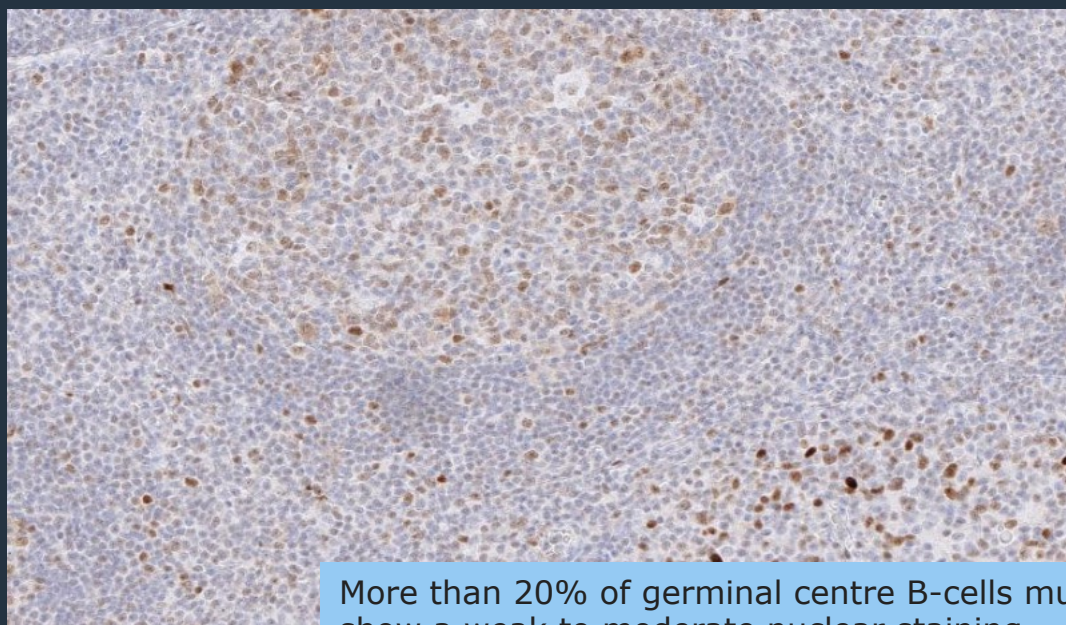# Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility

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

Antibodies	n	2-layer detection system		3-layer detection system	
		Sufficient	Optimal	Sufficient	Optimal
mAb conc **DO-7**	90	25% (1/4)	0% (0/4)	74% (64/86)	41% (35/86)
mAb RTU **BP53-11 760-2542*** Ventana/Roche	50	15% (2/13)	0% (0/13)	65% (24/37)	19% (7/37)
mAb clone RTU **DO-7 800-2912*** Ventana/Roche	92	8% (1/12)	8% (1/12)	83% (66/80)	50% (40/80)
mAb clone RTU **DO-7 IS/IR616*** Dako/Agilent	32	30% (3/10)	10% (1/10)	77% (17/22)	50% (11/22)
mAb clone RTU **DO-7 GA616*** Dako/Agilent	72	16% (3/17)	0% (0/17)	84% (46/55)	16% (9/55)

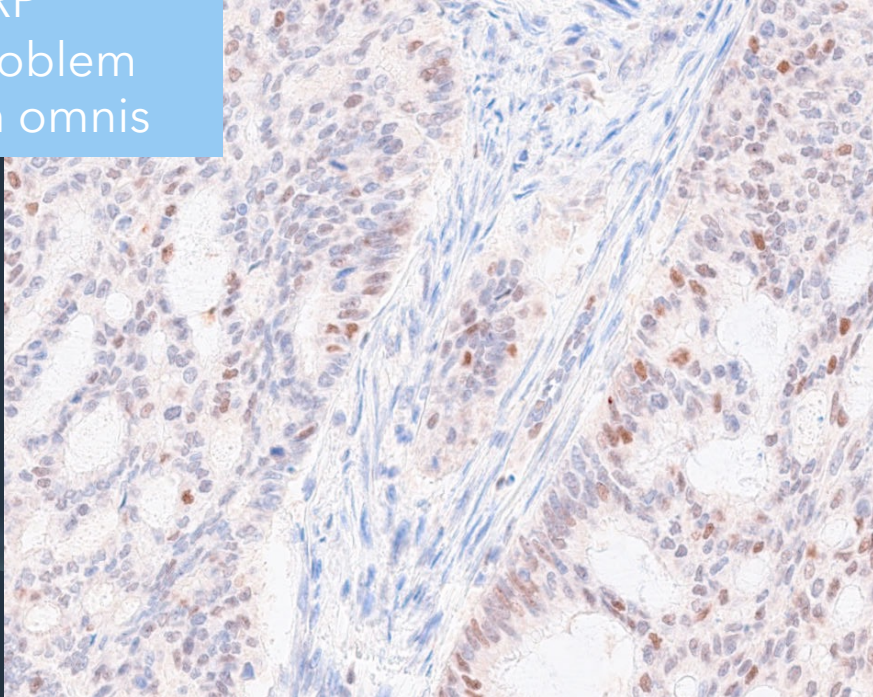| Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **DO-7**	53	Dako/Agilent						
	19	Leica Biosystems						
	7	Cell Marque						
	1	Diagnostic Biosystems	35	30	9	9	78%	42%
	1	Immunologic						
	1	Zeta Corporation						
	1	Epredia						
Conc total	90		35	33	12	10	76%	39%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **BP53-11 760-2542 (VRPS)**[3]	5	Ventana/Roche	-	3	2	-	60%	-
mAb clone **BP53-11 760-2542 (LMPS)**[4]	45	Ventana/Roche	7	16	16	6	51%	16%
mAb clone **DO-7 800-2912 (VRPS)**[3]	7	Ventana/Roche	1	3	2	1	57%	14%
mAb clone **DO-7 800-2912 (LMPS)**[4]	85	Ventana/Roche	40	23	16	6	74%	47%
mAb clone **DO-7 IS/IR616 (VRPS)**[3]	4	Dako/Agilent	1	1	-	2	-	-
mAb clone **DO-7 IS/IR616 (LMPS)**[4]	28	Dako/Agilent	11	7	4	6	64%	39%
mAb clone **DO-7 GA616 (VRPS)**[3]	10	Dako/Agilent	-	1	-	9	10%	-
mAb clone **DO-7 GA616 (LMPS)**[4]	62	Dako/Agilent	9	39	9	5	77%	15%
mAb clone **DO-7 PA0057 (VRPS)**[3]	13	Leica Biosystems	1	5	6	1	46%	8%
mAb clone **DO-7 PA0057 (LMPS)**[4]	12	Leica Biosystems	2	3	5	2	42%	17%
RTU total	282		73	101	66	42	62%	26%
Total	372		108	134	78	52		
Proportion			29%	36%	21%	14%	65%	

More than 20% of germinal centre B-cells must show a weak to moderate nuclear staining reaction, while less than 10% of the mantle zone B-cells should be demonstrated.

P53 – in general 2023 EnVision Flex problem

- For protocols performed on <u>Dako Omnis</u> a poor-signal-to-noise ratio or excessive background frequently was observed influencing the staining performance and in total 31 of the participants had issues where the extent and intensity of the background/cytoplasmic staining reaction impacted the interpretation of p53 status
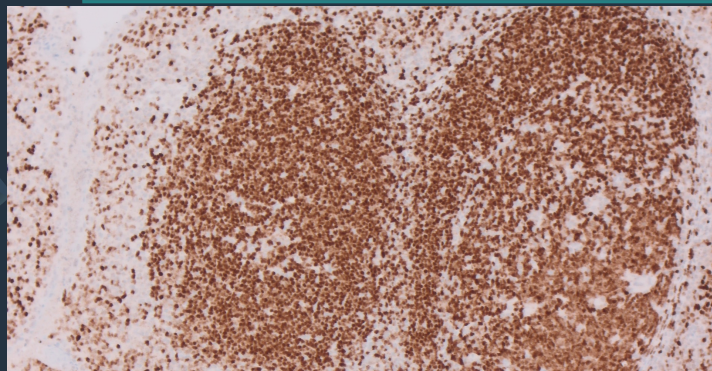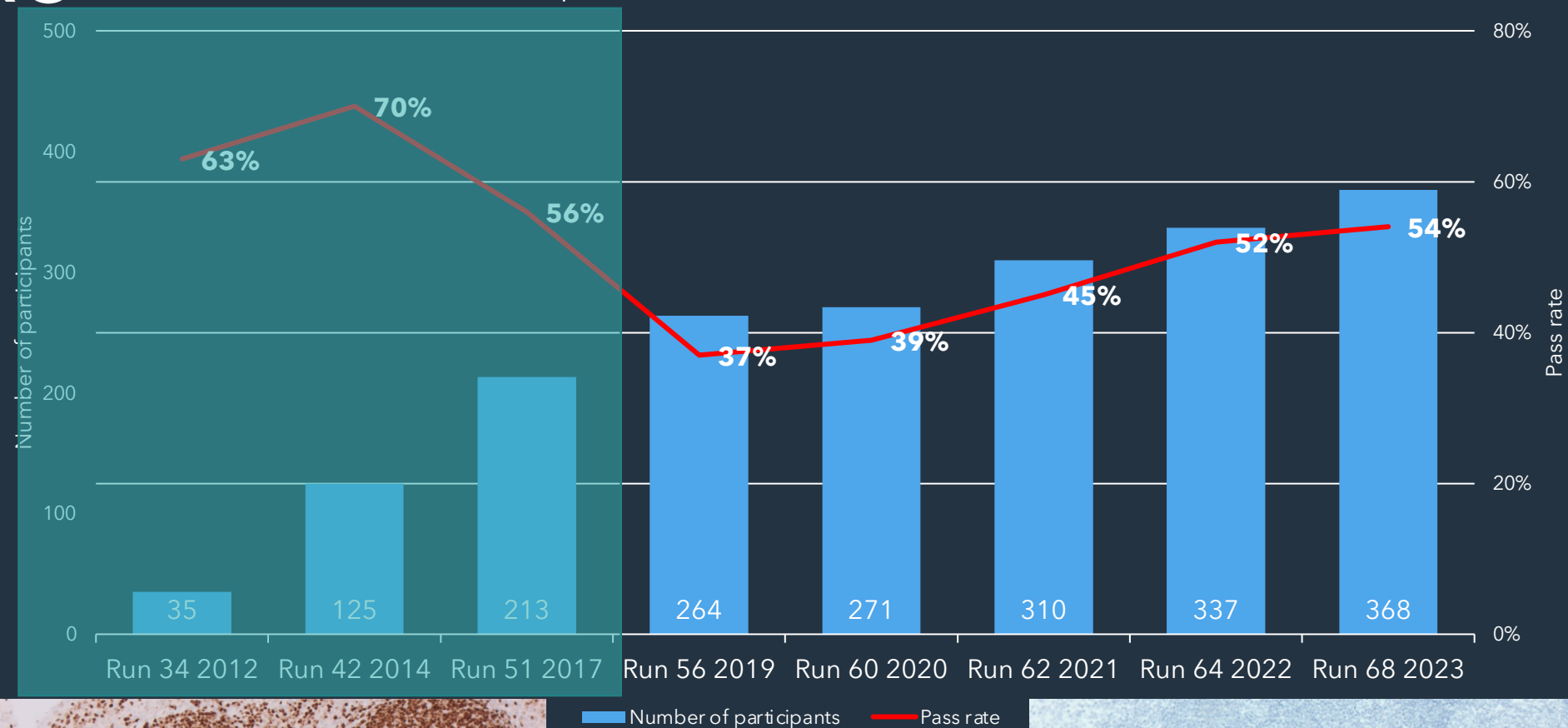

HRP problem on omnis

Same tumor with clean background
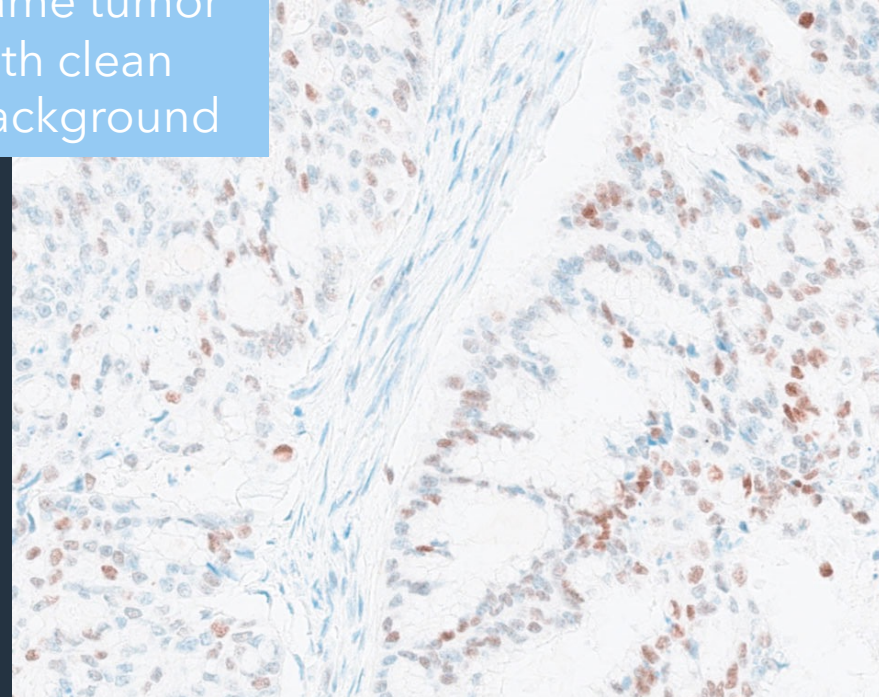
Pushing in the right direction

Modified table 1

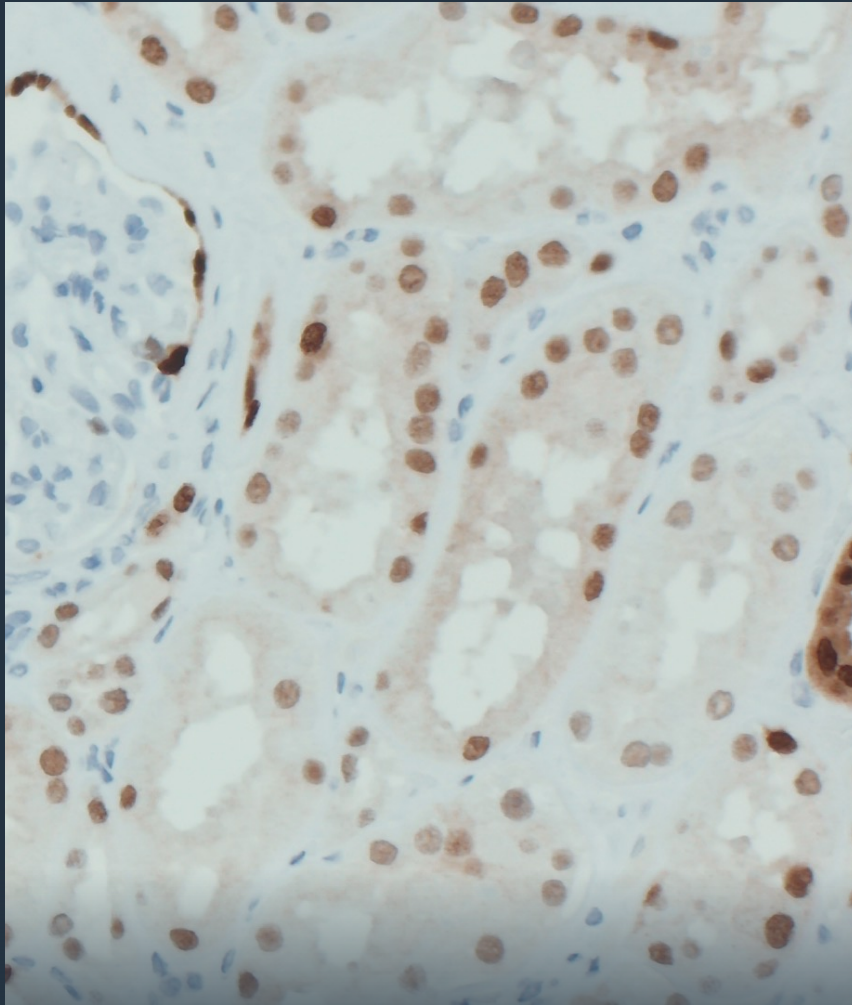| Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone BC12*	9 / 4	Biocare / Zytomed Systems	-	3	7	3	23%	-
mAb clone MRQ-50	16	Cell Marque	-	8	6	2	50%	-
rmAb clone EP298[5]*	1	Epitomics[5]	-	1	-	-	-	-
rmAb clone EP331*	10 / 4	Cell Marque / Epitomics	-	5	8	1	36%	-
rmAb clone SP348*	146	Abcam / Gennova / Spring Bioscience	102	31	9	4	91%	70%
rmAb clone ZR-1*	2 / 2	Zeta Corporation / BioSite	1	-	2	1	-	-
rmAb clone BP6157*	2	Biolynx	-	1	1	-	-	-
rmAb clone QR016*	7	Quartett	3	3	1	-	86%	43%
pAb, 10336-1-AP	11	Proteintech	-	1	3	7	9%	-
pAb, 363A-15	1	Cell Marque	-	-	1	-	-	-
pAb, CP379 AK	3	Biocare	-	-	1	2	-	-
pAb, RBK047	3	Zytomed Systems / Diagomics	-	-	3	-	-	-
Conc total	**223**		**106**	**54**	**43**	**20**	**72%**	**48%**
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone MRQ-50, 760-4618 (VRPS)[3]	6	Ventana/Roche	-	-	-	6	0%	0%
mAb clone MRQ-50, 760-4618 (LMPS)[4]	49	Ventana/Roche	-	3	34	12	6%	0%
rmAb clone, EP331* 760-6077(VRPS)[3]	3	Ventana/Cell Marque	-	1	2	-	-	-
rmAb clone, EP331* 760-6077(LMPS)[4]	11	Ventana/Cell Marque	-	4	6	1	36%	0%
mAb clone, BC12* API438	6	Biocare Medical	-	2	4	-	33%	0%
rmAb clone SP348* M6481	3	Spring Bioscience	2	1	-	-	-	-
rmAb clone GR002* GT210202	1	GeneTech	1	-	-	-	-	-
rmAb clone QR016* P-P008	2	Quartett	1	1	-	-	-	-
rmAb clone EP331* 363M/AC0338	12	Cell Marque	-	3	7	2	25%	0%
rmAb clone SP348* 363R-38	4	Cell Marque	2	1	1	-	-	-
rmAb clone RM436* 8257-C010	2	Sakura Finetek	1	1	-	-	-	-
Clone MXR013* RMA-1024	2	Fuzhou Maixin	2	-	-	-	-	-
Clone H5A8 DTBL0220101	1	DaTe Bioengineering Technology	1	-	-	-	-	-
RTU total	**145**		**10**	**27**	**77**	**31**	**26%**	**8%**
Total	368		116	81	120	51		
Proportion			32%	22%	32%	14%	54%	

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*

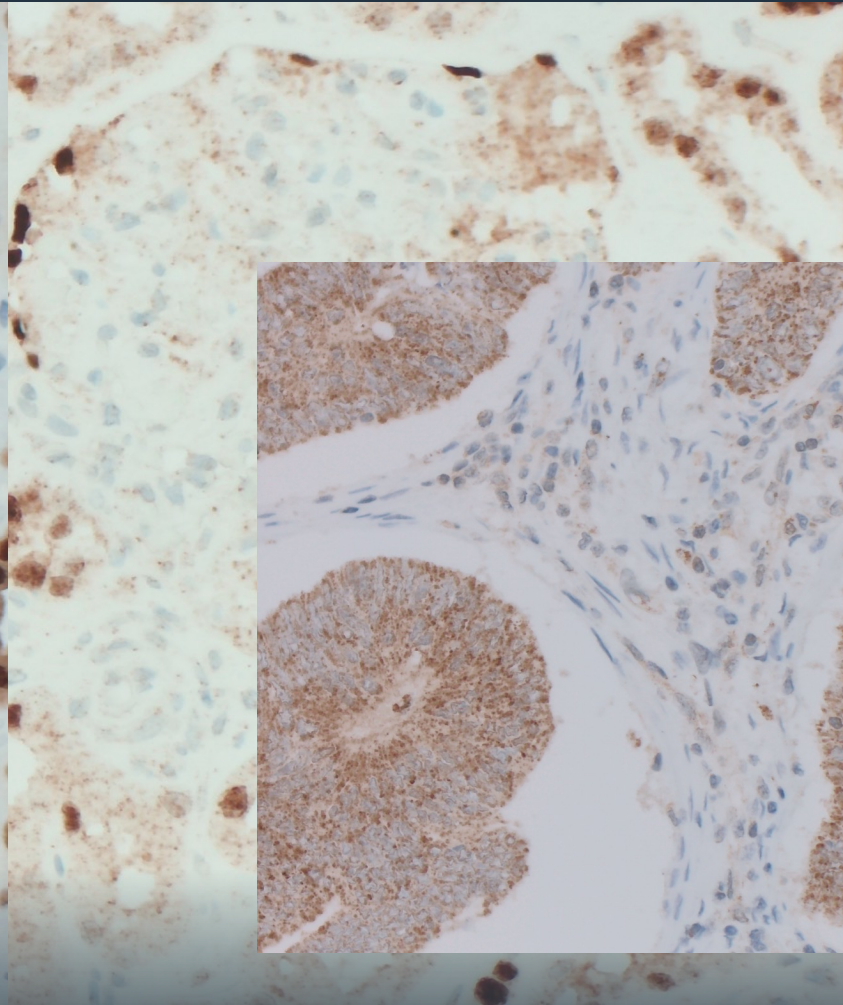| Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra			Leica Biosystems Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + P3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
rmAb SP348	3/7** (43%)	0/1	35/42 (83%)	2/2	60/81 (74%)	1/3	0/2	0/6	-
rmAb QR016	-	-	-	-	3/5 (60%)	-	-	0/2	-
rmAb SP331									
mAb B									
mAb M									
pAb 10 AP									

Ready-To-Use antibodies								Suff.[1]	OR.[2]
mAb clone, BC12* API438	6	Biocare Medical	-	2	4	-		33%	0%
rmAb clone SP348* M6481	3	Spring Bioscience	2	1	-	-		-	-
rmAb clone GR002* GT210202	1	GeneTech	1	-	-	-		-	-
rmAb clone QR016* P-P008	2	Quartett	1	1	-	-		-	-
rmAb clone SP348* 363R-38	4	Cell Marque	2	1	1	-		-	-
rmAb clone RM436* 8257-C010	2	Sakura Finetek	1	1	-	-		-	-
Clone MXR013* RMA-1024	2	Fuzhou Maixin	2	-	-	-		-	-
Clone H5A8 DTBL0220101	1	DaTe Bioengineering Technology	1	-	-	-		-	-

...b clone MRQ-50 on the four main IHC instruments in ...nd concentrate).

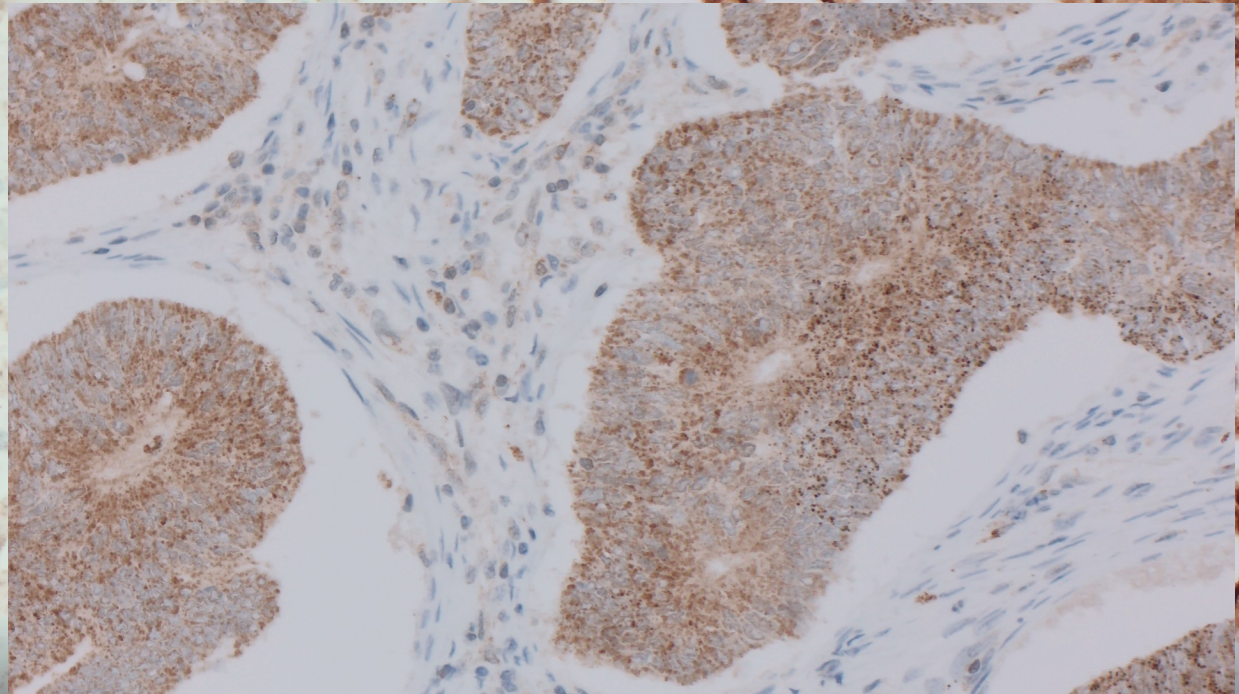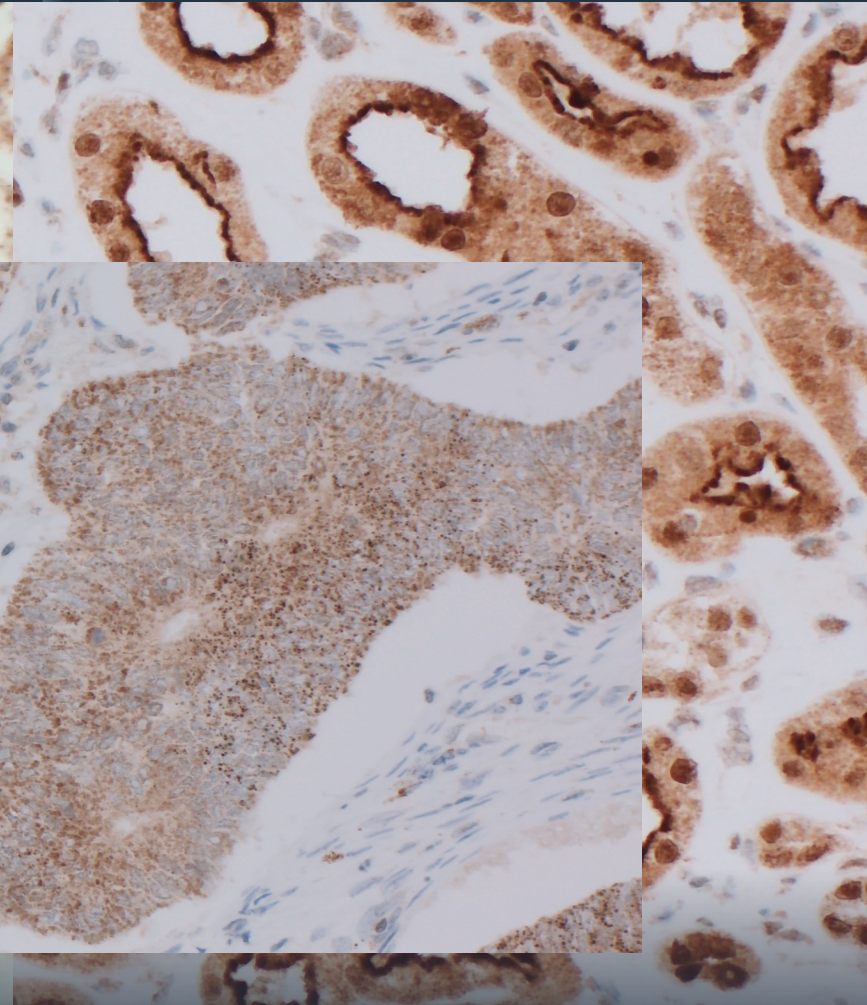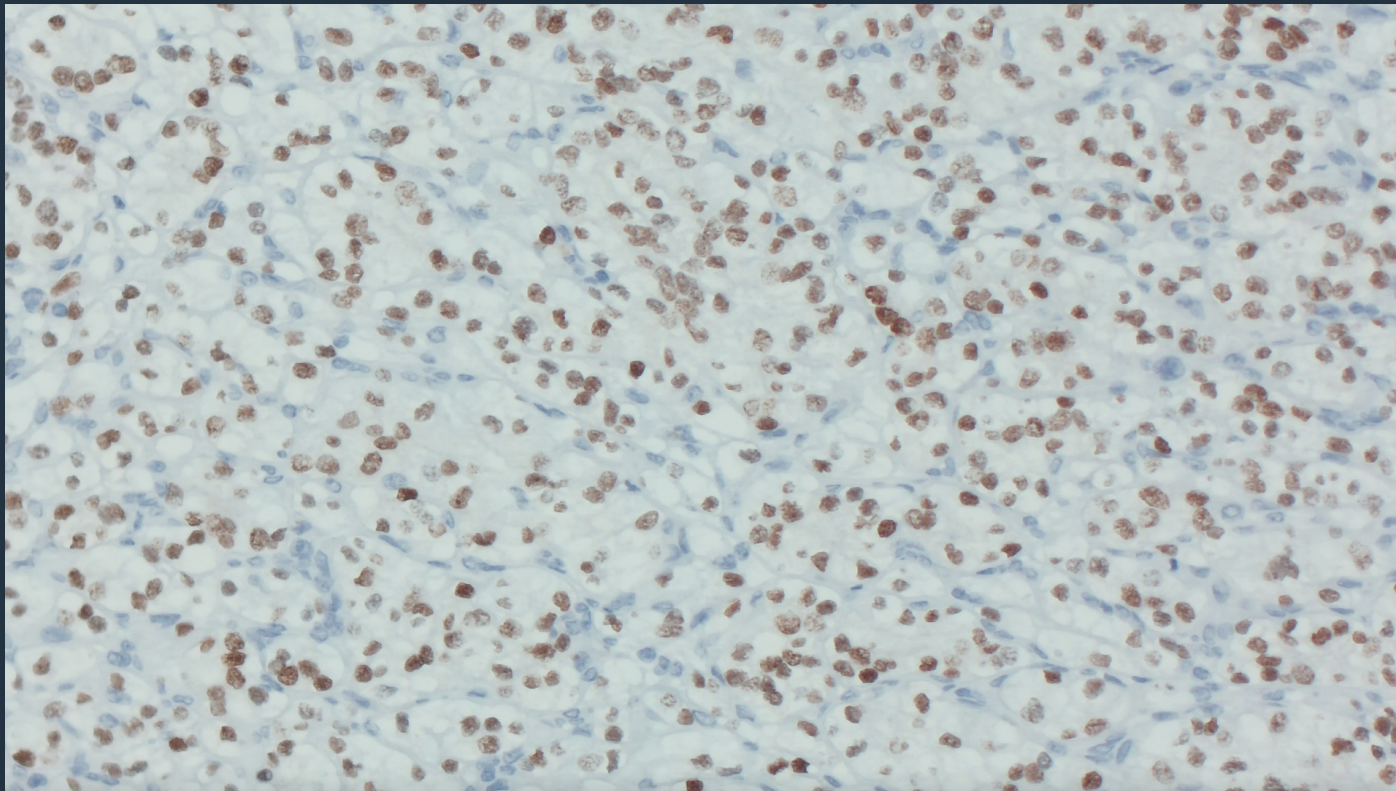|	...to/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max
	-	-	-
	-	12	37
Borderline	5	13	111	5
Poor	-	3	60	-
Total	29	16	183	40
Sufficient %	83%	0%	7%	93%

Kidney

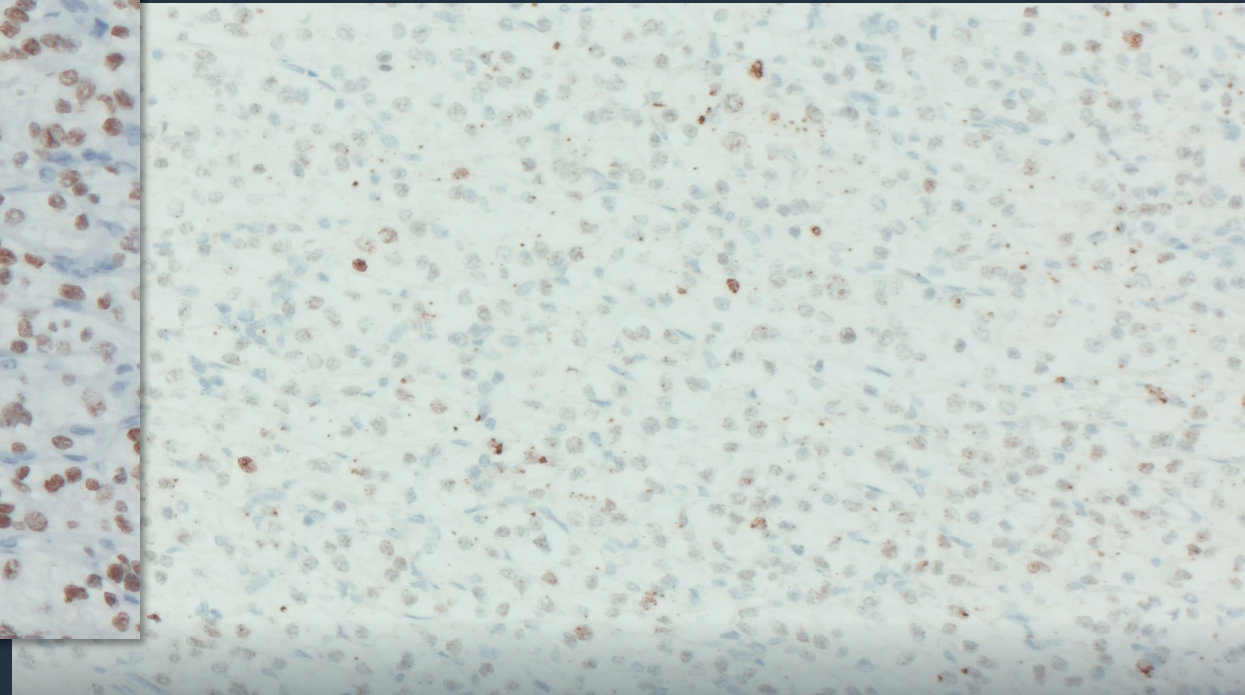SP348 - Ventana

MRQ-50 - Ventana

EP331 Autostainer

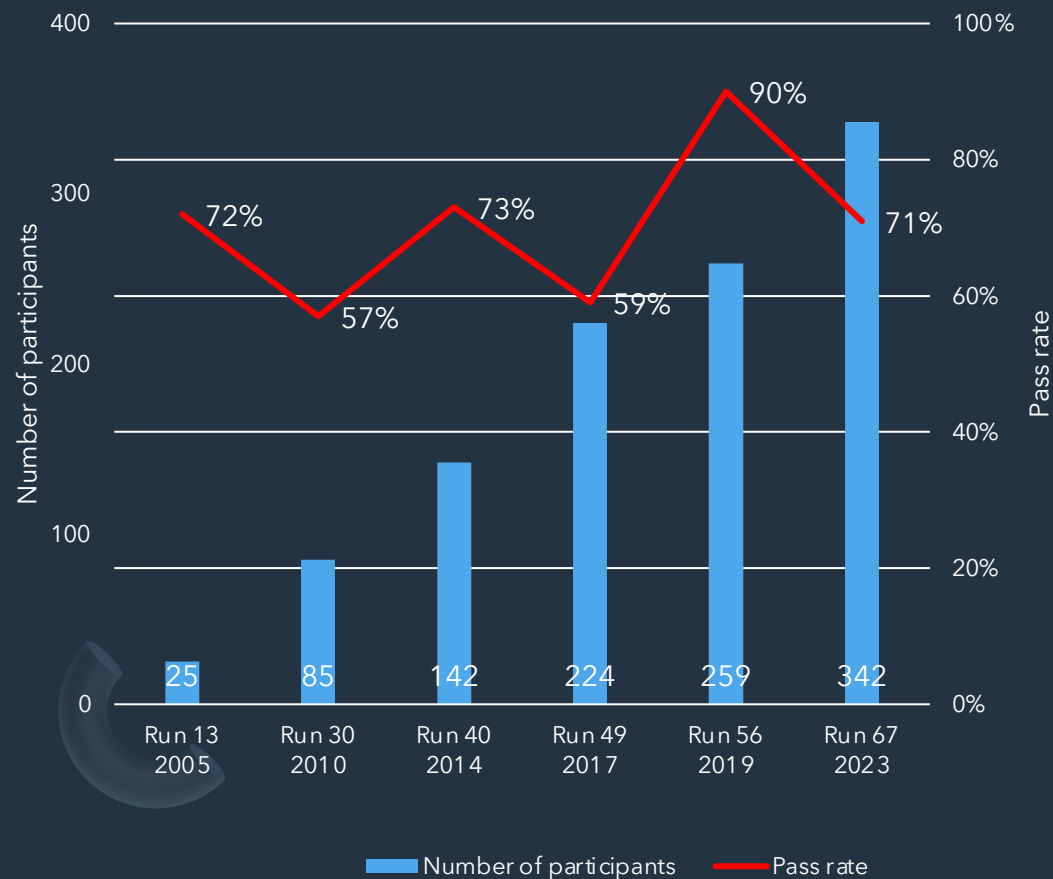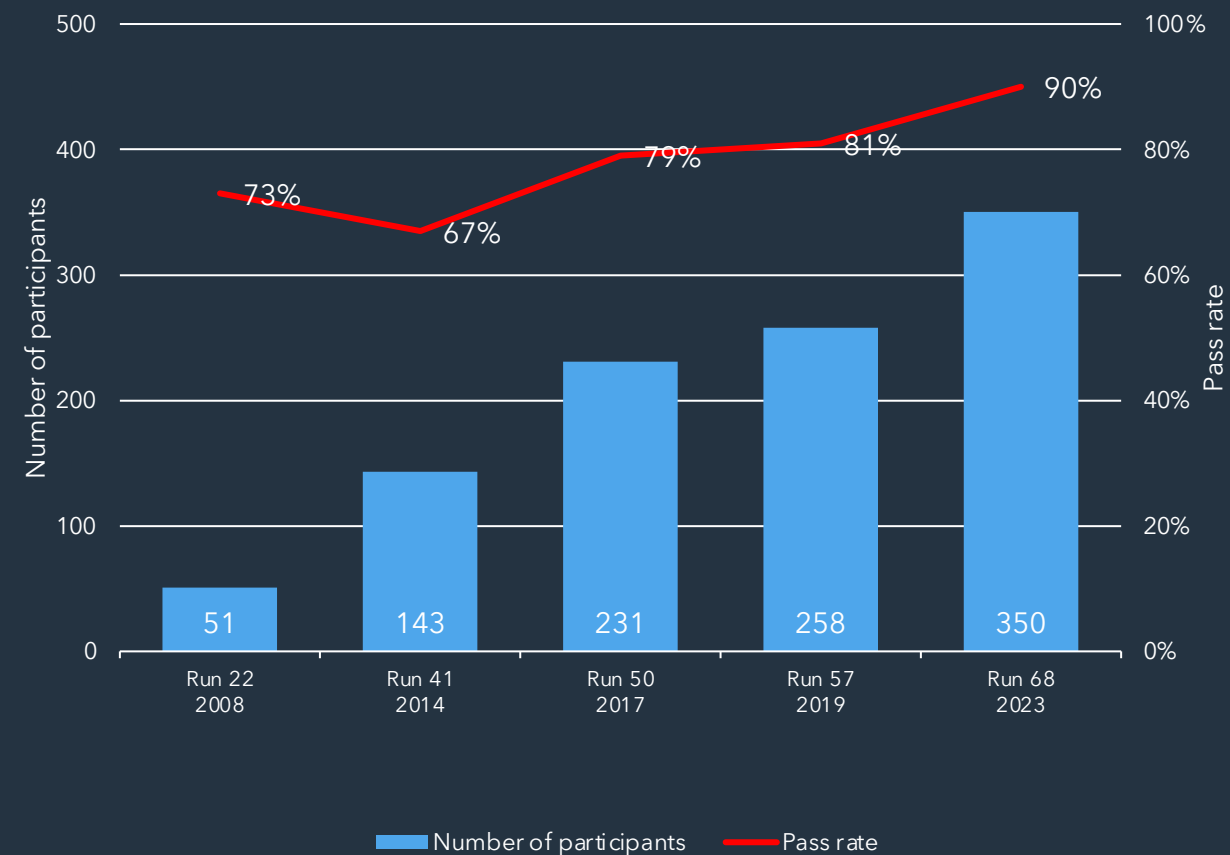# Renal clear cell carcinoma

SP348 ventana

MRQ-50 ventana

MLH1 and MSH2

MLH1 performance in NordiQC assessments

MSH2 performance in NordiQC assessments

MLH1

| Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **ES05**	20 28	Dako/Agilent Leica/Novocastra	22	14	9	3	75%	46%
mAb clone **G168-15**	7 2 1	BD Pharmingen Biocare Medical Thermo Scientific/ Epredia	3	4	2	1	70%	30%
Conc total	64		28	19	11	6	73%	44%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **M1 760-5091/ 790-5091/780-7140**[3]	30	Ventana/Roche	6	13	11	-	63%	20%
mAb clone **M1 760-5091/ 790-5091/780-7140**[4]	100	Ventana/Roche	41	29	29	1	70%	41%
mAb clone **ES05 IR/IS079**[3]	12	Dako/Agilent	8	-	3	1	67%	67%
mAb clone **ES05 IR/IS079**[4]	37	Dako/Agilent	15	4	13	5	51%	41%
mAb clone **ES05 GA079**[3]	41	Dako/Agilent	27	7	7	-	83%	66%
mAb clone **ES05 GA079**[4]	17	Dako/Agilent	8	7	1	1	88%	47%
mAb clone **ES05 PA0988**[3]	4	Leica Biosystems	1	1	2	-	-	-
mAb clone **ES05 PA0988**[4]	19	Leica Biosystems	15	1	3	-	84%	79%
mAb clone **GM011 8324-C010**[3]	2	Sakura Finetek	1	1	-	-	-	-
RTU total	278		130	65	73	10	70%	47%
Total	342		158	84	84	16		
Proportion			46%	25%	25%	5%	71%	

"Ventana RTU Optimal protocols could be obtained with OptiView+amplification"

MSH2

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **FE11**	4 2 2 1 1	Dako/Agilent BioCare Medical Calbiochem Biozol Zytomed Systems	4	3	3	0	70%	40%
mAb clone **G219-1129**	16 4 1 1	Cell Marque BD Biosciences Monosan Immunologic	8	6	7	1	64%	37%
mAb clone **79H11**	3	Leica Biosystems	2	1	0	0	-	-
mAb clone **BPM6143**	2	Biolynx Technologies	1	1	0	0	-	-
rmAb clone **RED2**	1 1	Epitomics Bejing Zhongshang	2	0	0	0	-	-
rmAb clone **QR010**	1	Quartett	1	0	0	0	-	-
rmAb clone **ZR260**	1	Zeta Corporation	1	0	0	0	-	-
Conc total	41		19	11	10	1	73%	46%
Ready-To-Use antibodies								
mAb clone **G219-1129 760-5093**[3]	33	Ventana/Roche	26	7	0	0	100%	79%
mAb clone **G219-1129 760-5093**[4]	119	Ventana/Roche	84	27	8	0	93%	71%
mAb clone **FE11 IR085**[3]	16	Dako/Agilent	11	4	1	0	94%	69%
mAb clone **FE11 IR085**[4]	34	Dako/Agilent	20	9	4	1	85%	59%
mAb clone **FE11 GA085**[3]	35	Dako/Agilent	16	19	0	0	100%	46%
mAb clone **FE11 GA085**[4]	16	Dako/Agilent	8	8	0	0	100%	50%
mAb clone **25D12 PA0048**#	4	Leica Biosystems	3	0	0	1	-	-
mAb clone **79H11 PA0989**[3]	13	Leica Biosystems	12	1	0	0	100%	92%
mAb clone **79H11 PA0989**[4]	10	Leica Biosystems	6	3	1	0	90%	60%
rmAb clone **RED2 8327-C010**	1	Sakura Finetek	0	1	0	0	-	-
RTU total	309		197	89	19	4	93%	64%
Total	350		216	100	29	5	-	
Proportion			62%	29%	8%	1%	91%	

Yes to VPRS

But don't overdo it

Colon adenocarcinoma with MLH1 loss
Ventana RTU OptiView with **amp 4+4**
CC1 64 min, Ab 24 min
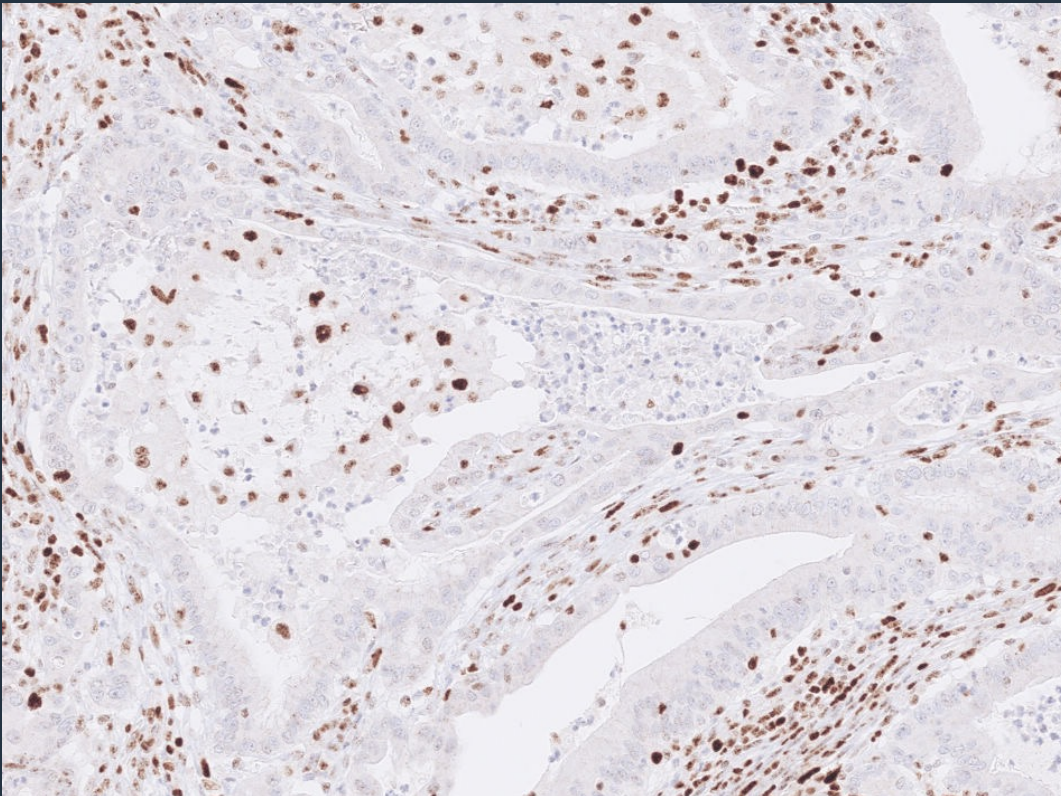
Colon adenocarcinoma with MLH1 loss
Ventana RTU OptiView with **amp 8+8**
CC1 64 min, Ab 36 min