NordiQC data: Antibody selection, protocols and controls

The generel module

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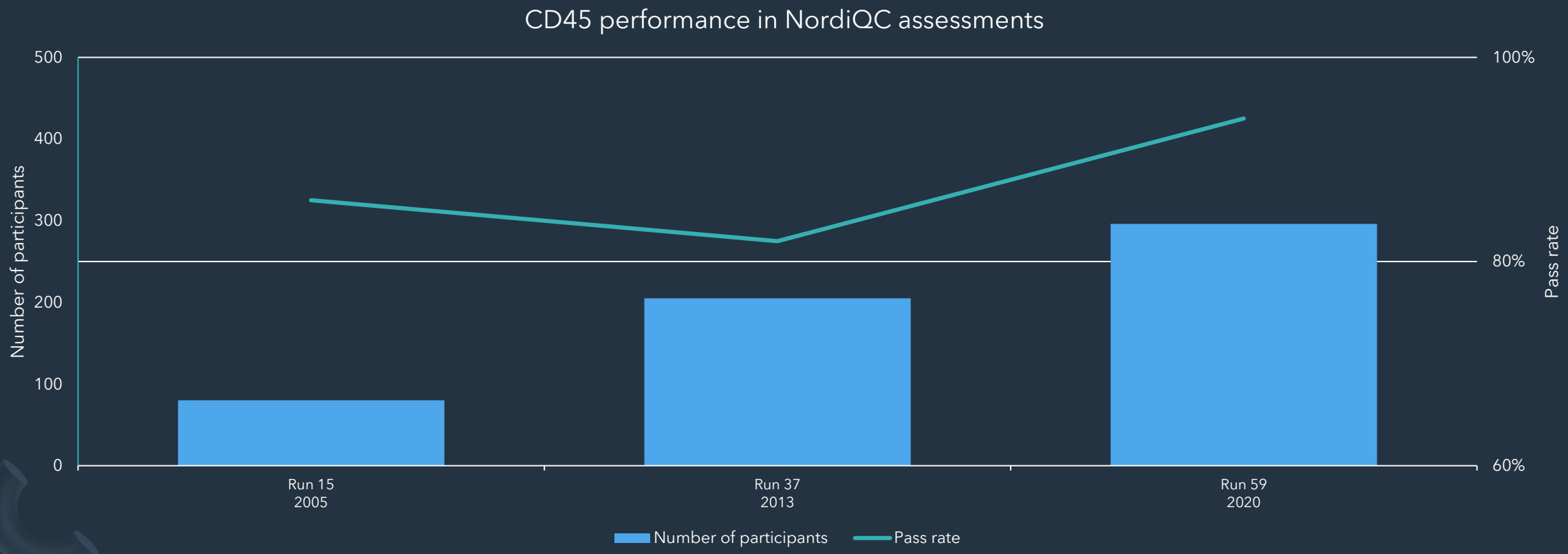
Primary panel for the unknown primary tumour

Is it as easy as it looks?!

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

CD45


CD45 performance in NordiQC assessments
500
400
300
200
100
0
Number of participants
100%
80%
60%
Pass rate
Run 15
2005
Run 37
2013
Run 59
2020
Number of participants
Pass rate

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

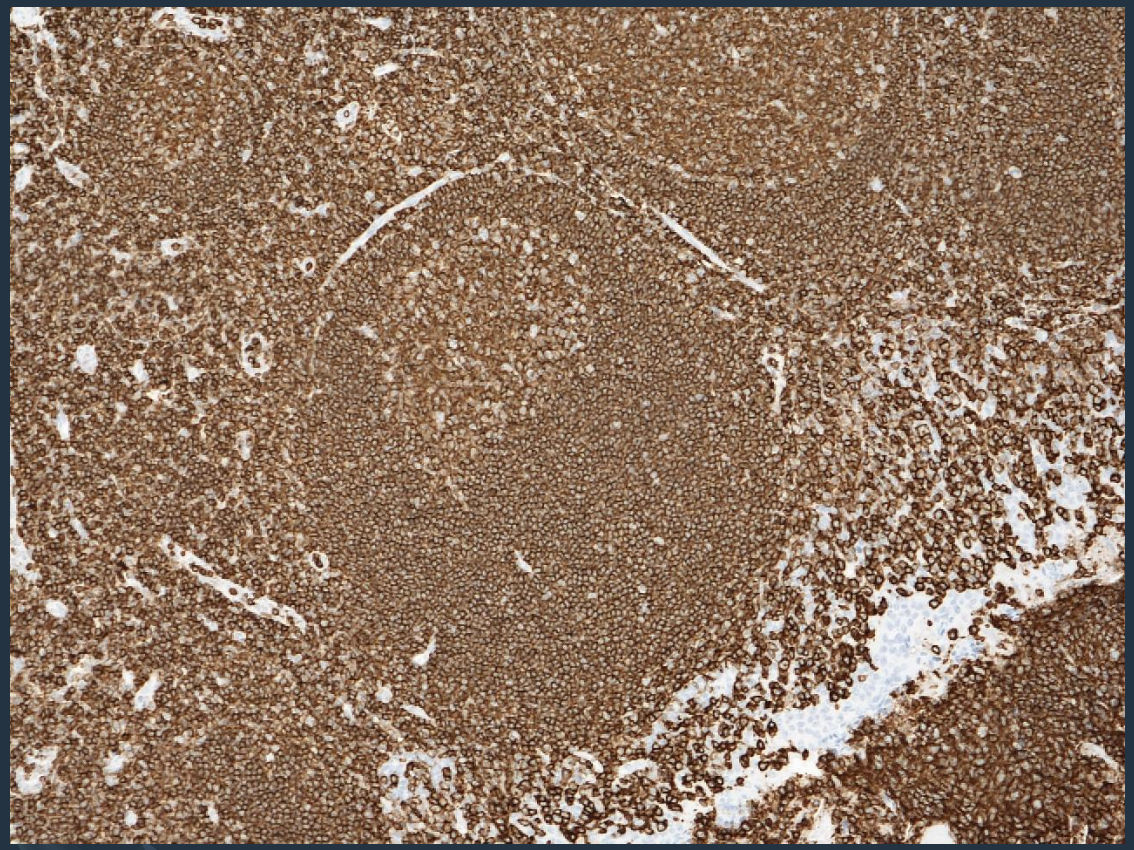
Table 1. Recommended Staining Protocols for CONFIRM anti-CD45, LCA (RP2/18)

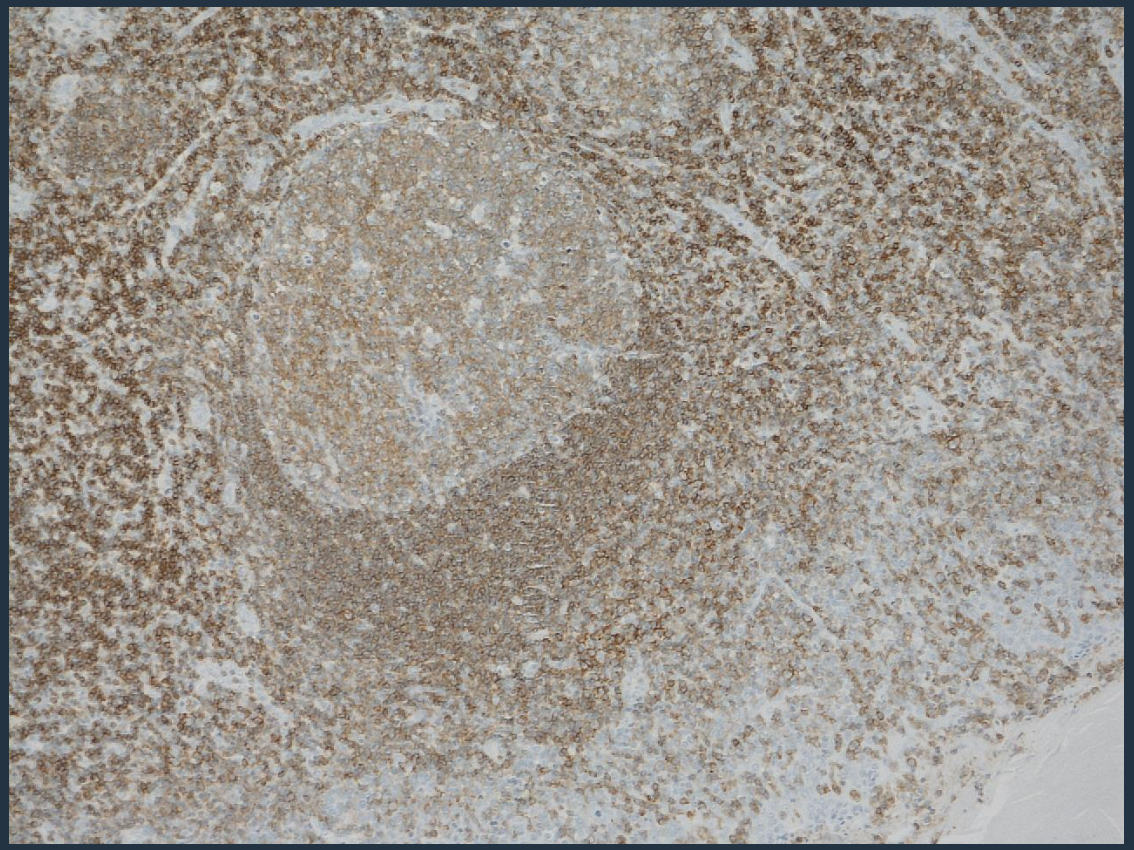
Procedure Type	Platform or Method	
	NexES IHC	**BenchMark Series**
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None required	None required
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37° C	Approximately 16 minutes, 37° C
A/B 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

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%

Controls – Tonsil

RP2/18 Ventana RTU

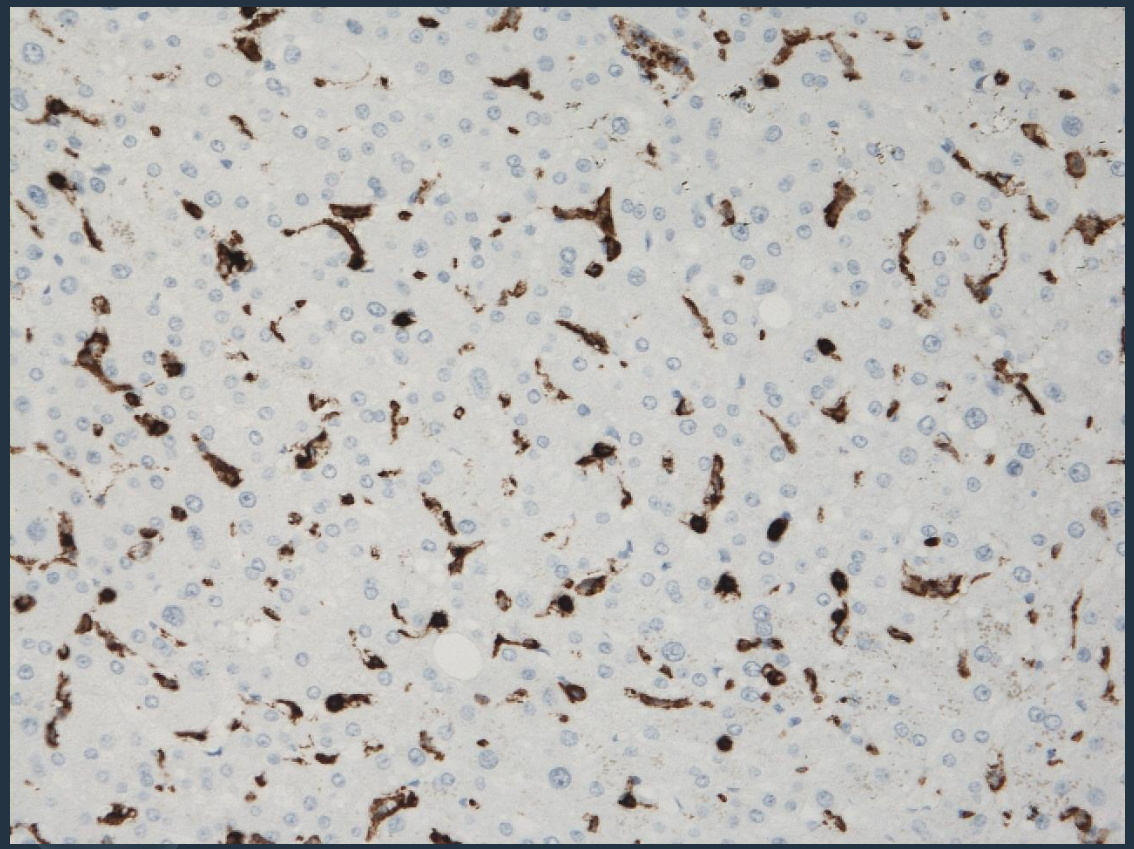


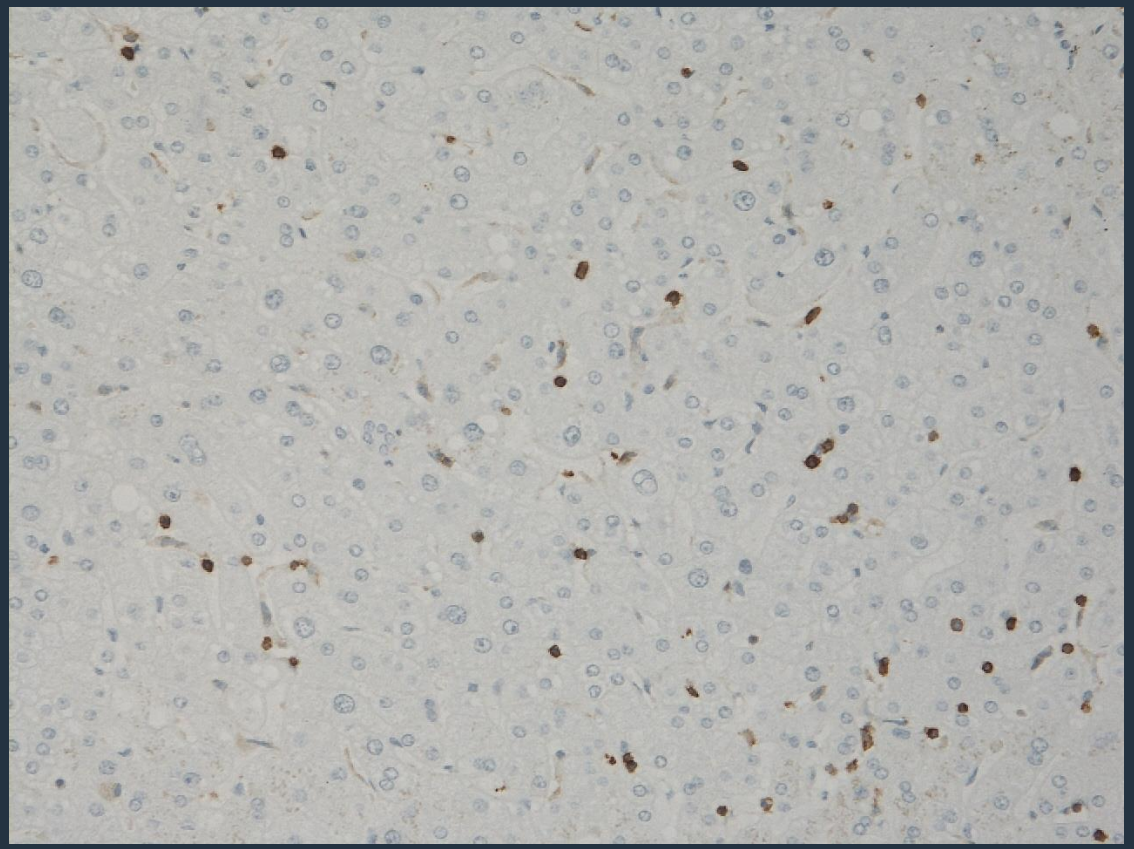


All lymphocytes (B- and T- cells) and histocytes must display a strong distinct membranous staining reaction. Squamous epithelial cells should be negative.

…. And Liver!

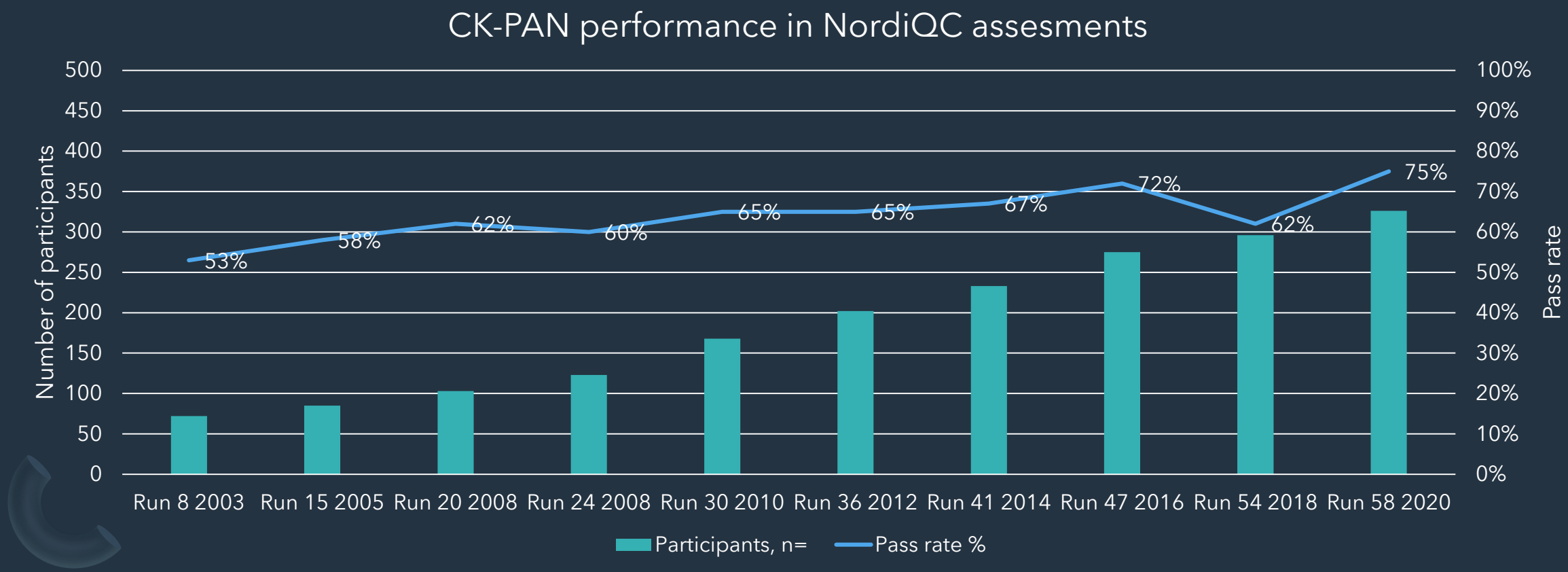
RP2/18 Ventana RTU





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

Pan-CK


CK-PAN performance in NordiQC assesments
Number of participants
Pass rate
500
450
400
350
300
250
200
150
100
50
0
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
53%
58%
62%
60%
65%
65%
67%
72%
62%
75%
Run 8 2003
Run 15 2005
Run 20 2008
Run 24 2008
Run 30 2010
Run 36 2012
Run 41 2014
Run 47 2016
Run 54 2018
Run 58 2020
Participants, n=
Pass rate %

Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone cocktail **AE1/AE3 IR053 (VRPS)[3]**	13	Dako/Agilent	12	-	-	1	92%	92%
mAb clone cocktail **AE1/AE3 IR053 (LMPS)[4]**	14	Dako/Agilent	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%
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	-	-	-	-

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.

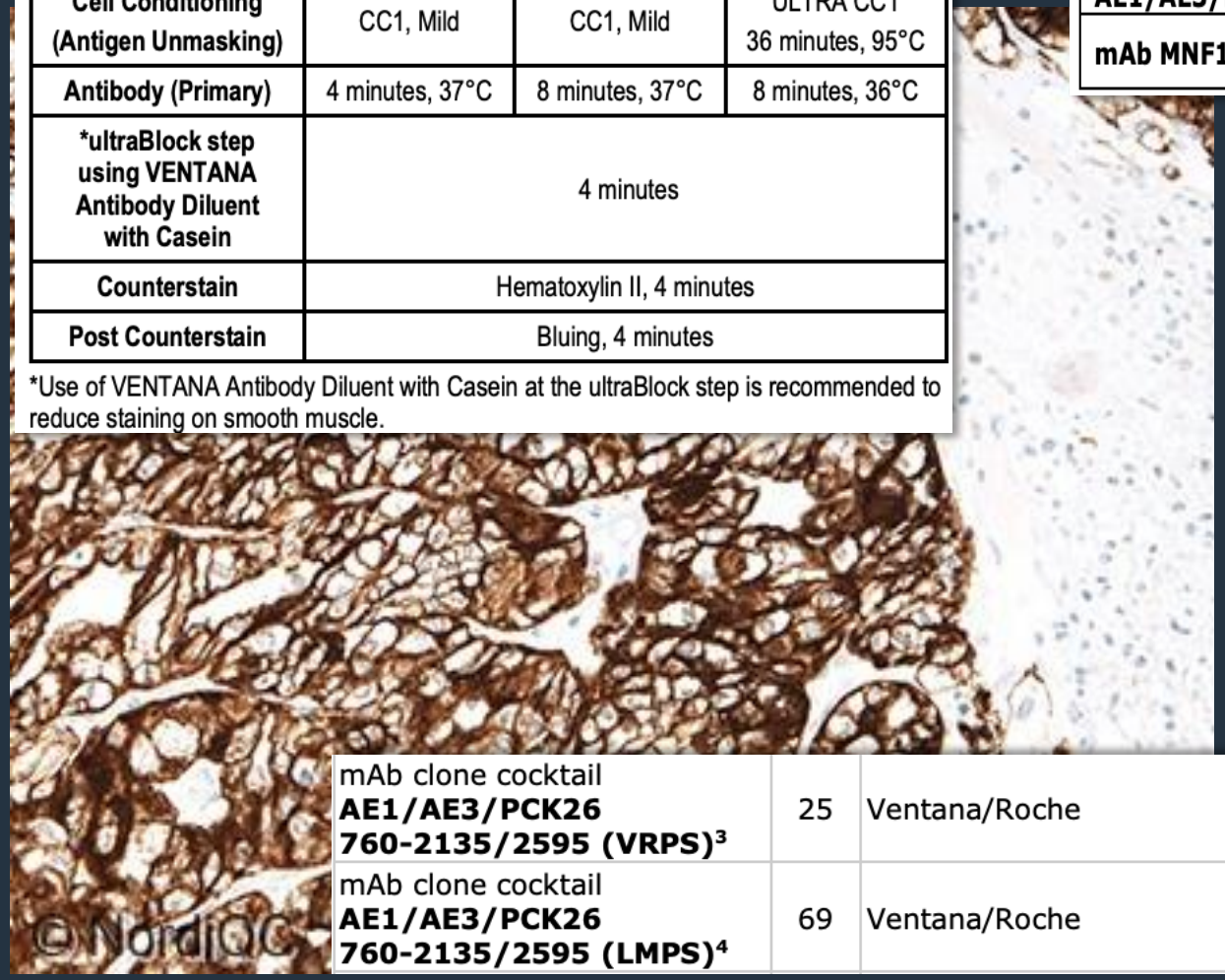
Table 2. Recommended staining protocol for Anti-Pan Keratin (AE1/AE3/PCK26) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

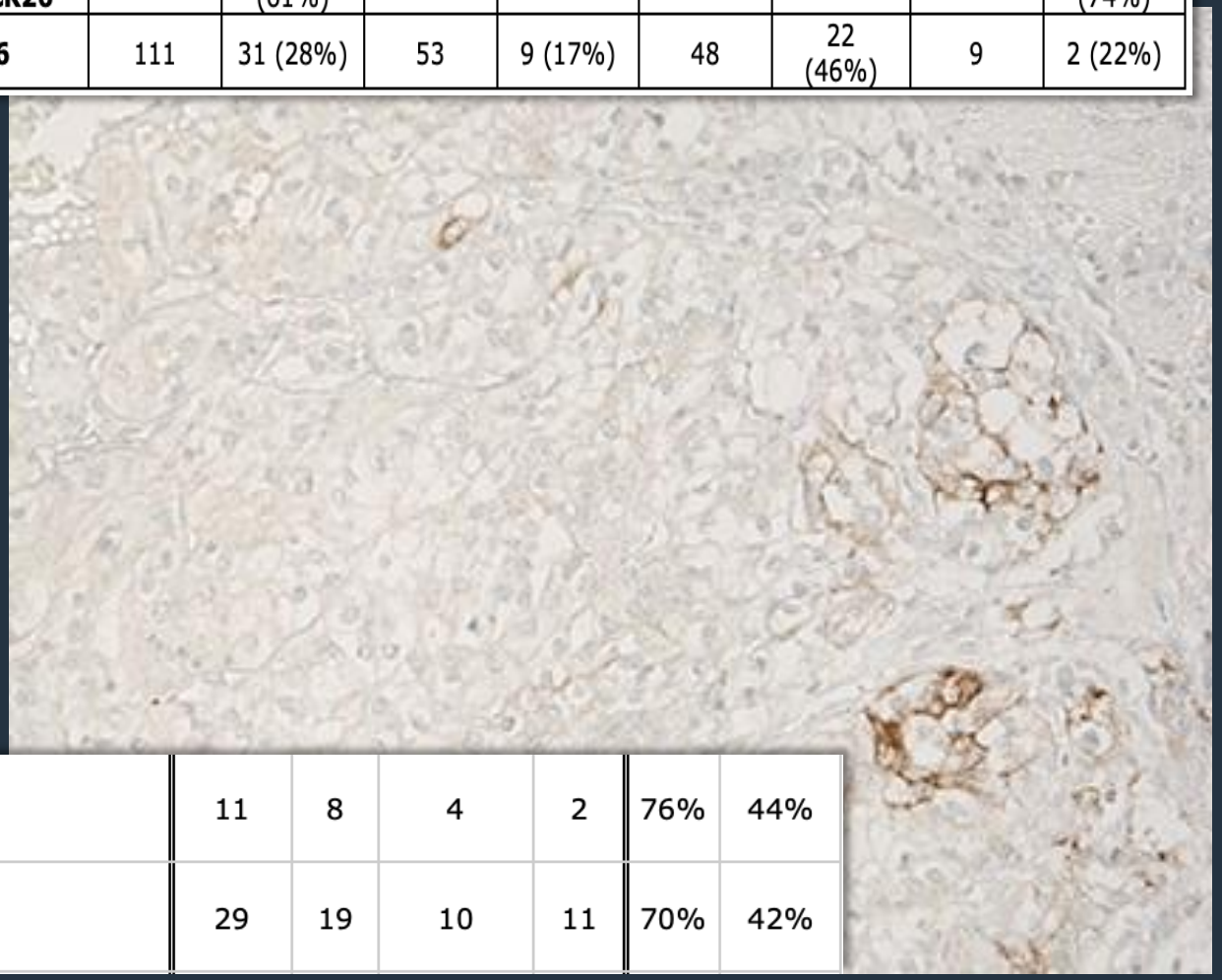
Procedure Type	Method		
	GX	XT	ULTRA
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1 36 minutes, 95°C
Antibody (Primary)	4 minutes, 37°C	8 minutes, 37°C	8 minutes, 36°C
***ultraBlock step using VENTANA Antibody Diluent with Casein**	4 minutes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

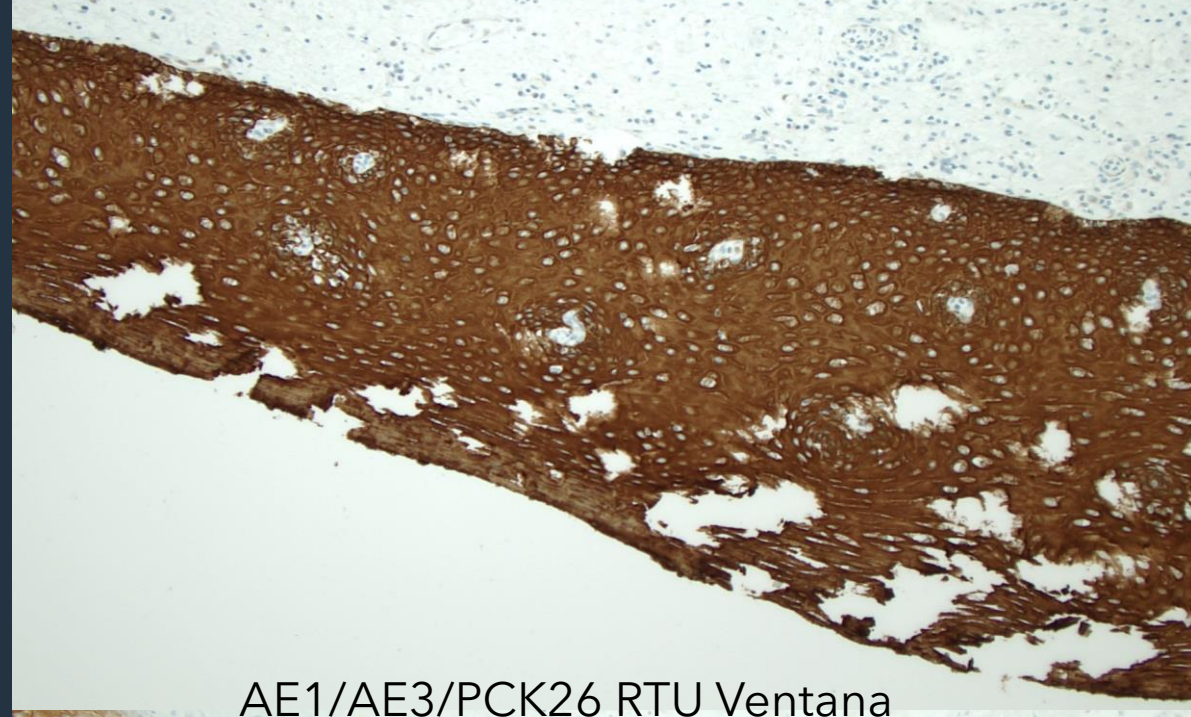




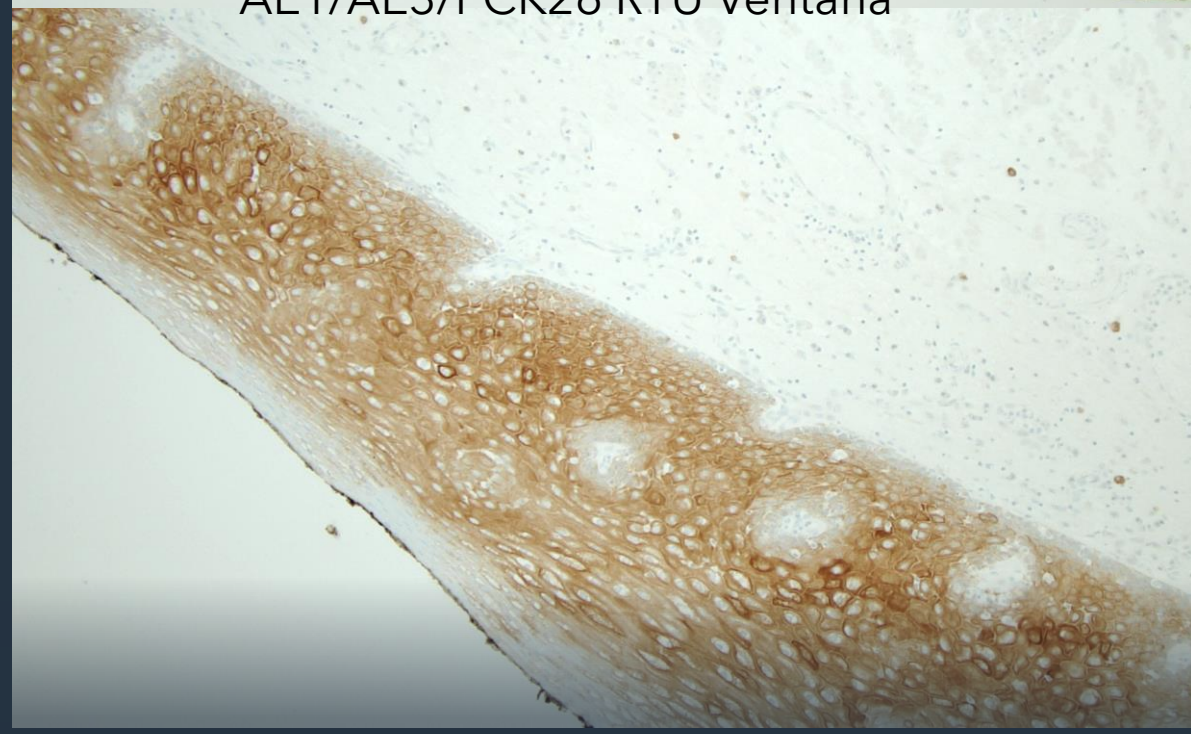
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (VRPS)[3]**	25	Ventana/Roche	11	8	4	2	76%	44%
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (LMPS)[4]**	69	Ventana/Roche	29	19	10	11	70%	42%

Control - Esophagus

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.



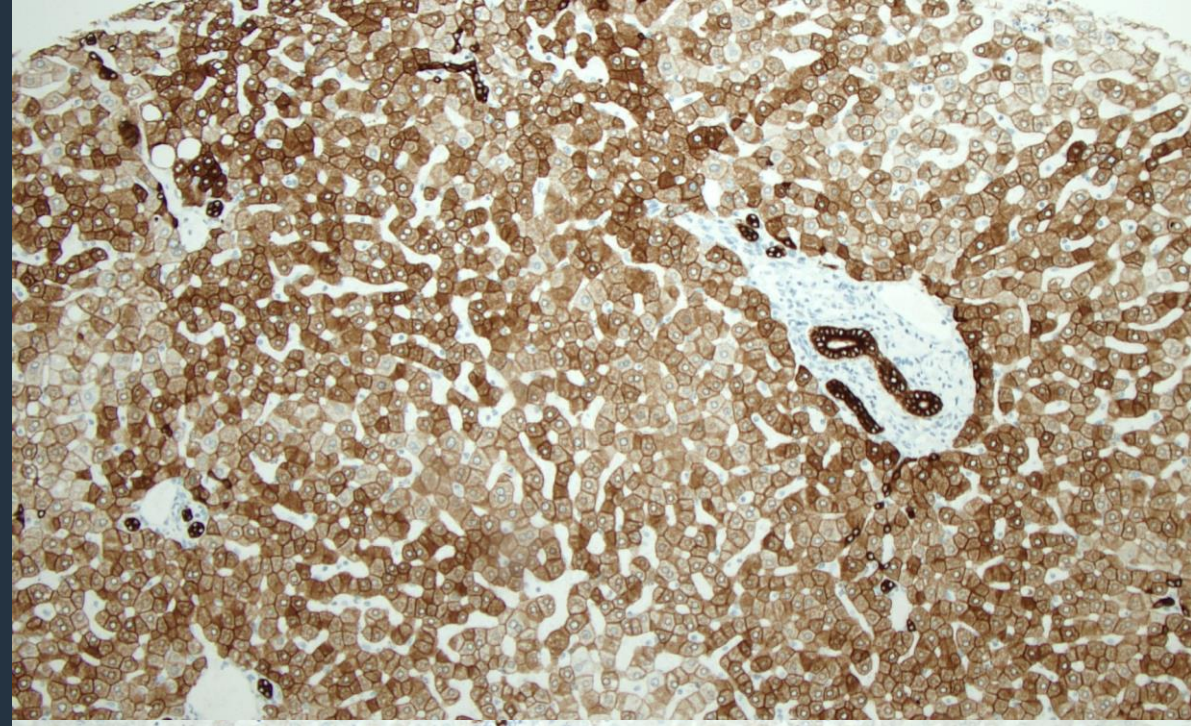
AE1/AE3/PCK26 RTU Ventana



AE1/AE3/PCK26 RTU Ventana

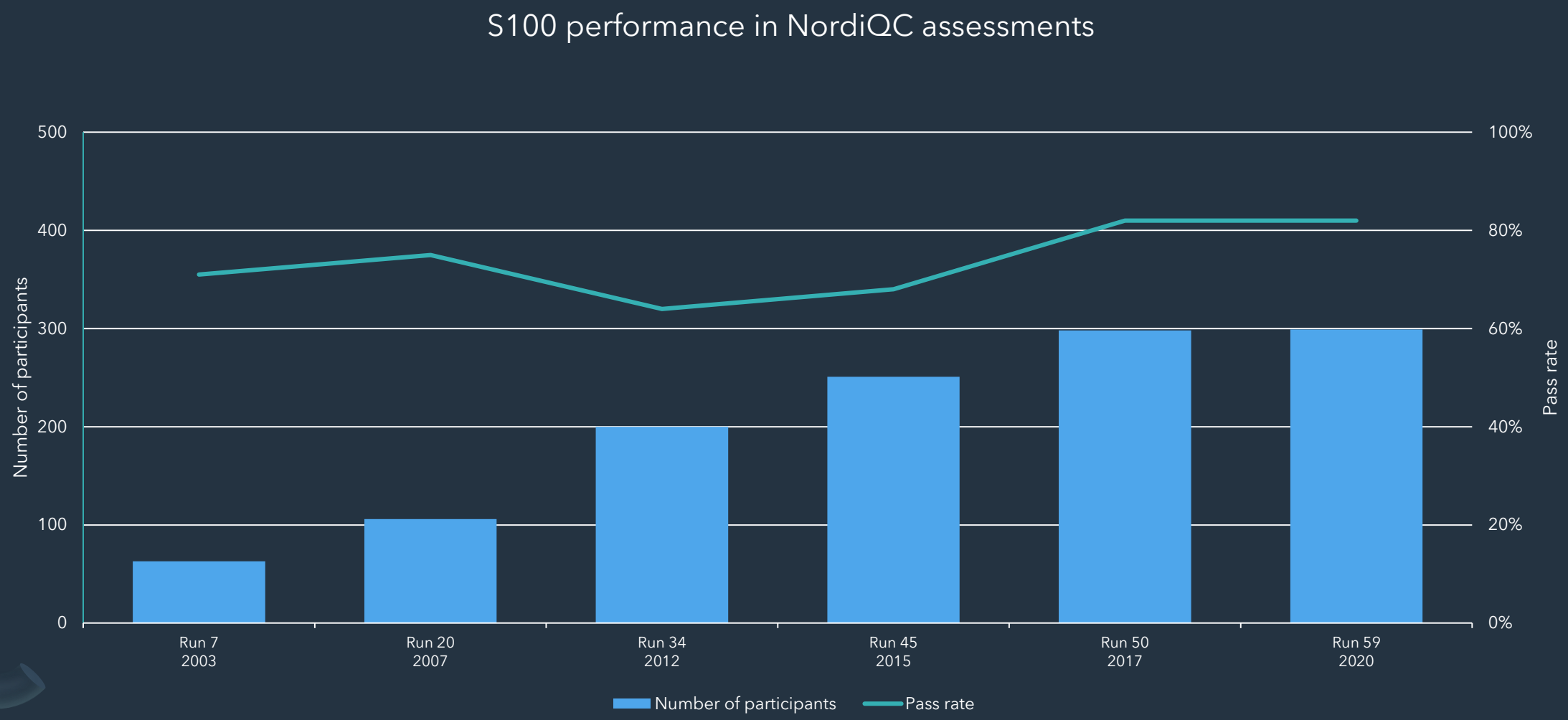
And Liver

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 leat moderate, distinct cytoplasmic and membranous staining reaction. No staining should be seen in stromal cells in the liver.



AE1/AE3 conc. Ventana

S100


S100 performance in NordiQC assessments
Number of participants
Pass rate
500
400
300
200
100
0
100%
80%
60%
40%
20%
0%
Run 7
2003
Run 20
2007
Run 34
2012
Run 45
2015
Run 50
2017
Run 59
2020
Number of participants
Pass rate

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%)

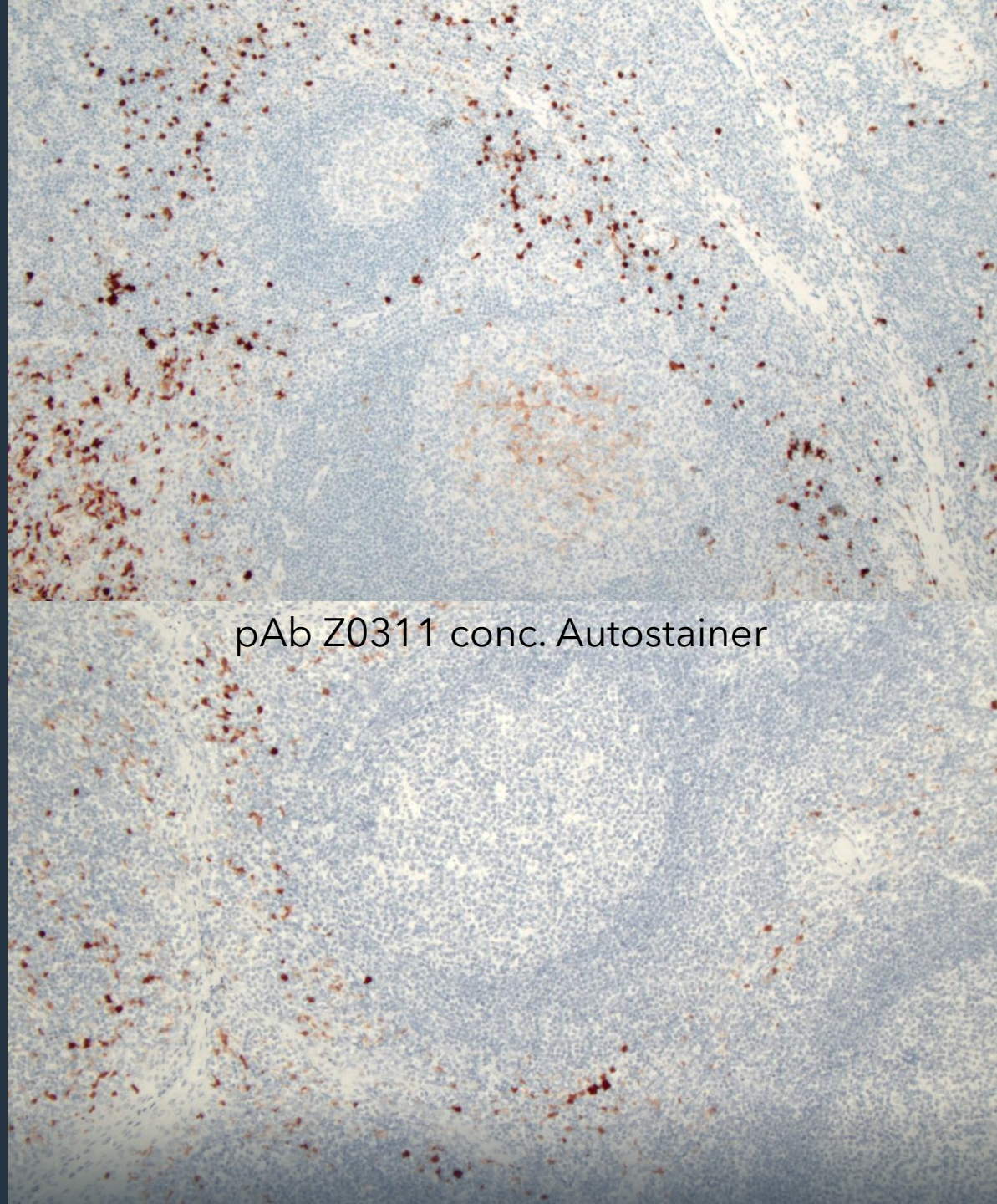
The clone Z0311 which was used by 57% both as concentrate and RTU is now terminated from vendor as a concentrate.

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **4C4.9**	1 1 2 1 1 2 2 1	Thermoscientific Immunologic Cell Marque Diagnostic BioSystems DCS BioCare Medical Zytomed Systems Zeta Corporation	2	5	2	2	63%	18%
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 **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 **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 **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%	

Controls

Only Z0311

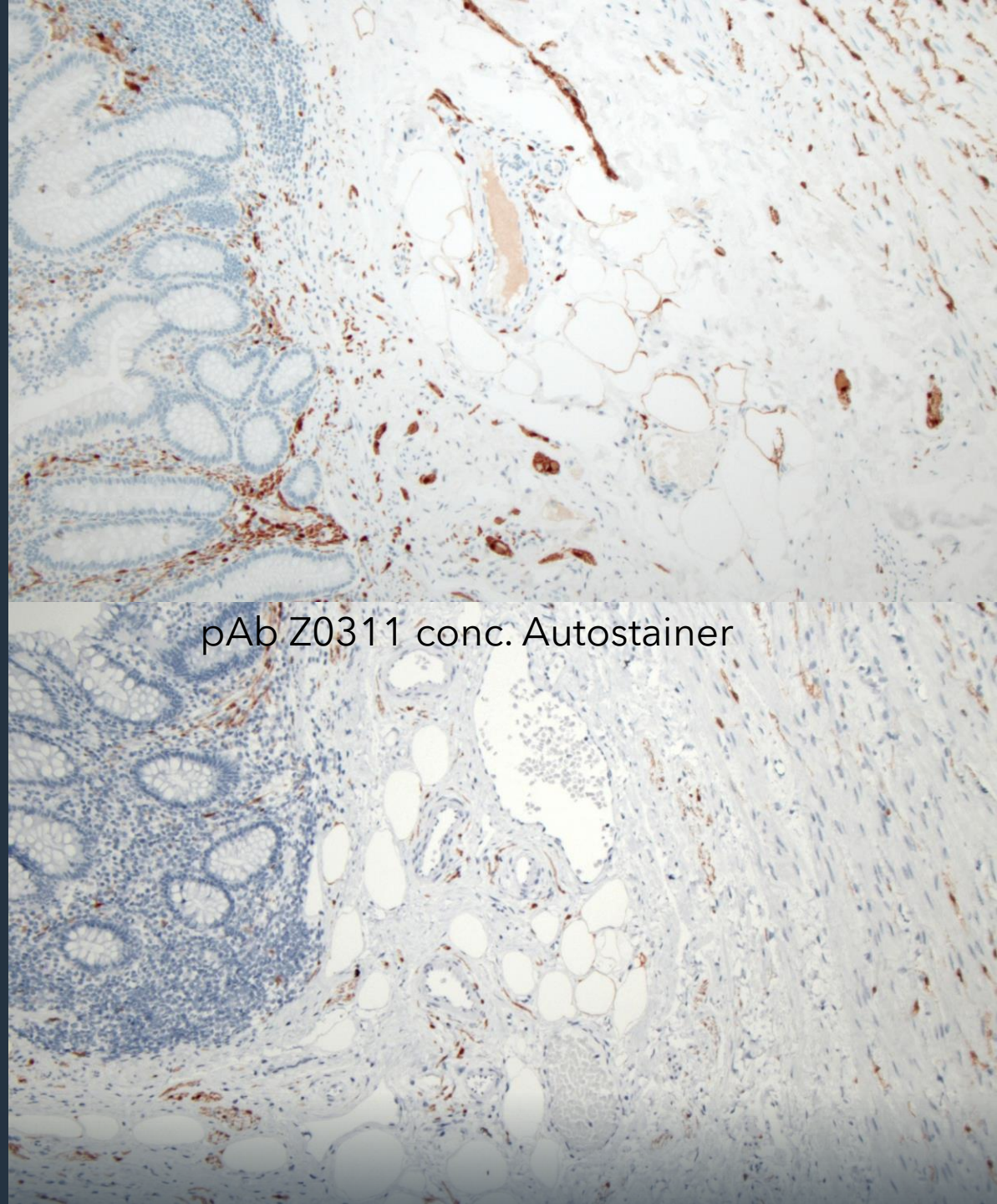
In the tonsil, interfollicular dendritic cells and Langerhans cells of the squamous epithelium, must display a moderate to strong staining intensity whereas the follicular dendritic cell meshwork of the germinal centres should show an at least weak to moderate nuclear and cytoplasmic staining reaction.



pAb Z0311 conc. Autostainer

Appendix

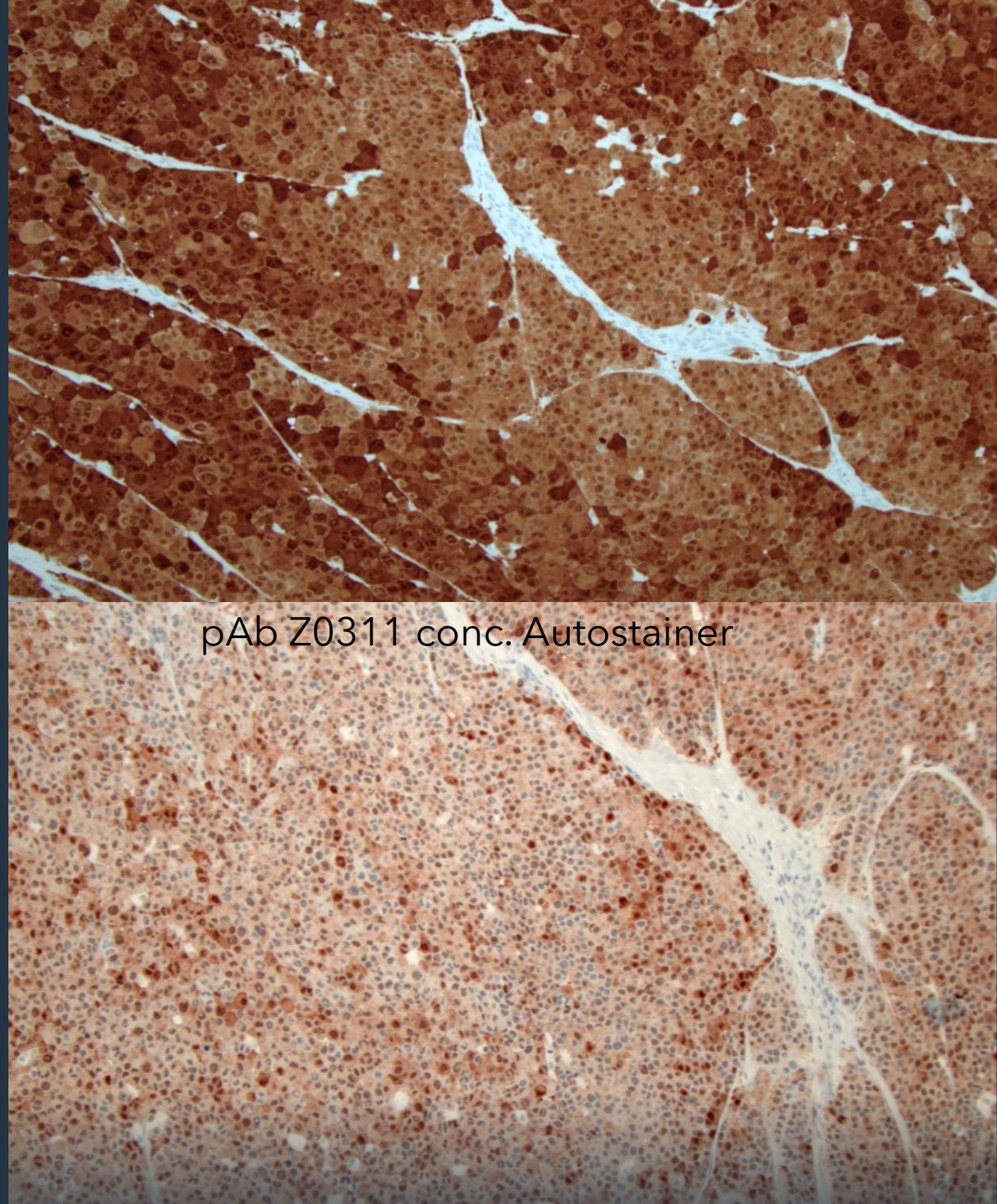
Virtually all adipocytes and Schwann cells of peripheral nerves, must show an as strong as possible nuclear and cytoplasmic staining reaction without any staining reaction of the smooth muscle or epithelial cells.



pAb Z0311 conc. Autostainer

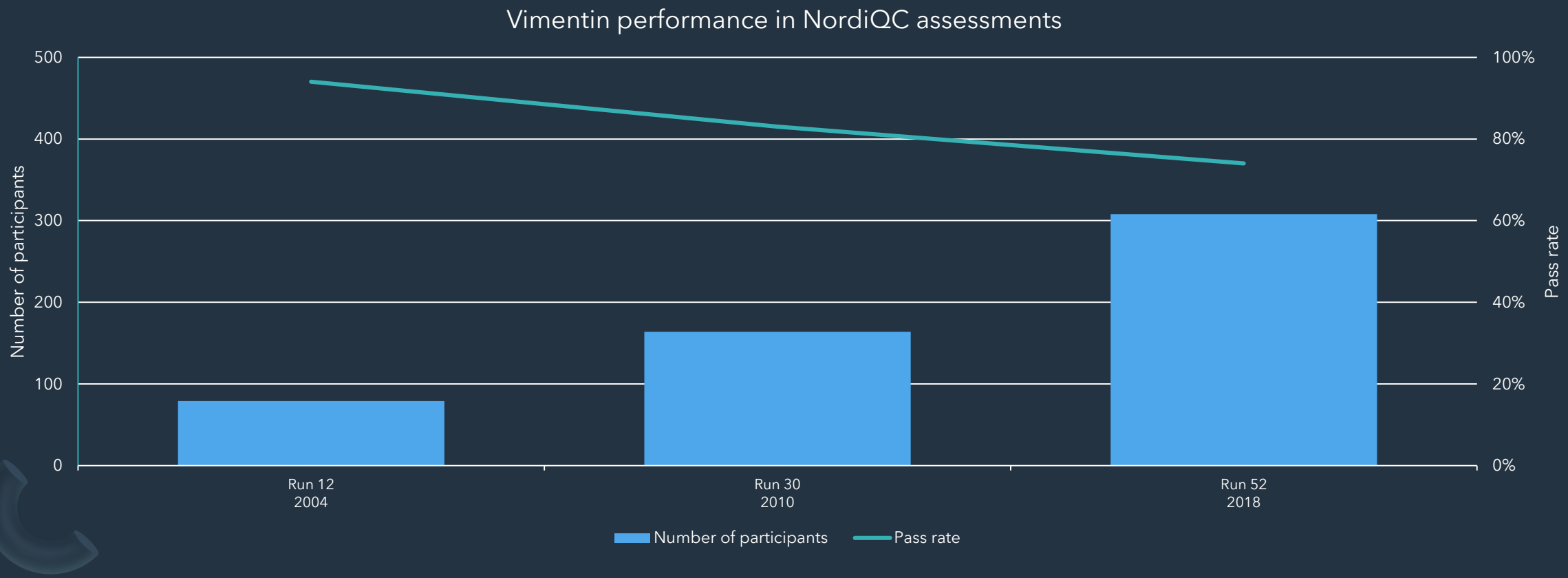
In addition

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



pAb Z0311 conc. Autostainer

Vimentin


Vimentin performance in NordiQC assessments
Number of participants
Pass rate
500
400
300
200
100
0
100%
80%
60%
40%
20%
0%
Run 12
2004
Run 30
2010
Run 52
2018
Number of participants
Pass rate

Tonsil is out

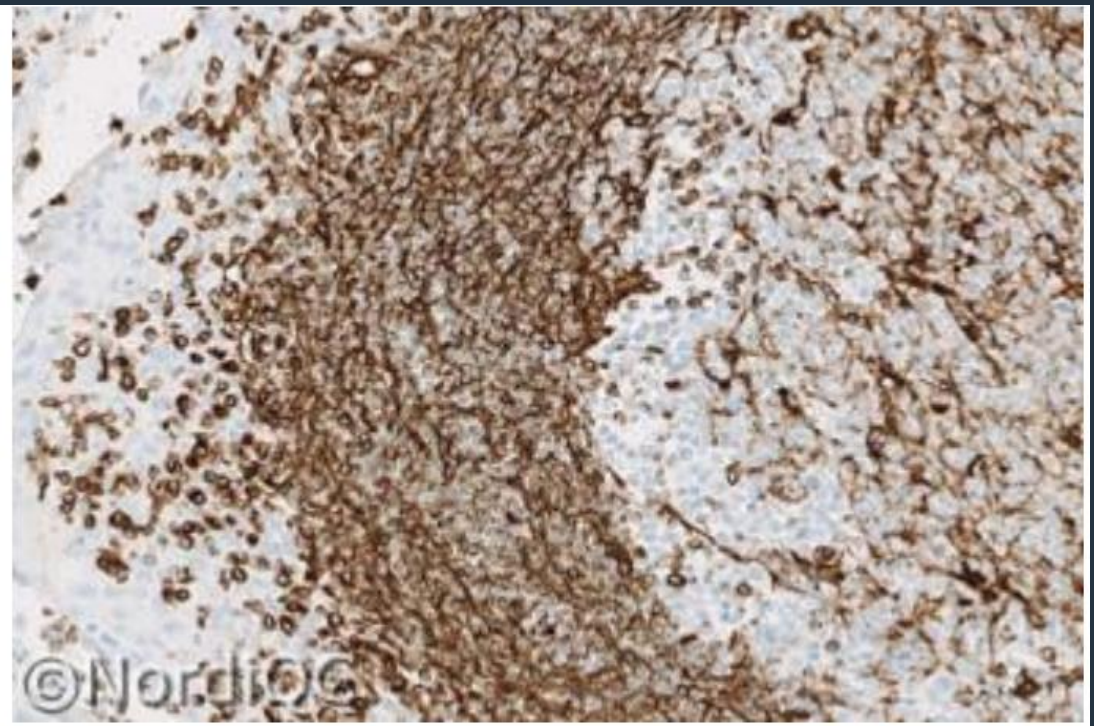
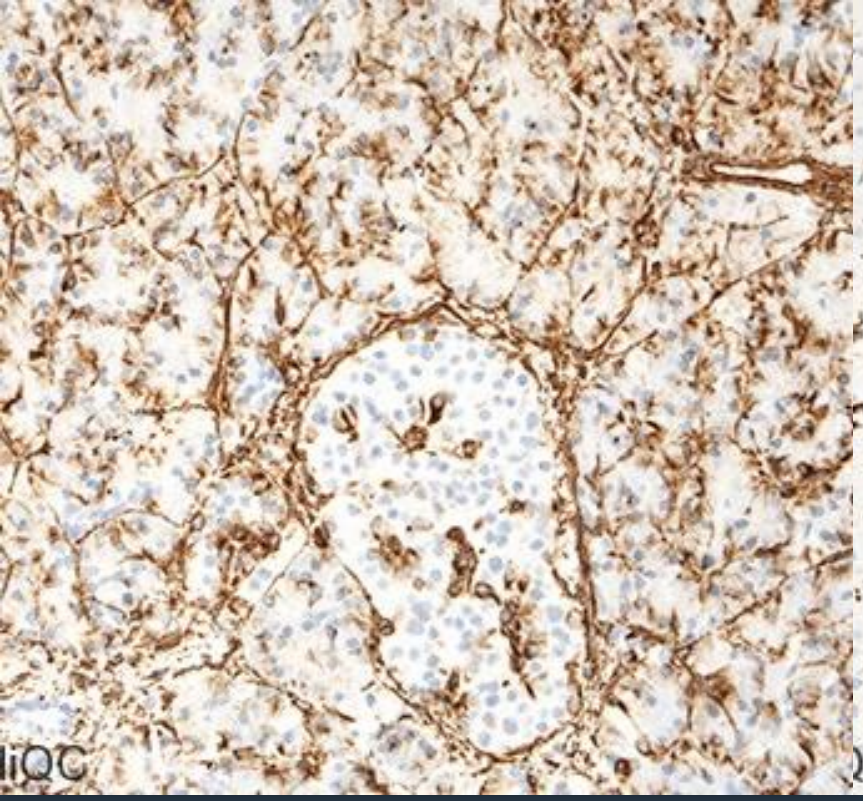
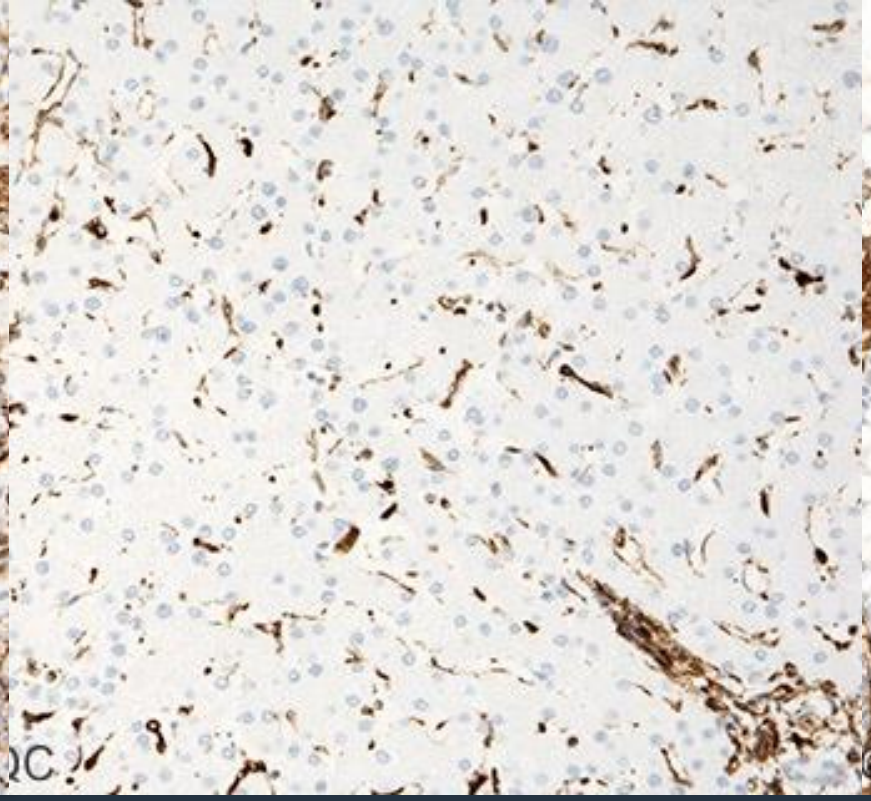


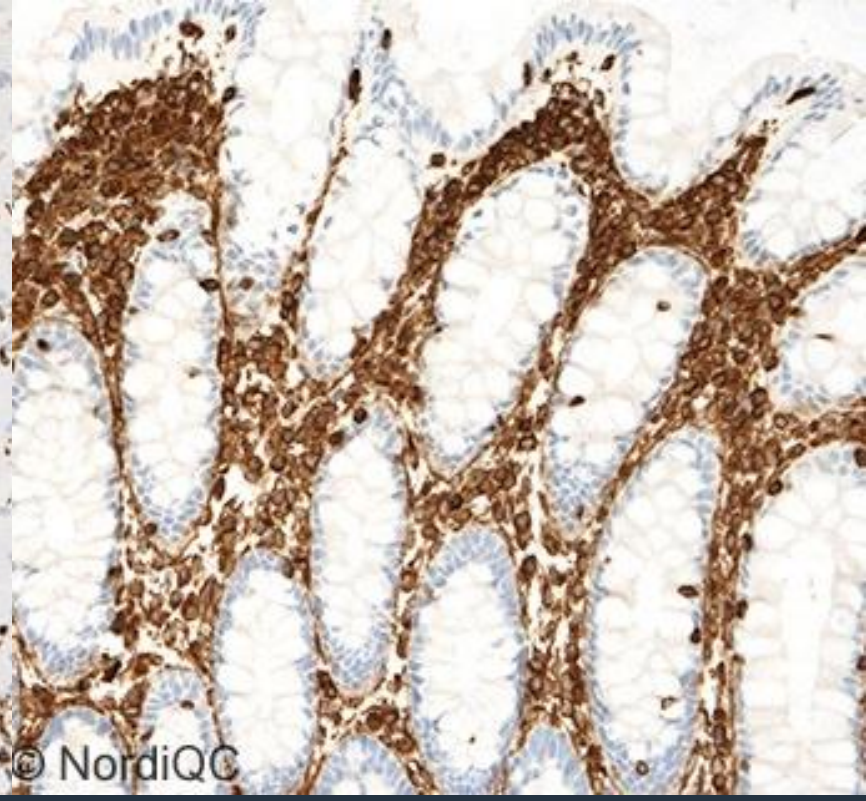
Fig. 1a
Optimal VIM staining of the tonsil using the mAb clone V9 carefully calibrated after HIER. The intraepithelial lymphocytes, the mantle zone B-cells and the germinal centre macrophages show a strong and distinct staining. No staining is is seen in the squamous epithelial cells.



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 IR630**[3]	5	Agilent/Dako	5	0	0	0	-	-
mAb clone **V9 GA630**	29	Agilent/Dako	23	2	4	0	86%	79%
mAb clone **V9 GA630**[3]	2	Agilent/Dako	1	0	1	0	-	-
mAb clone **V9 790-2917**	100	Roche/Ventana	21	51	19	9	72%	21%
mAb clone **V9 347M-10**	2	Cell Marque	0	1	1	0	-	-
mAb clone **V9 PA0640**	7	Leica/Novocastra	5	2	0	0	100%	71%
mAb clone **V9 PA0640**[3]	1	Leica/Novocastra	0	0	0	1	-	-

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 strictly: 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 is outdated and needs to be revised.

ed Staining Protocols for CONFIRM anti-Vimentin (V9)

e	Platform or Method	
	ES or NexES IHC	**BenchMark Series**
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	10 mM sodium citrate (pH 6.0), 2 minutes, Decloaking Chamber, 120° C	Cell Conditioning 1, Standard
Enzyme (Protease)	None required	None required
Antibody (Primary)	Approximately 16 minutes, 37° C	Approximately 16 minutes, 37° C
A/B Block (Biotin Blocking)	Optional	Optional
Amplify (Amplification)	Optional	Optional
Counterstain (Hematoxylin)	Hematoxylin, 2 to 4 minutes	Hematoxylin, 2 to 4 minutes
Post Counterstain	Bluing, 2 to 4 minutes	Bluing, 2 to 4 minutes

The procedures for staining on the Ventana automated slide stainers are as follows. For more detailed instructions and additional protocol options, refer to your Operator's Manual.

Overview

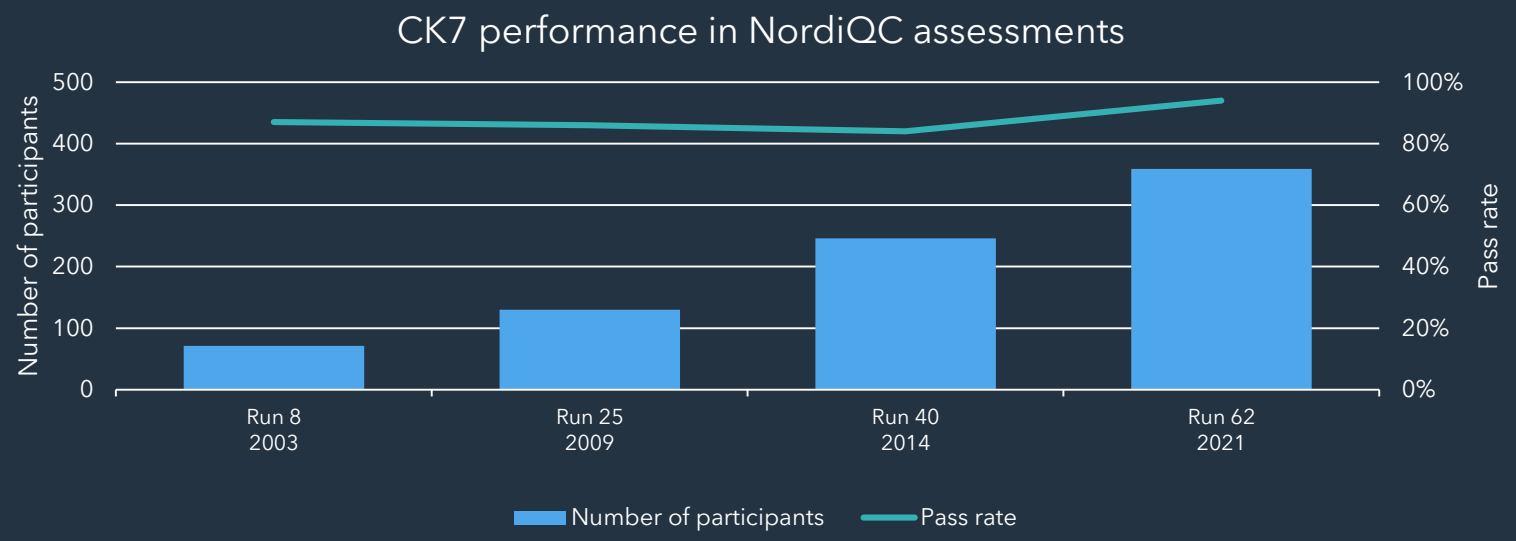
Marker	Last run	Pass rate/optimal	No. of labs
CD45	Run 59 2020	94% / 79%	296
PAN-CK	Run 58 2020	75% / 52%	326
S100	Run 59 2020	82% / 48%	299
Vimentin	Run 52 2018	74% / 43%	308

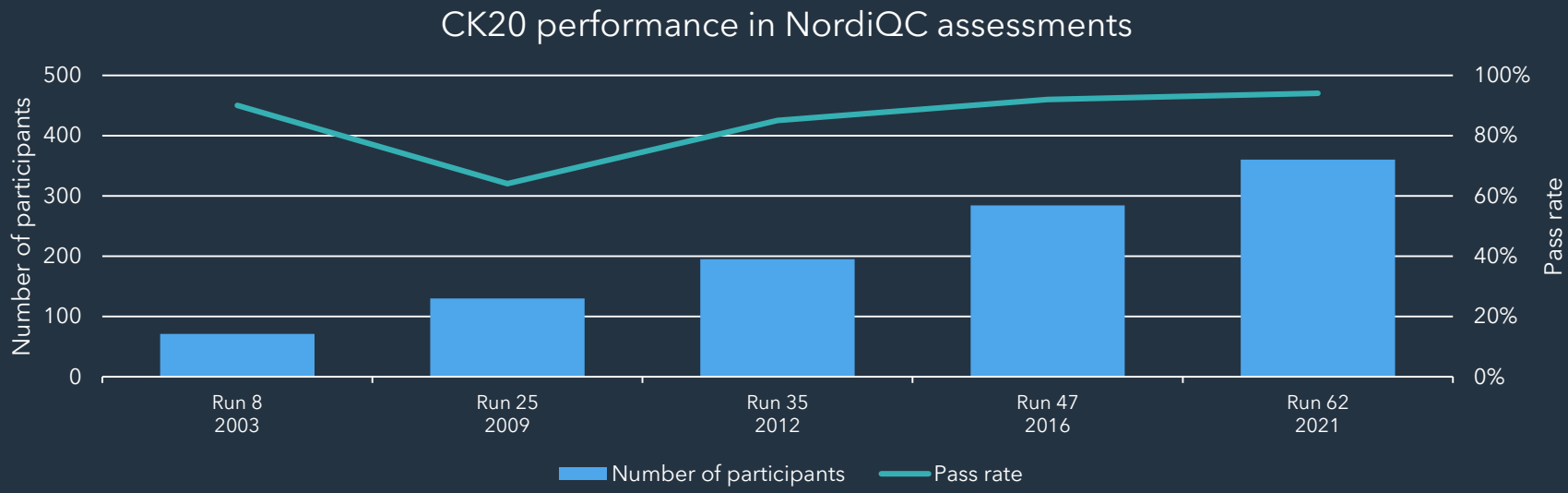
What else do you have?

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	58 2020*	58% / 33%	105
CDX2	Pancreas, tonsil	61 2021	91% / 79%	325
SMAD4	Tonsil	57 2019*	42% / 31%	52
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, fallowpian tube	62 2021	45% / 19%	310
NKX3,1	Testis, prostate	58 2020	82% / 55%	107
P16	Tonsil	59 2020	83% / 50%	291
ASMA	Appendix, liver	59 2020	69% / 28%	260
CD117	Appendix	56 2019	87% / 48%	312
CD31	Liver, tonsil	62 2021	79% / 56%	342
BRAF	Positive and negative tumors	62 2021*	72% / 35%	135

*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, IR619**[3]	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone **OV-TL 12/30, IR619**[4]	12	Dako/Agilent	11	1	0	0	100%	92%
mAb clone **OV-TL 12/30, GA619**[3]	32	Dako/Agilent	31	1	0	0	100%	97%
mAb clone **OV-TL 12/30, GA619**[4]	30	Dako/Agilent	27	2	1	0	97%	90%
mAb clone **RN7, PA0942**[3]	6	Leica Biosystems	1	5	0	0	100%	17%
mAb clone **RN7, PA0942/PA0138**[4]	11	Leica Biosystems	5	6	0	0	100%	45%
rmAb clone **SP52, 790-4462**[3]	16	Ventana/Roche	11	5	0	0	100%	69%
rmAb clone **SP52, 790-4462**[4]	101	Ventana/Roche	86	12	2	1	97%	85%
Total	359		265	71	20	3	-	
Proportion			74%	20%	5%	1%	94%	

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

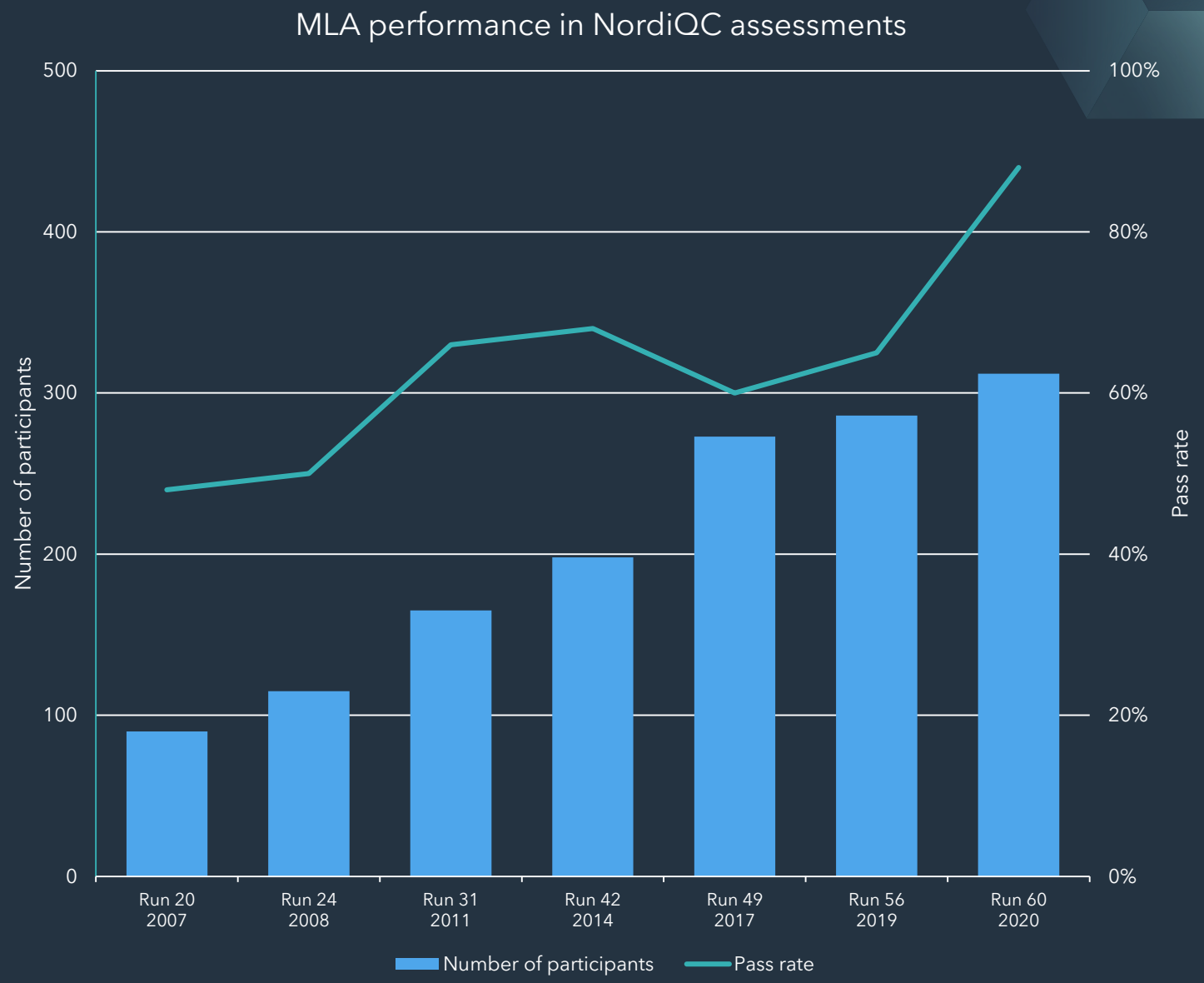
Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
rmAb clone **SP33 790-4431**[3]	19	Ventana/Roche	16	3	-	-	100%	84%
rmAb clone **SP33 790-4431**[4]	105	Ventana/Roche	89	14	2	-	98%	85%
mAb clone **Ks20.8 IR/IS777**[3]	18	Dako/Agilent	14	4	-	-	100%	78%
mAb clone **Ks20.8 IR/IS777**[4]	16	Dako/Agilent	12	3	1	-	94%	75%
mAb clone **Ks20.8 GA777**[3]	33	Dako/Agilent	31	2	-	-	100%	94%
mAb clone **Ks20.8 GA777**[4]	27	Dako/Agilent	19	7	1	-	96%	70%
mAb clone **Ks20.8 PA0022**[3]	5	Leica Biosystems	4	1	-	-	100%	80%
mAb clone **Ks20.8 PA0022**[4]	10	Leica Biosystems	7	3	-	-	100%	70%
Total	360		266	72	21	1		
Proportion			74%	20%	6%	-	94%	

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	58 2020*	58% / 33%	105
CDX2	Pancreas, tonsil	61 2021	91% / 79%	325
SMAD4	Tonsil	57 2019*	42% / 31%	52
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	62 2021	45% / 19%	310
NKX3,1	Testis, prostate	58 2020	82% / 55%	107
P16	Tonsil	59 2020	83% / 50%	291
ASMA	Appendix, liver	59 2020	69% / 28%	260
CD117	Appendix	56 2019	87% / 48%	312
CD31	Liver, tonsil	62 2021	79% / 56%	342
BRAF	Positive and negative tumors	62 2021*	72% / 35%	135

*First NordiQC run

Melan A

Last time the pass-rate was 65% (run 56 2019) in the latest assessment it was 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]	OR[2]
mAb clone **A103**	57	Dako/Agilent	21	57	9	0	90%	24%
	19	Novocastra/Leica						
	6	Cell Marque						
	1	Abcam						
	1	Biocare						
	1	Monosan						
	1	Biogenex						
	1	Zeta Corporation						
rmAb clone **EP43**	9	Nordic Biotite	18	1	0	0	100%	95%
	9	Epitomics						
	1	Cell Marque						
Ready-To-Use antibodies								
mAb clone **A103 790-2990**[3]	3	Ventana/Roche	0	0	3	0	-	-
mAb clone **A103 790-2990**[4]	94	Ventana/Roche	6	74	11	3	85%	6%
mAb clone **A103, IR633/IS633**[3]	14	Dako/Agilent	1	13	0	0	100%	7%
mAb clone **A103, IR633/IS633**[4]	56	Dako/Agilent	12	36	7	1	85%	21%
mAb clone **A103, PA0233/PA0044**[3]	7	Leica Biosystems	2	5	0	0	100%	29%
mAb clone **A103, PA0233/PA0044**[4]	10	Leica Biosystems	6	4	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:	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:	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 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

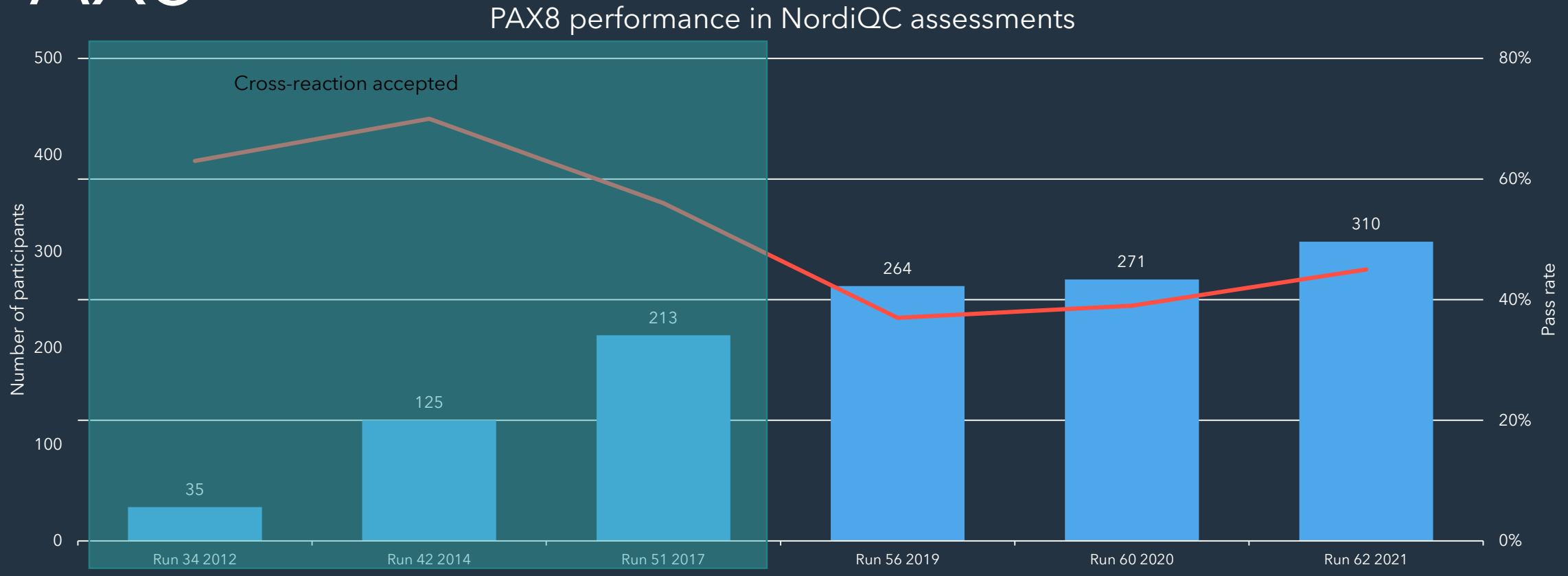
Epitope retrieval, HIER

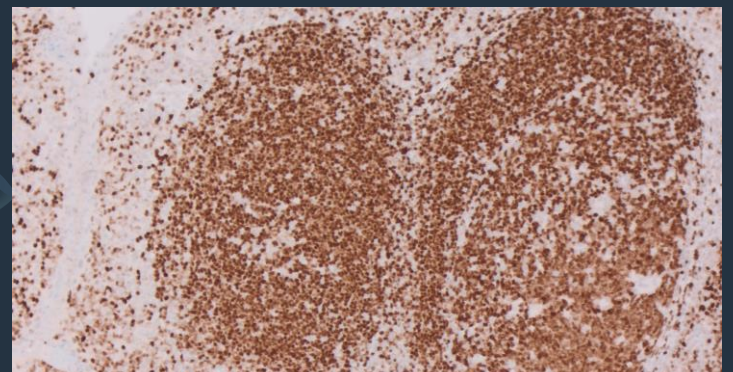
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	99°C

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
Cross-reaction accepted
Number of participants
Pass rate
500
400
300
200
100
0
80%
60%
40%
20%
0%
35
125
213
264
271
310
Run 34 2012
Run 42 2014
Run 51 2017
Run 56 2019
Run 60 2020
Run 62 2021
Number of participants
Pass rate



Pushing in the right direction

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC12***	4 1 1	Biocare Zytomed Systems Diagnostic Biosystems	-	3	1	2	50%	-
mAb clone **MRQ-50**	34	Cell Marque	-	18	8	8	55%	-
rmAb clone **EP298***	7 4 3 1	Cell Marque Epitomics[5] BIO SB Nordic Biosite	4	4	6	1	53%	27%
rmAb clone **EP331***	8 4 1	Cell Marque Epitomics Abcam	-	5	7	1	39%	-
rmAb clone **SP348***	55 5	Abcam Gennova	47	10	1	2	95%	78%
rmAb clone **ZR-1***	5 1 1	Zeta Corporation Abcam Bio SB	3	1	3	-	57%	43%
pAb, **10336-1-AP**	21	Proteintech	-	8	9	4	38%	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
rmAb clone, **EP331* 760-6077(VRPS)[3]**	2	Ventana/Cell Marque	-	1	1	-	-	-
rmAb clone, **EP331* 760-6077(LMPS)[4]**	14	Ventana/Cell Marque	-	1	11	2	7%	-
mAb clone **MRQ-50, 760-4618 (VRPS)[3]**	1	Ventana/Roche	-	-	1	-	-	-
mAb clone **MRQ-50, 760-4618 (LMPS)[4]**	68	Ventana/Roche	-	7	44	17	10%	-
Total	310		60	79	121	50	-	
Proportion			19%	26%	39%	16%	45%	

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***

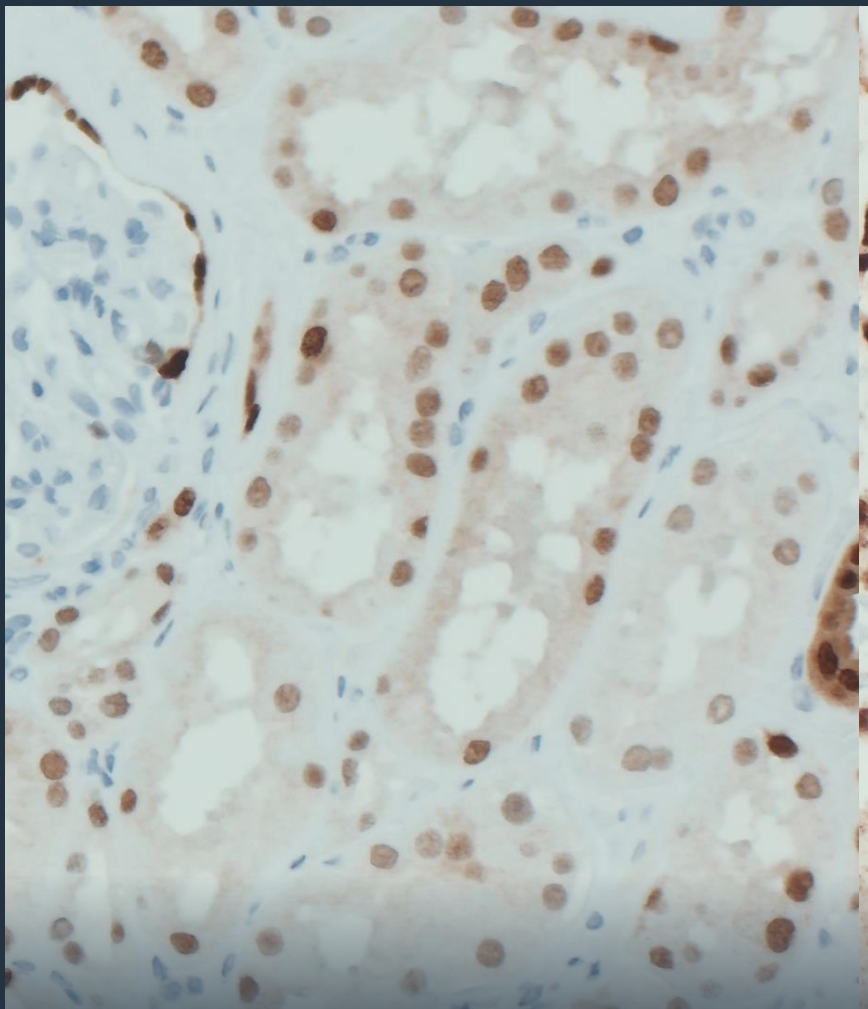
Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra		Leica Biosystems Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
rmAb **EP298**	1/1	-	3/7 (43%)	-	0/6	-	-	-
rmAb **SP348**	0/3	-	17/21 (81%)	-	29/35 (83%)	0/1	-	-
rmAB **ZR-1**	2/2	-	-	-	0/2	-	1/1	-
rmAb **EP331**	0/2	-	0/6	-	0/4	-	0/1	-

Table 5. Overview of the assessment marks for mAb clone MRQ-50 on the four main IHC instruments.

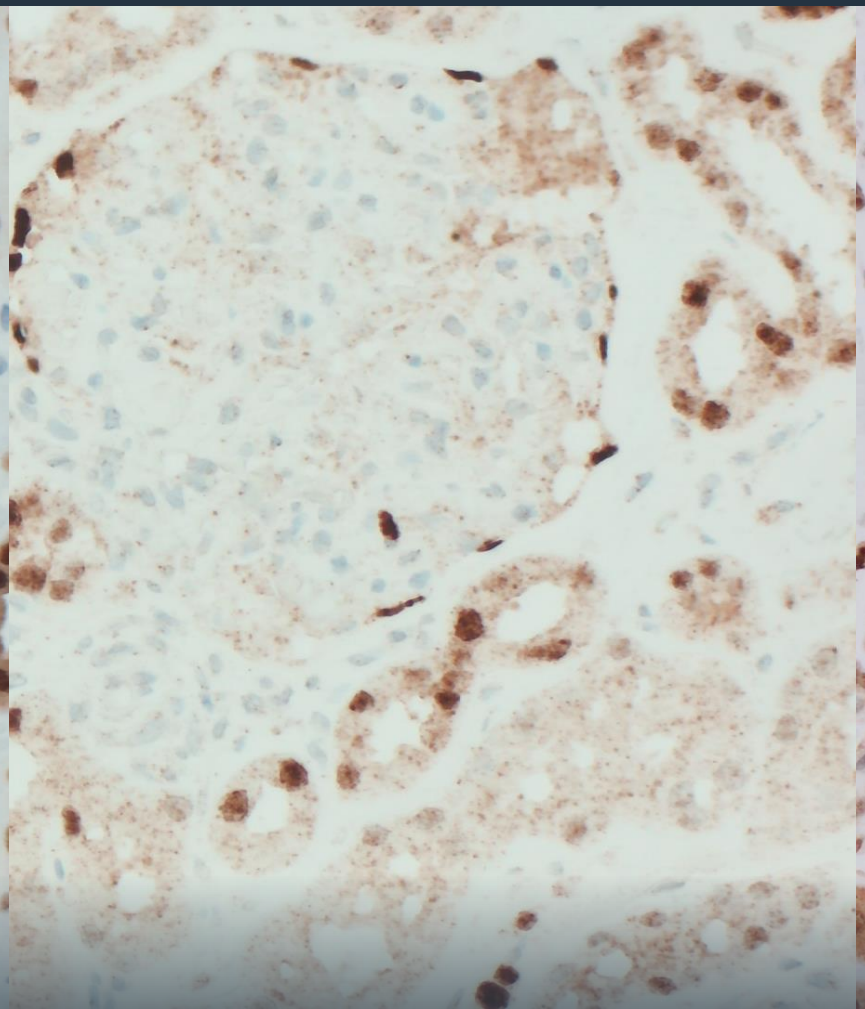
MRQ-50 score	Dako/Agilent Autostainer	Dako/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max
Optimal	-	-	-	-
Good	11	-	9	14
Borderline	3	5	59	3
Poor	-	3	31	-
Total	14	8	99	17
Sufficient %	79%	0%	9%	82%

Kidney

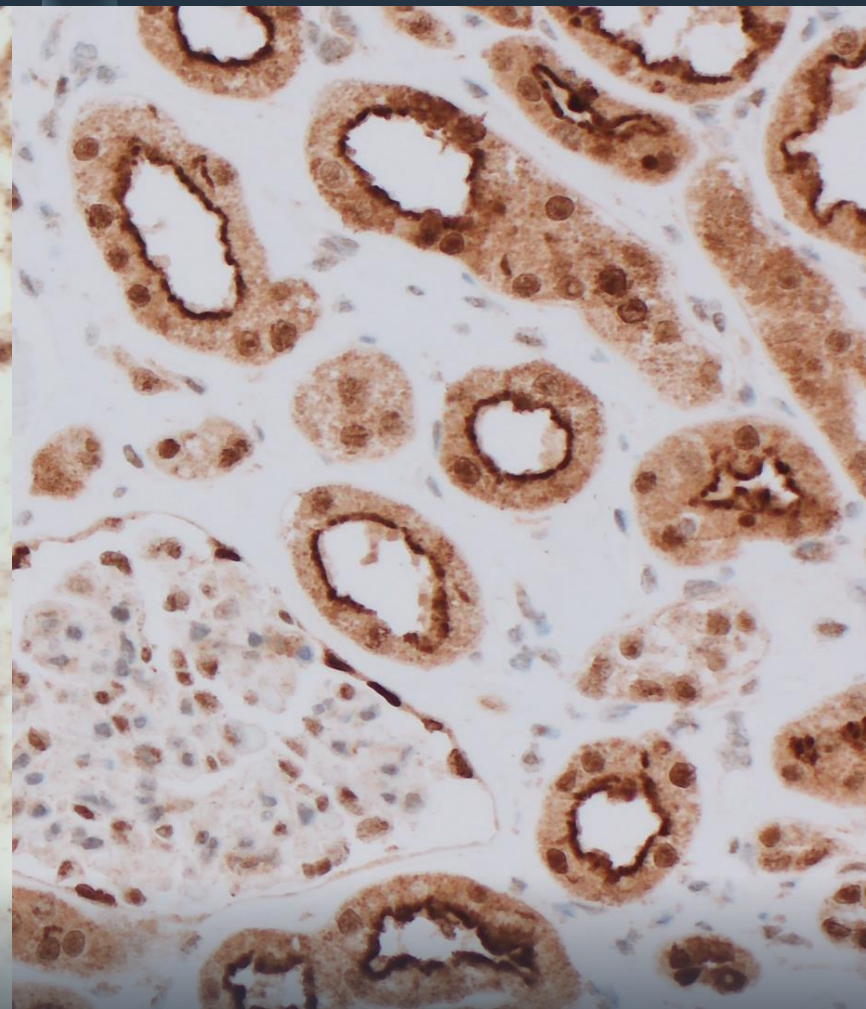
SP348 - Ventana



MRQ-50 - Ventana

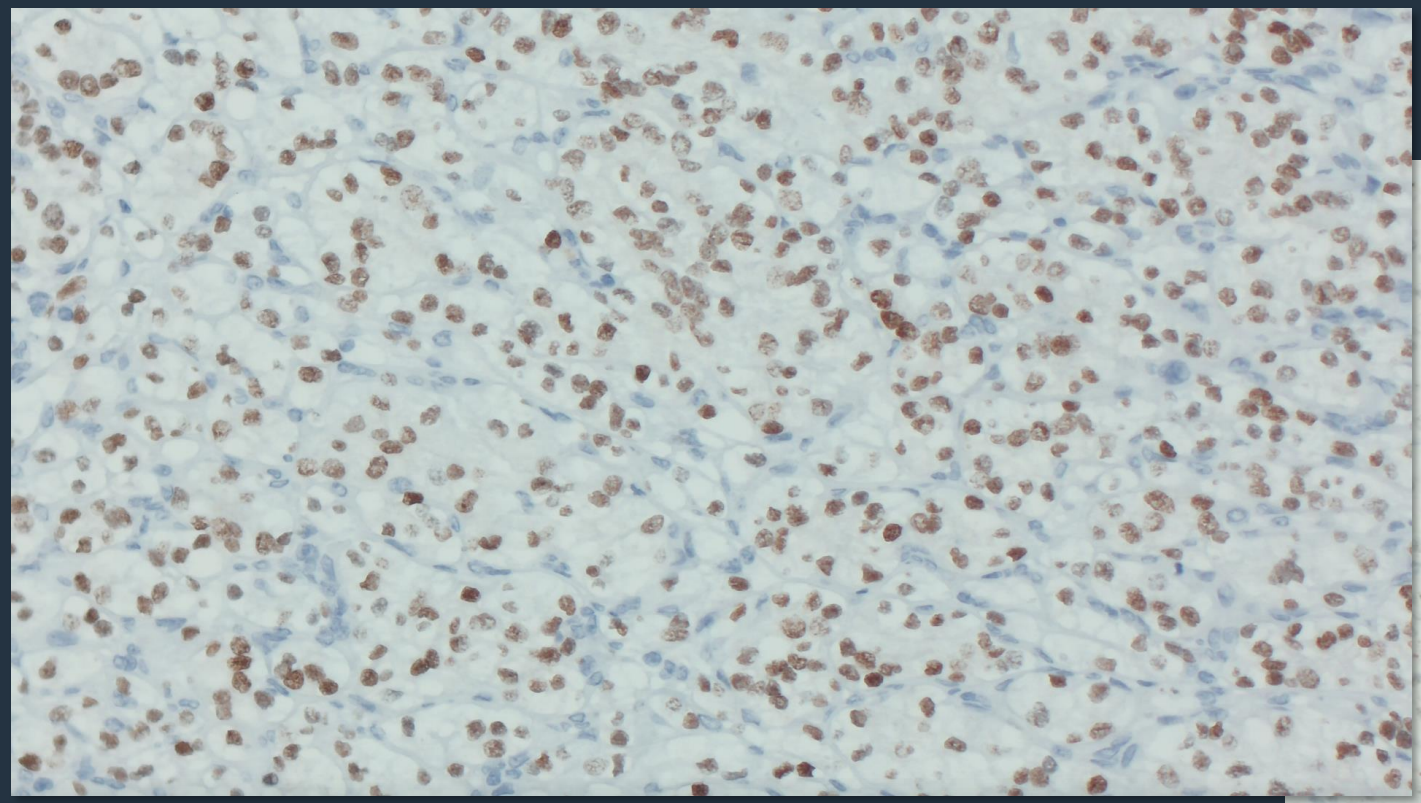


EP331 Autostainer

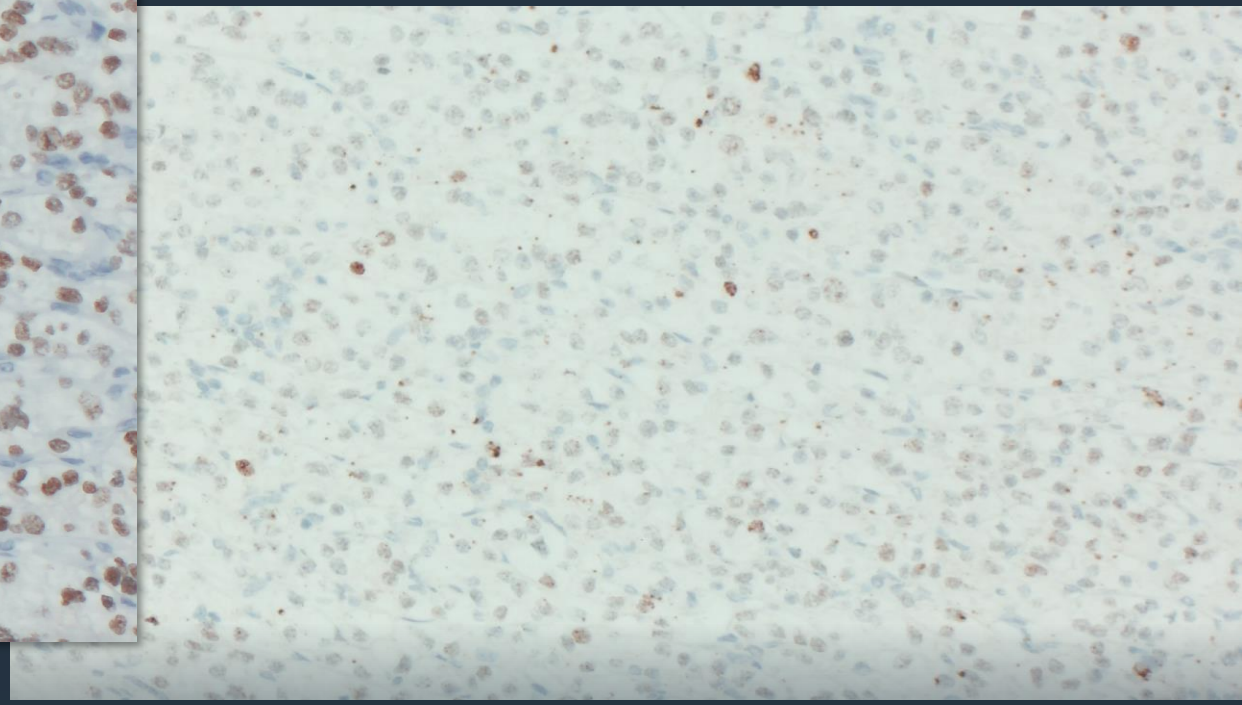


Renal clear cell carcinoma

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:	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:	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 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

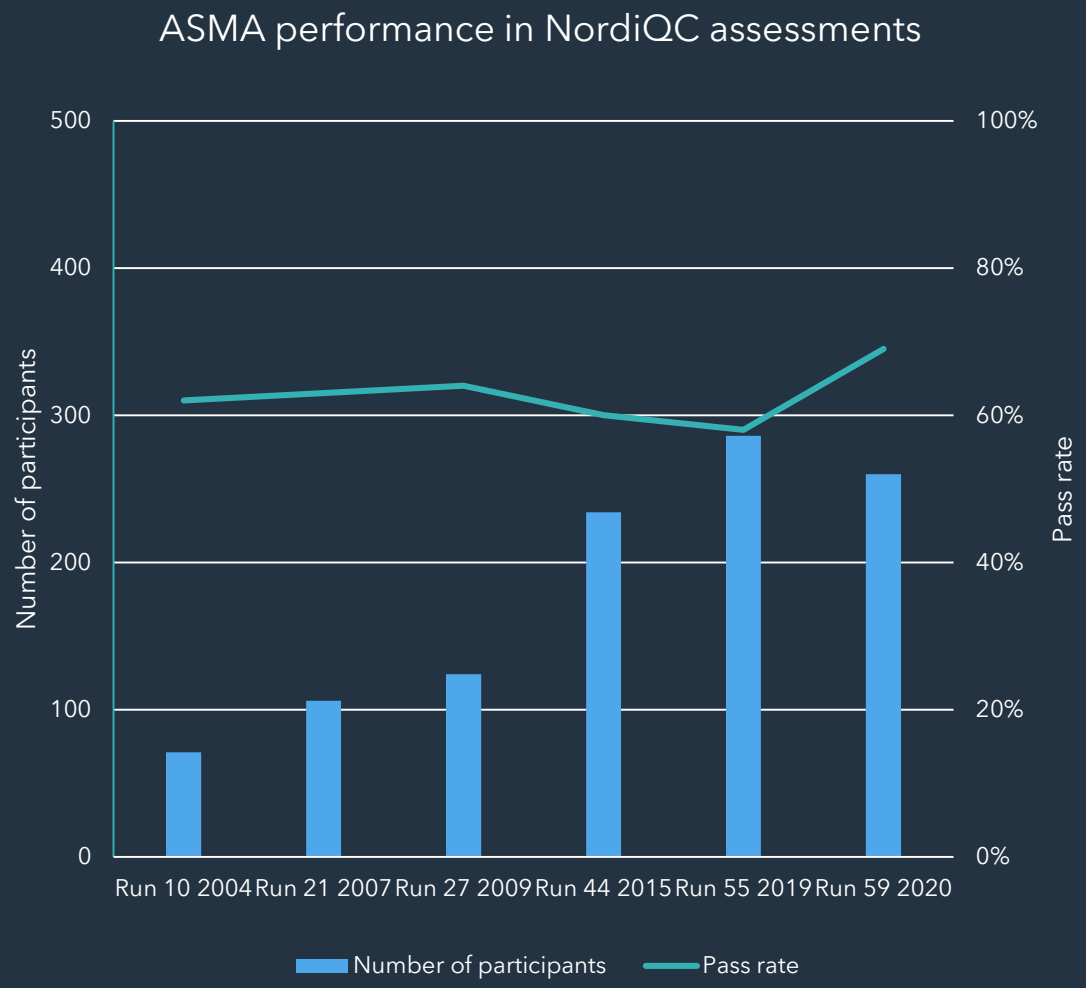
Device:	PT-link / PT-module
Buffer:	Dako TRS High pH (3-1)
Heating time at max. temp.:	20 min.
Maximum heating temp.:	97°C

Visualization system

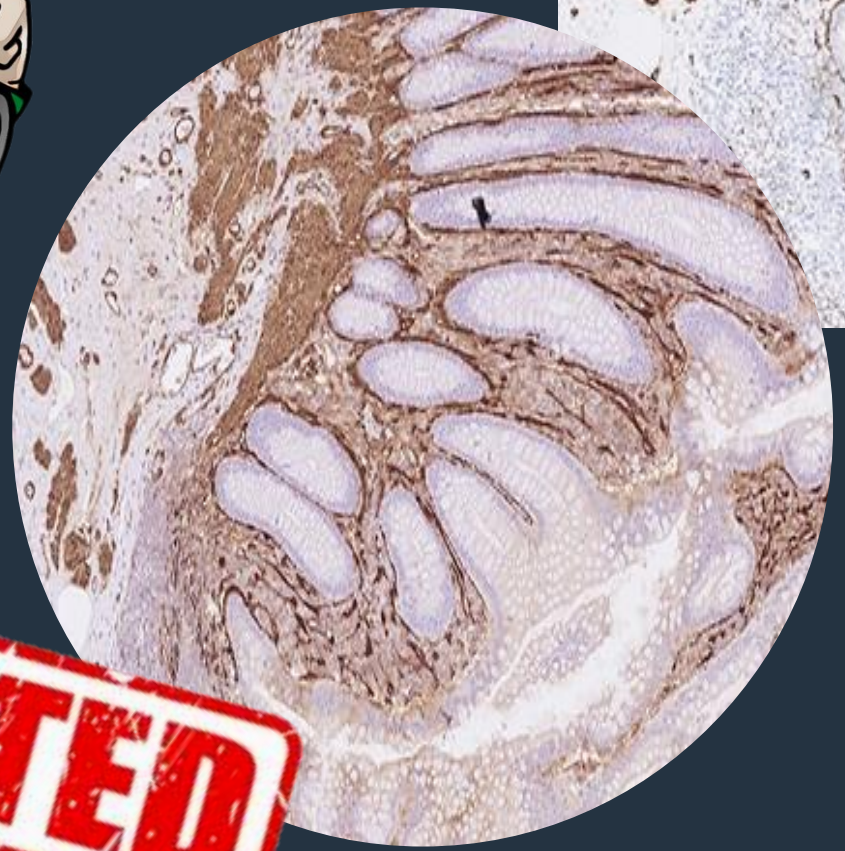
Producer:	Dako
Product / no:	EnVision FLEX+ / K8002/SM802
Linker:	Linker, Rabbit
Incubation time linker:	10 min.
Incubation time polymer:	20 min.
Incubation temperature:	23°C

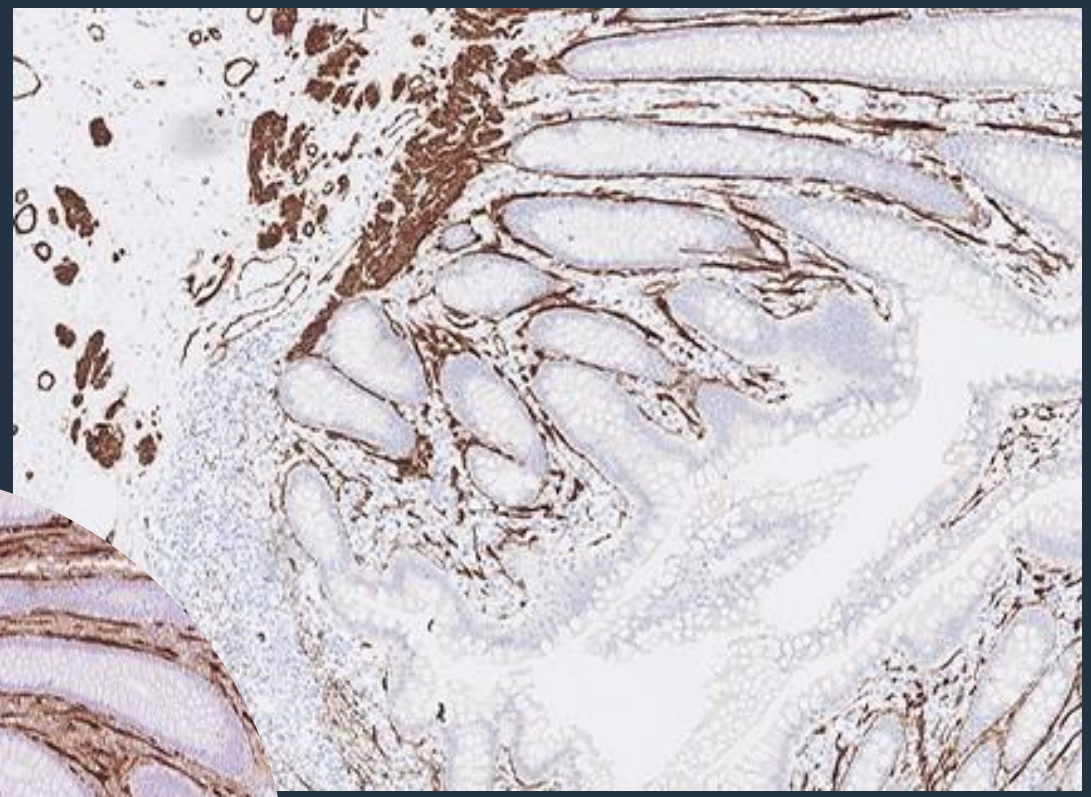
ASMA

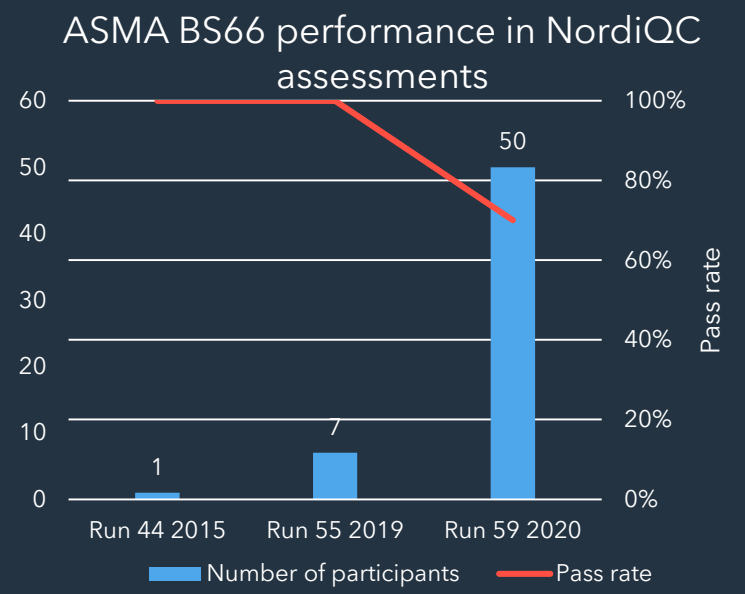
Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1A4**	62 6 4 1 1 2 1 1 1 1 1	Agilent/Dako Cell Marque Sigma Aldrich Thermo Fisher Scientific Zytomed Systems Biocare Genemed Diagnostic Biosystems Spring Bioscience Abcam Zeta Corporation	28	34	14	5	77%	35%
mAb clone **BS66**	50	Nordic Biosite	19	16	15	-	70%	38%
rmAb clone **EP188**	11 2	Epitomics Cell Marque	1	6	6	-	54%	8%
Ready-To-Use antibodies								
mAb clone **1A4 IR/IS611 (VRPS)**[3]	7	Agilent/Dako	4	2	-	1	86%	57%
mAb clone **1A4 IR/IS611 (LMPS)**[4]	17	Agilent/Dako	3	10	4	-	77%	18%
mAb clone **1A4 GA611 (VRPS)**[3]	12	Agilent/Dako	7	5	-	-	100%	58%
mAb clone **1A4 GA611 (LMPS)**[4]	12	Agilent/Dako	4	6	1	1	83%	33%
mAb clone **1A4 760-2833 (VRPS)**[3]	4	Ventana/Roche	-	-	3	1	-	-
mAb clone **1A4 760-2833 (LMPS)**[4]	40	Ventana/Roche	-	18	13	9	45%	0%
mAb clone **asm-1 PA0943 (VRPS)**[3]	4	Leica Biosystems	4	-	-	-	-	-
mAb clone **asm-1 PA0943 (LMPS)**[4]	6	Leica Biosystems	2	3	-	1	83%	33%
Total	260		74	106	60	20		
Proportion			28%	41%	23%	8%	69%	



BS66 – Why oh why!







Type:	Ventana Benchmark Ultra
Primary antibody	
Clone:	EP188
Producer:	Epitomics
Product no. / lot no.:	AC-0167 / EO62901
Diluent:	Antibody Diluent
Dilution factor:	1:200
Incubation time / temperature:	16 min. / 36°C
Epitope retrieval, HIER	
Device:	On Board / On Machine
Buffer:	Ventana Ultra CC1
Heating time at max. temp.:	32 min.
Maximum heating temp.:	95°C
Epitope retrieval, proteolysis	
Enzyme:	Protease 2
Enzyme producer / no:	Ventana / 760-2019
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.
Amplifier:	OptiView Amplification Kit
Incubation time amplifier:	4 min.

Immunostainer	
Type:	Leica BOND III
Primary antibody	
Clone:	?sm-1
Producer:	Leicabiosystems
Product no. / lot no.:	PA0943 / 65028
Format:	Ready-To-Use (prediluted)
Incubation time / temperature:	15 min. / 20°C
Visualization system	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time linker:	8 min.
Incubation time polymer:	8 min.
Incubation temperature:	20°C
Chromogen	
Producer:	Leica
Product / no:	Bond Refine / DS9800
Incubation time / temperature:	10 min. / 20°C
Enhancement:	CuSO4

Immunostainer	
Type:	Dako Omnis
Primary antibody	
Clone:	1A4
Producer:	Dako/Agilent
Product no. / lot no.:	GA611 / 20075900
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.:	99°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:	1A4
Producer:	Dako/Agilent
Product no. / lot no.:	IS611/IR611 / 20078645
Format:	Ready-To-Use (prediluted)
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:	None
Incubation time polymer:	20 min.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