The unknown primary tumour: Antivody selection, protocols and controls

Workshop in Diagnostic Immunohistochemistry 2-4. October 2019.

Rasmus Røge, MD, NordiQC scheme organizer

Primary panel in Unknown Primary Tumours

- Pan-CK
  - Epithelial and mesothelial neoplasms
- S100
  - (Non-neuronal) Neuroepithelial neoplasms
- CD45
  - Haemato-lymphoid neoplasms
- Vimentin
  - Mesenchymal neoplasms (but also many epithelial, mesothelial, neuroepithelial neoplasms)

Primary panel

	Latest run	Pass rate
Pan-CK	Run 54, 2018	62%
S100	Run 50, 2017	82%
CD45	Run 37, 2013 (planned 2020)	82%
Vimentin	Run 52, 2018	74%

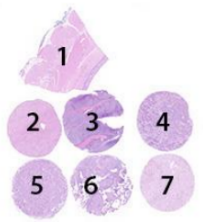
Pan cytokeratin

Assessment Run 54 2018

Pan Cytokeratin (CK-PAN)

Material
The slide to be stained for CK-PAN comprised:

1. Esophagus, 2. Liver, 3. Tonsil, 4. Small cell lung carcinoma (SCLC),
5. Lung adenocarcinoma, 6. Lung squamous cell carcinoma,
7. Clear cell renal cell carcinoma (CCRCC).



Criteria for assessing a CK-PAN staining as optimal were:

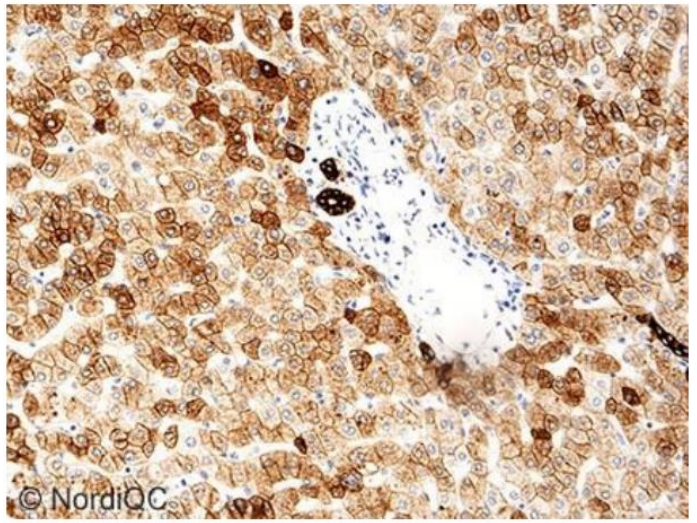
- A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and an at least moderate cytoplasmic staining reaction with membrane accentuation of the vast majority of hepatocytes.
- A strong, distinct cytoplasmic staining reaction of all squamous epithelial cells throughout all cell layers in the esophagus.
- A strong, distinct cytoplasmic staining reaction of the majority of neoplastic cells in the lung adenocarcinoma and squamous cell carcinoma.
- An at least weak to moderate, distinct cytoplasmic, dot-like staining reaction of the majority of neoplastic cells in the SCLC.
- A moderate to strong, distinct cytoplasmic staining reaction of the majority of neoplastic cells in the CCRCC.
- No more than a weak to moderate, focal reaction of smooth muscle cells of muscularis propria in the esophagus. All other cells including lymphocytes and stromal cells should be negative.

Table 2. **Proportion of sufficient results for CK-PAN in the nine NordiQC runs performed**

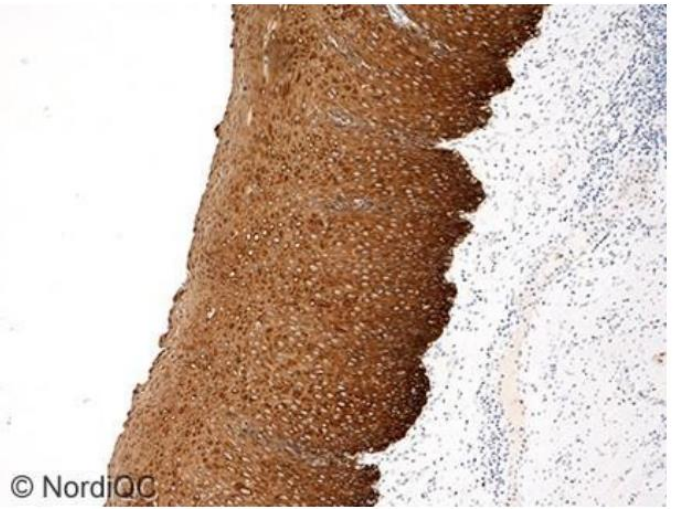
	Run 8 2003	Run 15 2005	Run 20 2008	Run 24 2008	Run 30 2010	Run 36 2012	Run 41 2014	Run 47 2016	Run 54 2018
Participants, n=	72	85	103	123	168	202	233	275	296
Sufficient results	53%	58%	62%	60%	65%	65%	67%	72%	62%

Pan cytokeratin - controls

- Liver: Hepatocytes should be weakly to moderate positive (CK 8 and 18), while bile ducts (CK7) should be strongly positive. Stromal cells should be negative



- Esophagus: All squamous epithelial cells should be strongly positive (CK5 and 14), while stroma should be negative.



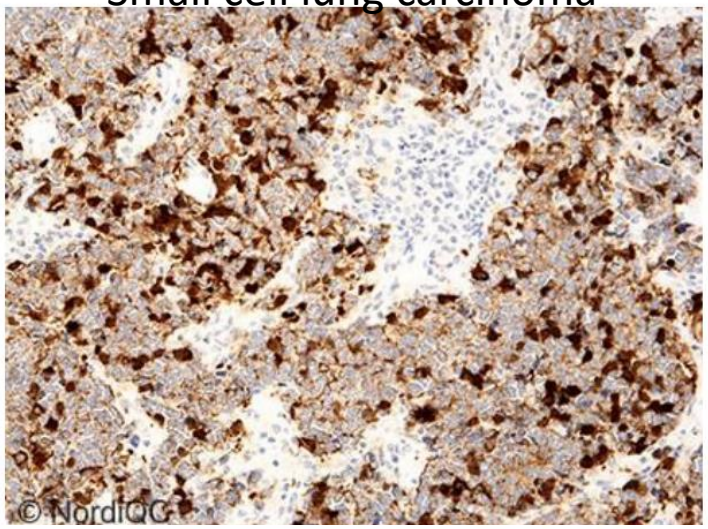
Pan cytokeratin

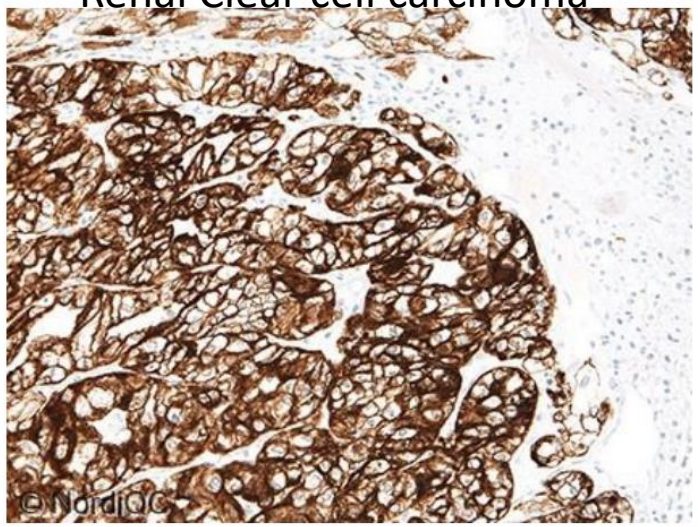
Small cell lung carcinoma

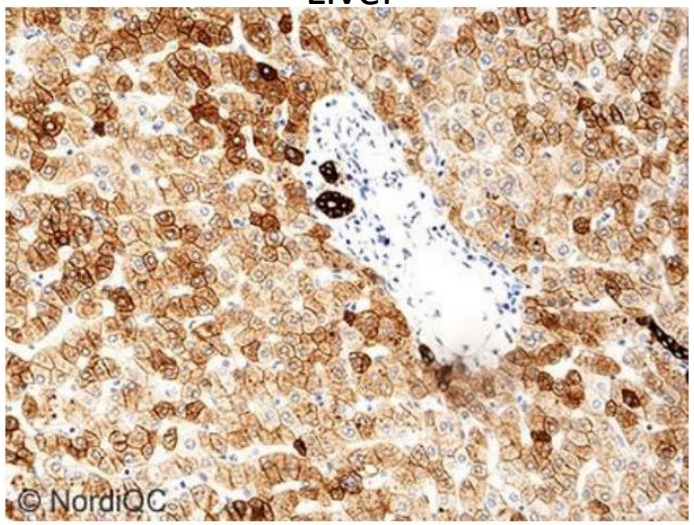
Renal Clear cell carcinoma

Liver

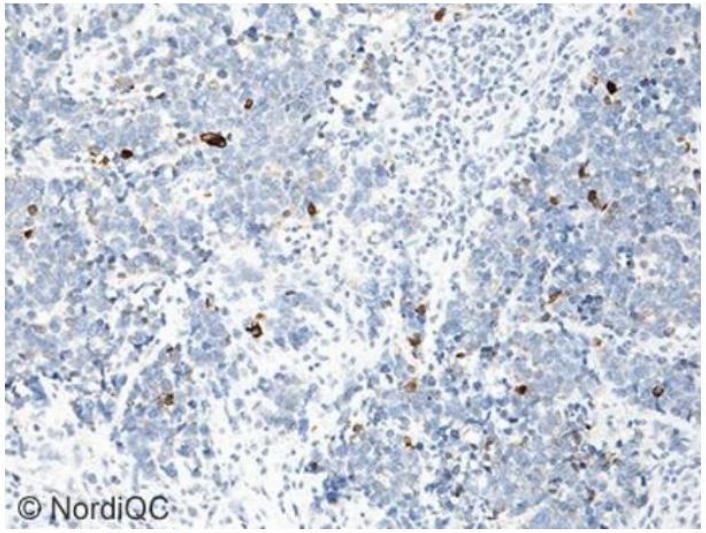
Optimal

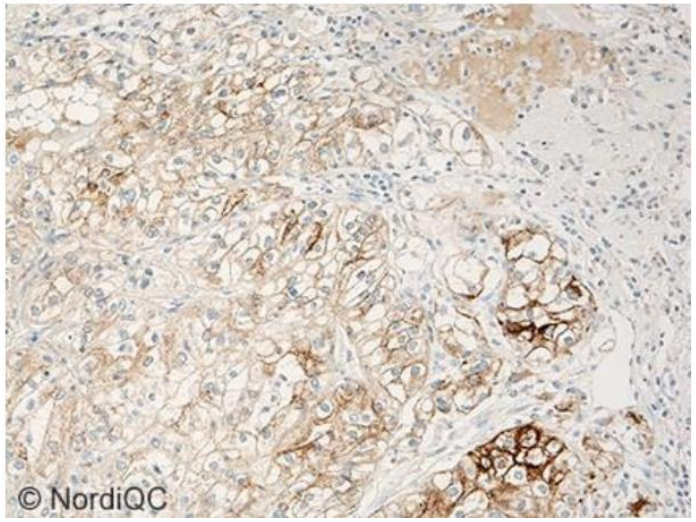


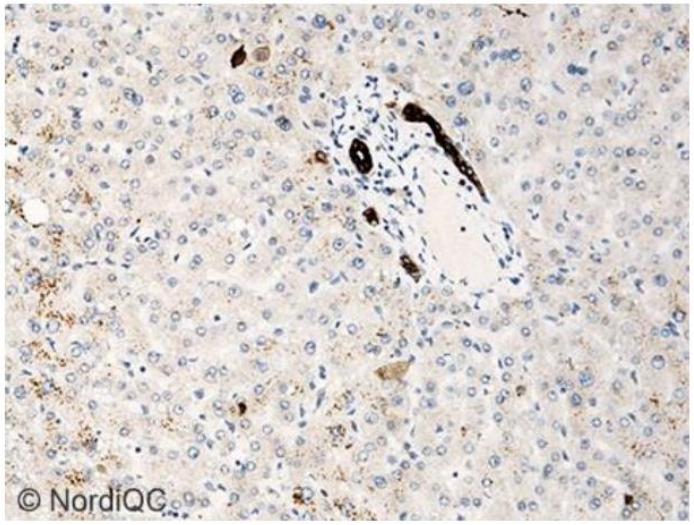




Insufficient







Pan cytokeratin – results run 54 – conc.

Table 1. **Antibodies and assessment marks for CK-PAN, run 54**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone cocktail **AE1/AE3**	77	Dako/Agilent	30	31	21	24	58%	74%
	5	Thermo/NeoMarkers						
	8	Cell Marque						
	9	Leica/Novocastra						
	1	Biocare Medical						
	1	Zytomed						
	1	Diagnostic Biosystems						
	1	Genemed						
	1	Immunologic						
	1	DCS Diagnostics						
	1	Invitrogen						
mAb clone cocktail **AE1/AE3/5D3**	3	Biocare Medical	4	1	0	1	-	-
	2	Zytomed						
	1	Abcam						
mAb clone cocktail **PAN-CK (Ab-C2562)**	1	Sigma Aldrich	1	0	0	0	-	-
mAb clone **BS5**	4	Monosan	5	0	0	0	-	-
	1	Nordic Biosite						
mAb clone **MNF116**	11	Dako/Agilent	0	1	2	8	9%	-
mAb clone **OSCAR**	1	"In-house"	0	0	1	0	-	-

Pan cytokeratin - RTU

- AE1/AE3: technically challenging
  - Optimal results could not be obtained on the Leica platform.
  - HIER is mandatory in alkaline buffer
  - No apparent difference between 2 and 3-step visiualization
- MNF116: Have not provided sufficient results in several assessment – should be substitued with another product
- BS5: Although data is limited, this clone seems like a robust alternative

Pan cytokeratin – results 54 - RTU

Readv-To-Use antibodies								
mAb clone cocktail **AE1/AE3 IR053**	24	Dako/Agilent	18	6	0	0	100%	100%
mAb clone cocktail **AE1/AE3 IR053**[3]	5	Dako/Agilent	3	0	1	1	-	-
mAb clone cocktail **AE1/AE3 GA053**	33	Dako/Agilent	22	10	1	0	97%	100%
mAb clone cocktail **AE1/AE3 GA053**[3]	2	Dako/Agilent	1	1	0	0	-	-
mAb clone cocktail **AE1/AE3 313M-18**	3	Cell Marque	0	1	1	1	-	-
mAb clone cocktail **AE1/AE3 MAD 001000QD**	1	Master Diagnostica	0	0	0	1	-	-
mAb clone cocktail **AE1/AE3 PA0909**	3	Leica/Novocastra	0	0	1	2	-	-
mAb clone cocktail **AE1/AE3 PA0094**	2	Leica/Novocastra	0	1	1	0	-	-
mAb clone cocktail **AE1/AE3**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone cocktail **AE1/AE3 PDM072**	1	Diagnostic Biosystems	0	1	0	0	-	-
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595**	83	Ventana/Roche	24	18	24	17	51%	83%
mAb clone cocktail **AE1/AE3/5D3 PM162**	1	Biocare Medical	0	0	1	0	-	-
m&rmAb clone cocktail **B22.1/B23.1 EP24/EP67 MAD-000680QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **Lu-5 PM043**	1	Biocare Medical	0	0	0	1	-	-
mAb clone **MX005 MAB-0671**	1	Maixin	1	0	0	0		
mAb clone **OSCAR Z-465-26-Y**	1	Zytomed Systems	0	0	0	1		
Total	296		112	72	55	57	-	
Proportion			38%	24%	19%	19%	62%	

Table 4. **Proportion of sufficient and optimal results for CK-PAN in the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb AE1/AE3 **IR053**	100% (10/10)	60% (6/10)	100% (10/10)	90% (9/10)
Dako Omnis mAb AE1/AE3 **GA053**	100% (26/26)	69% (18/26)	83% (5/6)	50% (3/6)
VMS Ultra/XT/GX mAb AE1/AE3/PCK26 **760-2135/2595**	70% (7/10)	20% (2/10)	50% (35/73)	30% (22/73)

* 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.

Table 5. **Pass rates for antibody cocktails combined with epitope retrieval methods in eight NordiQC runs**

Pass rate for compiled data from run 15, 20, 24, 30, 36, 41, 47 & 54								
	Total		HIER		Proteolysis		HIER + proteolysis	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb AE1/AE3	949	679 (72%)	882	670 (76%)	47	5 (11%)	8	3 (40%)
mAb AE1/AE3/5D3	44	39 (89%)	43	39 (91%)	1	0	0	0
mAb AE1/AE3/PCK26	267	152 (57%)	37	16 (43%)	41	2 (5%)	182	132 (73%)
mAb MNF116	102	31 (30%)	48	9 (19%)	48	22 (46%)	5	2 (40%)

Pan cytokeratin - Conc

- AE1/AE3: RTU systems from Dako had the highest pass rate. HIER is mandatory in alkaline buffer. Both vendor recommended protocols and LDT can be used.
- AE1/AE3/PCK26: HIER (in alkaline buffer) has to be combined with enzymatic pretreatment (Protease 3!). Other enzymes provides a significant lower pass rate.

CD45

Assessment Run 37 2013

CD45

Leucocyte Common Antigen (LCA)

Material
The slide to be stained for CD45 comprised:

1. Tonsil, 2. Liver, 3. Brain, 4. B-CLL

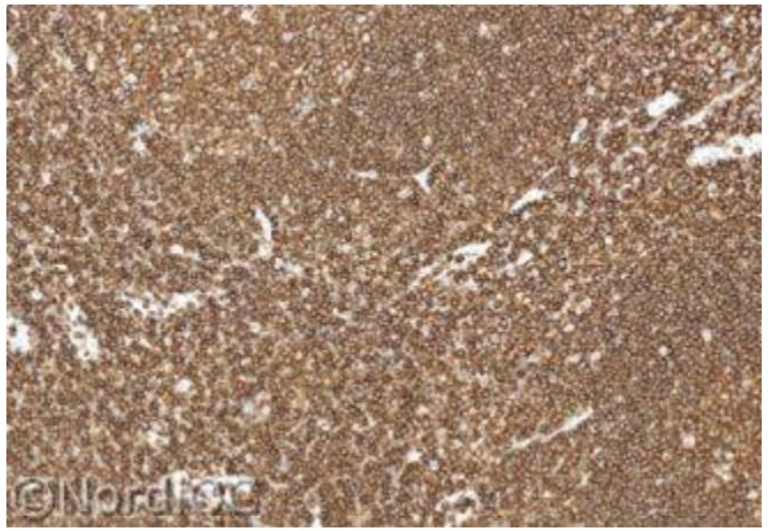
All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD45 staining as optimal included:

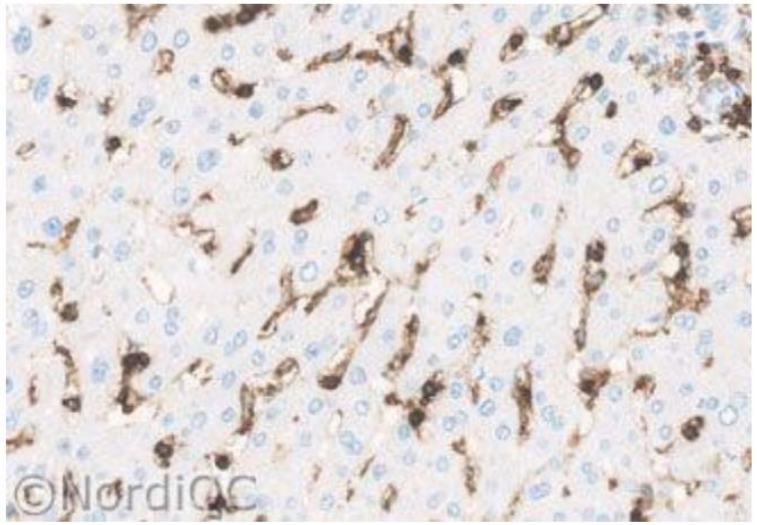
- A moderate to strong and distinct predominantly membranous staining reaction of all lymphocytes in all four tissues tested. In the tonsil both the B- and T-cells should be distinctively demonstrated.
- An at least weak to moderate and distinct staining reaction of the Kupffer cells in the liver and the microglial cells of the brain.
- An at least weak to moderate predominantly membranous staining reaction of virtually all the neoplastic cells of the B-CLL
- No staining of squamous epithelial cells in the tonsil or hepatocytes in the liver.

CD45 - controls

- Tonsil:
  - B- and T-zones should be moderate to strongly positive (high expressors). Squamous epithelium should be negative.



- Liver (or brain):
  - Kuppfer cells (or mikroglia) are CD45 low expressors and will function as a sensitivity indicator. Hepatocytes should be negative.



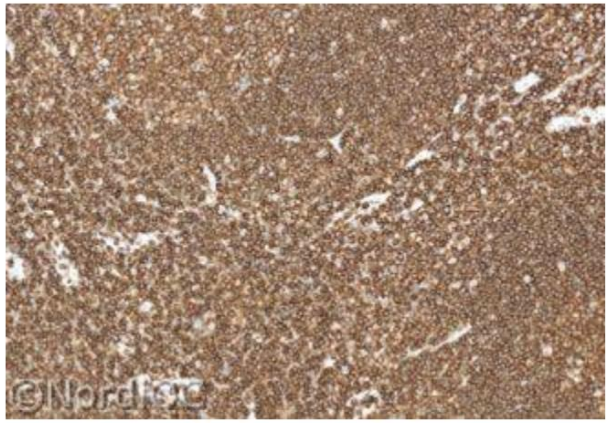
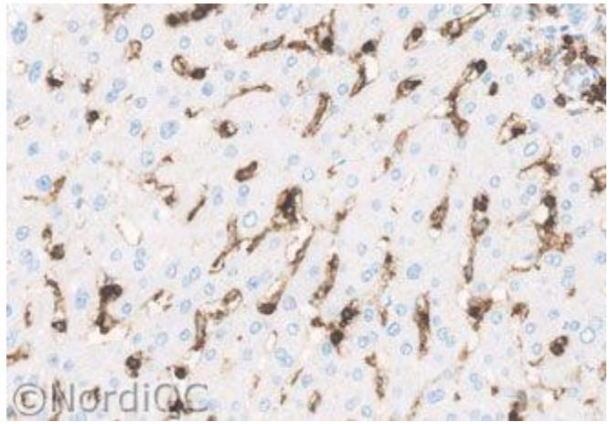
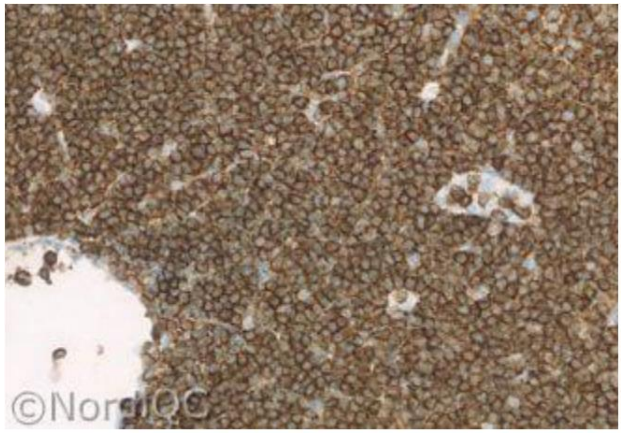
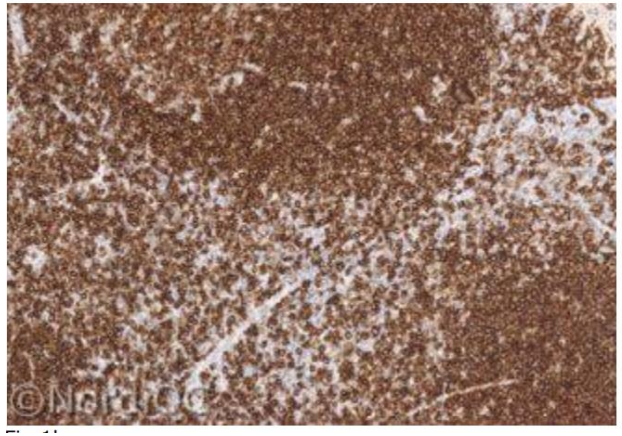
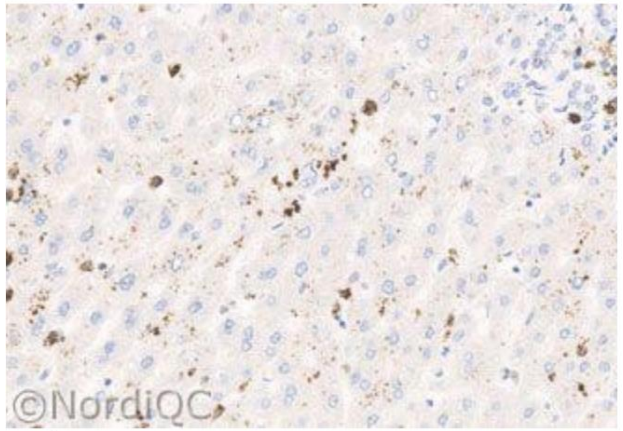
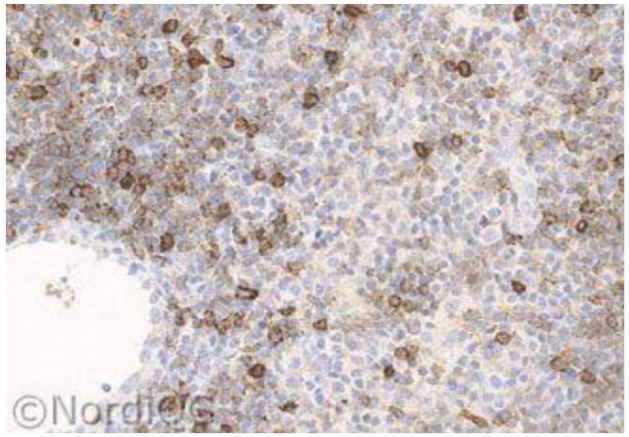
CD45 – results run 37

Table 1. **Antibodies and assessment marks for CD45, run 37**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clones **MEM28/MEM56 /MEM55**	1	Invitrogen	0	1	0	0	-	-
mAb clones **PD7/26/26+2B11**	3	Thermo/Neomarkers	0	1	2	0	-	-
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
rmAb clone **EP68**	1	Epitomics	0	0	0	1	-	-
Ready-To-Use Antibodies								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100%	100%
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clones **2B11+PD7/26 148M-98**	2	Cell Marque	2	0	0	0	-	-
mAb clones **2B11+PD7/26 N1514**	1	Dako	1	0	0	0	-	-
mAb clones **2B11+PD7/26 E005**	1	Linaris	0	0	1	0	-	-
mAb clones **2B11+PD7/26 MAD-004010QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11 PM-016**	1	Biocare	0	1	0	0	-	-
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	%
Total	205		115	54	30	6	-	
Proportion			56 %	26 %	15 %	3 %	82 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

CD45

	Tonsil	Liver	B-CLL
Optimal			
Insufficient			

CD45 - conclusions

- Antibody clones: Most antibody clones could be used to obtain an optimal result
- HIER: Mandatory for optimal results. Both High and Low pH could provide optimal results.
- Antibody concentration: Careful calibration mandatory

The most frequent causes of insufficient stainings were:
- Omission of HIER
- Too low concentration of the primary antibody

Table 2. **Optimal results for CD45 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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 clones **2B11+PD7/26**	64 % 18/28**	100 % 3/3	48 % 21/44	33 % 1/3	90 % 9/10	100 % 1/1
mAb clone **X16/99**	-	100 % 1/1	100 % 2/2	-	50 % 1/2	100 % 2/2

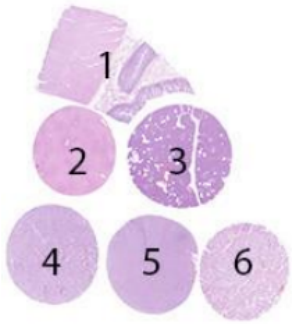
*Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Vimentin

Assessment Run 52 2018

Vimentin (VIM)

Material
The slide to be stained for VIM comprised:



1. Colon, 2. Liver, 3. Pancreas, 4. Seminoma, 5. Malignant melanoma, 6. Renal cell carcinoma (RCC).

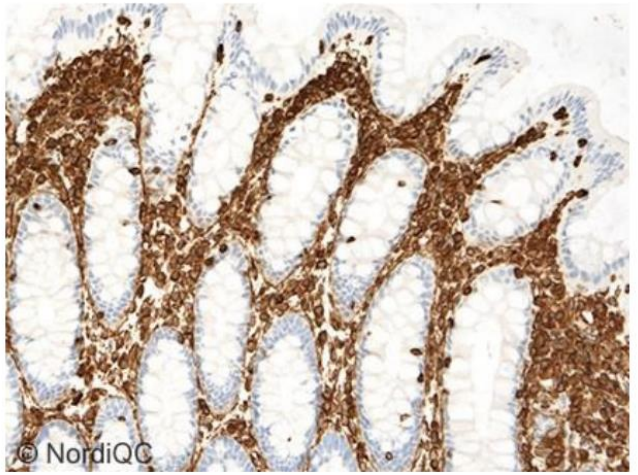
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing VIM staining as optimal included:

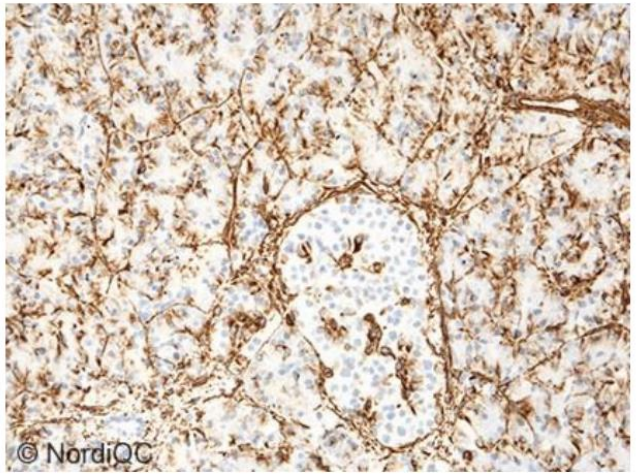
- An at least moderate, distinct cytoplasmic staining reaction of most endothelial cells, stromal cells, macrophages, and lymphocytes.
- An at least weak to moderate, distinct cytoplasmic staining reaction of virtually all endothelial and Kupffer cells of the sinusoids in the liver.
- An at least weak, distinct cytoplasmic staining reaction of the vast majority of epithelial cells of exocrine acini in the pancreas.
- A strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells of the malignant melanoma and the seminoma (dot-like and/or complete cytoplasmic staining reaction).
- An at least moderate, distinct cytoplasmic staining reaction of virtually all neoplastic cells of the RCC.
- No staining reaction of epithelial cells in the colon and of hepatocytes in the liver.

Vimentin - controls

- Liver: Strong staining of Kuppfer-cells, endothelial cells of sinusoids should be weakly positive. Hepatocytes should be completely negative.
- Colon: Dispersed lymphocytes should be strongly positive. Endothelial cells of vessels and stromal cells should positive (cytoplasmic). Epithelial cells should be negative.
- Pancreas: Exocrine cells should be postive (basolateral)



Colon



Pancreas

Vimentin

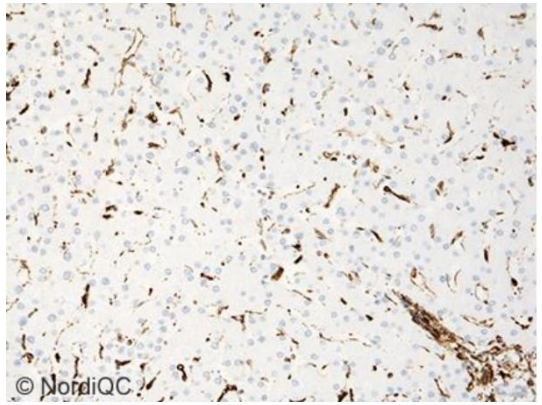


Fig. 1a (x200)
Optimal VIM staining of liver using the mAb clone 3B4, optimally calibrated, HIER in BERS2 pH 9 (Leica) and Bond Refine (Leica) as detection system.
The Kupffer cells show a moderate to strong, distinct cytoplasmic staining reaction, whereas the endothelial cells of the sinusoids display weak staining intensity.
Same protocol used in Figs. 2a - 6a.

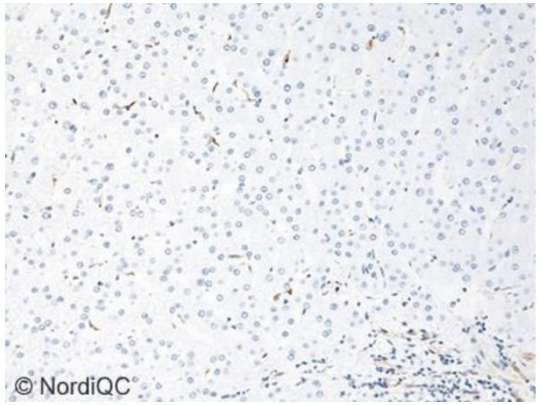


Fig. 1b (x200)
Insufficient VIM staining of liver using the mAb clone 3B4, too diluted, less efficient HIER in BERS1 pH 6 and Bond Refine (Leica) as detection system– same field as in Fig. 1a.
Only scattered Kupffer cells display a too weak staining intensity and the endothelial cells of the sinusoids are completely negative (compare Figs.1a - 6b). Same protocol used in Figs. 2b - 6b.

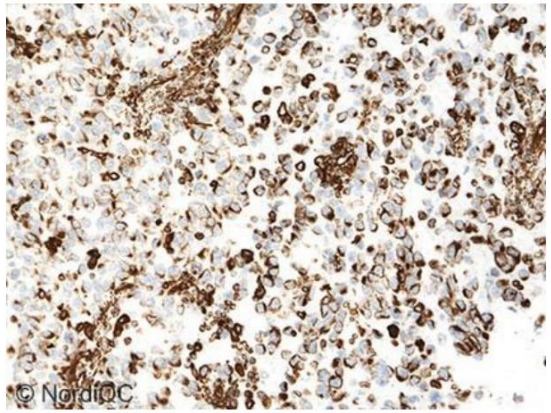


Fig. 5a (x200)
Optimal VIM staining of the seminoma using same protocol as in Figs. 1a – 4a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction (dot-like and/or complete cytoplasmic staining pattern).

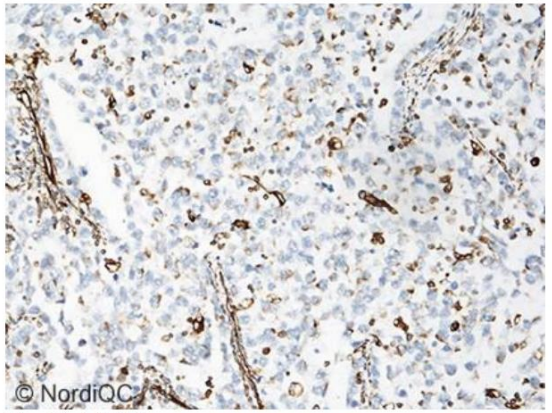


Fig. 5b (x200)
Insufficient VIM staining of the seminoma using same protocol as in Figs. 1b - 4b. The neoplastic cells only display a faint dot-like staining reaction or are completely negative – same field as in Fig. 5a.

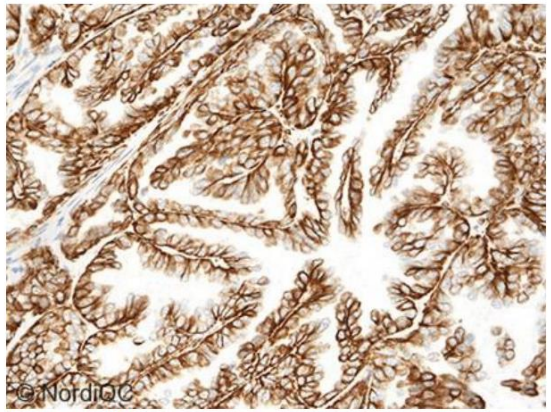


Fig. 6a (x200)
Optimal VIM staining of the RCC using same protocol as in Figs. 1a – 5a. All the neoplastic cells show a strong and distinct cytoplasmic staining reaction

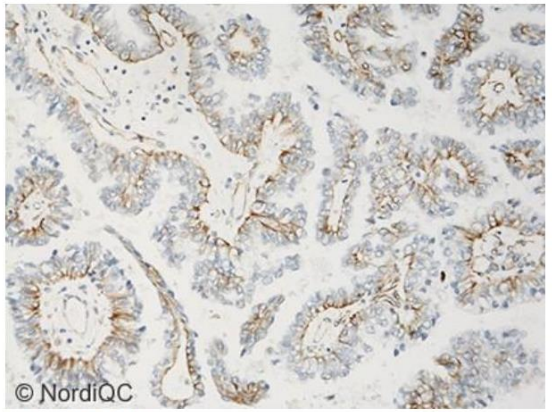


Fig. 6b (x200)
Insufficient VIM staining of the RCC using same protocol as in Figs. 1b - 5b. The neoplastic cells display too weak staining intensity or are completely negative.

Vimentin – run 52 results

Table 1. **Antibodies and assessment marks for VIM, run 52**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. ORS[2]
mAb clone **V9**	57 10 6 3 2 2 1 1 1 1	Agilent/Dako Leica/Novocastra BioGenex Cell Marque GeneMed Linaris Diagnostic Biosystems Zymed/Invitrogen Zytomed Systems Thermo S/ Neomarkers	32	23	18	11	65%	83%
mAb clone **3B4**	29	Agilent/Dako	10	13	2	4	79%	100%
mAb clone **SRL33**	2	Leica/Novocastra	0	0	1	1	-	-
mAb clone **BS13**	1	Nordic Biosite	0	1	0	0	-	-
rmAb clone **SP20**	2 2 1	Cell Marque Thermo S./Neomarkers Diagnostic Biosystems	2	2	0	1	-	-
Ready-To-Use antibodies								
mAb clone **V9 IR630**	31	Agilent/Dako	27	1	3	0	90%	95%
mAb clone **V9 IR630**[3]	5	Agilent/Dako	5	0	0	0	-	-
mAb clone **V9 GA630**	29	Agilent/Dako	23	2	4	0	86%	100%
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%	78%
mAb clone **V9 347M-10**	2	Cell Marque	0	1	1	0	-	-
mAb clone **V9 PA0640**	7	Leica/Novocastra	5	2	0	0	100%	100%
mAb clone **V9 PA0640**[3]	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **V9 KIT-0019**	1	Maixin	1	0	0	0	-	-
mAb clone **V9 8336-C010**	1	Sakura FineTek	1	0	0	0	-	-
mAb clone **V9 AM074-10M**	1	BioGenex	1	0	0	0	-	-
mAb clone **V9 ILM52311 R25**	1	Immunologic	0	0	0	1	-	-
mAb clone **3B4 760-2512**	3	Roche/Ventana	2	0	0	1	-	-
rmAb clone **SP20 347R-18**	1	Cell Marque	0	0	0	1	-	-
rmAb clone **SP20 MAD-000326QD**	2	Master Diagnostica	2	0	0	0	-	-
Total	308		133	96	49	30	-	
Proportion			43%	31%	16%	10%	74%	

1) Proportion of sufficient stains (optimal or good). 2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

Vimentin - conclusions

- Ab clone: V9, 3B4 and SP20 recommendable
- Ab format: RTU products from Dako and Leica performed better that LDT and the RTU from Roche.
- HIER: Mandatory, better performance in alkaline buffer
- Antibody titer: Relative high concentrations (1:100-1:500) in optimal results

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.

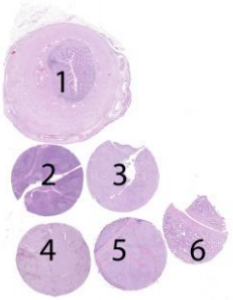
S100

Assessment Run 50 2017

S100

Material
The slide to be stained for S100 comprised:

1. Appendix, 2. Tonsil, 3. Schwannoma, 4-5. Malignant melanoma,
6. Colon adenocarcinoma.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of Schwann cells of peripheral nerve fibres and ganglionic satellite cells in the muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of adipocytes and macrophages in all specimens.
- A strong, distinct nuclear and cytoplasmic staining reaction of virtually all neoplastic cells of the malignant melanomas (cores 4-5) and the Schwannoma.
- A weak to moderate, cytoplasmic and nuclear staining reaction of the follicular dendritic cells in the germinal centres of the tonsil and the Peyer's plaques in the appendix.
- No staining of other cells. The neoplastic cells in the colon adenocarcinoma, squamous epithelial cells in the tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative.

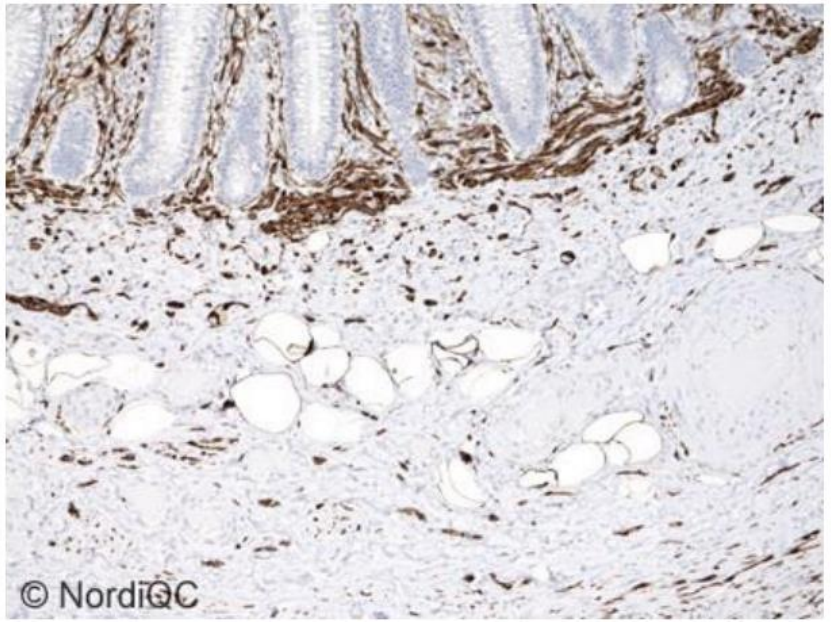
Table 2. **Proportion of sufficient results for S100 in the five NordiQC runs performed**

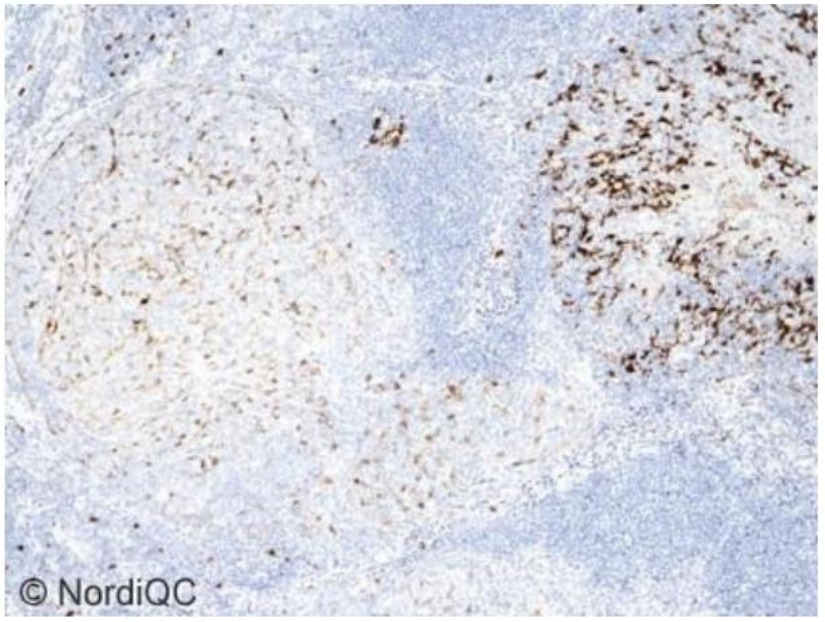
	Run 7 2003	Run 20 2007	Run 34 2012	Run 45 2015	Run 50 2017
Participants, n=	63	106	200	251	299
Sufficient results	71%	75%	64%	68%	82%

S100 controls

- Appendix: Adipocytes, Schwann cells and dendritic cells should be stained as strong as possible (without introducing false positive staining)

- Tonsil: Strong positive staining of interfollicular dendritic cells and Langerhans cells of the squamous epithelium, while germinal center dendritic cells must display an at least weak to moderate staining reaction





S100 – run 50 results

Table 1. **Antibodies and assessment marks for S100, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C4.9**	2 2 2 1	Immunologic Zytomed Systems Cell Marque Thermo/NeoMarkers	0	3	4	0	43%	-
mAb clone **15E2E2**	1 1	Biogenex Biocare	0	1	1	0	-	-
mAb clone **15E2E2+4C4.9**	1	Biocare	0	1	0	0	-	-
pAb **Z0311**	137	Agilent/Dako	62	60	14	1	89%	97%
pAb **NCL-L-S100p**	10	Leica/Novocastra	1	6	3	0	70%	100%
pAb **RB-9018-P**	1	Thermo/NeoMarkers	0	0	1	0	-	-
pAb **RP035**	1	Diagnostic Biosystems	0	0	1	0	-	-
Unknown	1	-	0	1	0	0	-	-

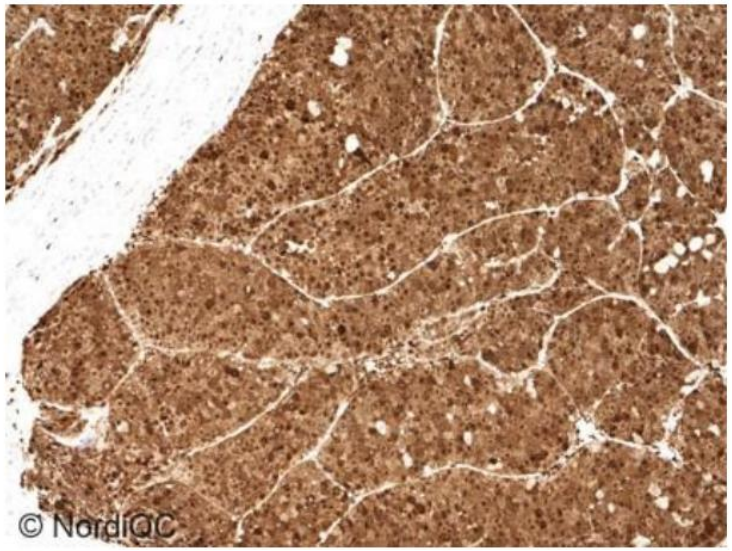
Ready-To-Use antibodies								
mAb clone **4C4.9 790-2914**	36	Roche/Ventana	0	20	16	0	56%	-
mAb clone **4C4.9 330M-18**	2	Cell Marque	0	2	0	0	-	-
mAb clone **4C4.9 MAD-001221QD**	3	Master Diagnostica	0	2	1	0	-	-
mAb clone **4C4.9 MON-RTU1191**	1	Monosan/Sanbio	0	1	0	0	-	-
mAb clone **4C4.9 KIT-0007**	1	Maixin	0	0	1	0	-	-
mAb clone **15E2E2+4C4.9 PM089**	1	Biocare	0	1	0	0	-	-
rmAb clone **EP32 AN713**	1	Biogenex	0	1	0	0	-	-
rmAb clone **EP32 8442-C010**	1	Sakura	0	1	0	0	-	-
pAb **IS/IR504**	26	Agilent/Dako	0	22	4	0	85%	-
pAb **IS/IR504**[3]	5	Agilent/Dako	0	5	0	0	100%	-
pAb **GA504**	21	Agilent/Dako	1	19	1	0	95%	100%
pAb **GA504**[4]	6	Agilent/Dako	3	2	0	1	83%	-
pAb **760-2523**	28	Roche/Ventana	0	23	2	3	82%	-
pAb **PA0900**	6	Leica/Novocastra	0	6	0	0	100%	-
pAb **E031**	1	Linaris	0	1	0	0	-	-
Total	299		67	178	49	5	-	
Proportion			23%	59%	16%	2%	82%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Agilent/Dako semi-automatic system (Autostainer) but used by laboratories on different platforms (e.g. Leica BOND III).
4) RTU system developed for the Agilent/Dako full-automated systems (Omnis) but used by laboratories on different platforms (e.g. Ventana Benchmark) or manually.

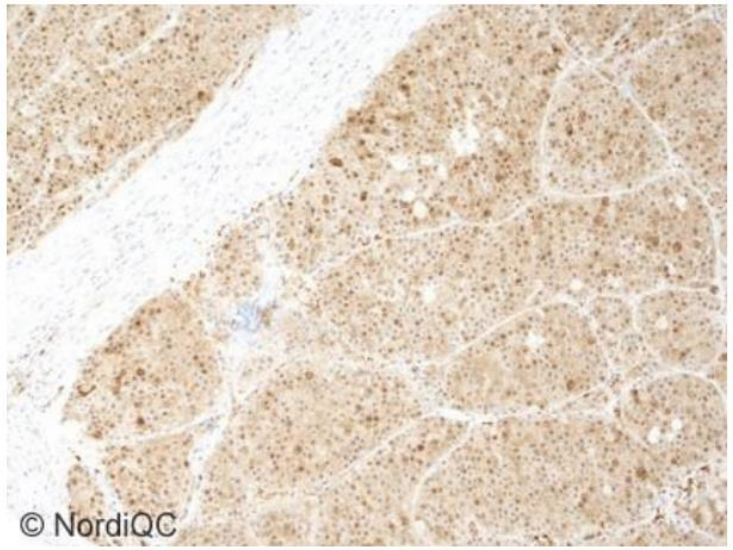
S100

Melanoma

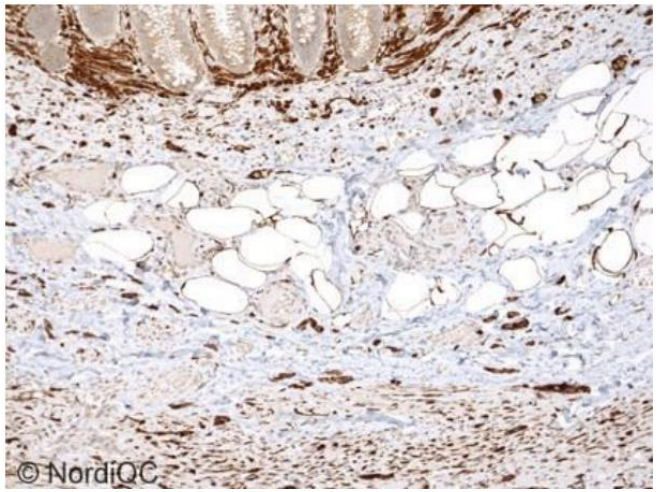
Optimal



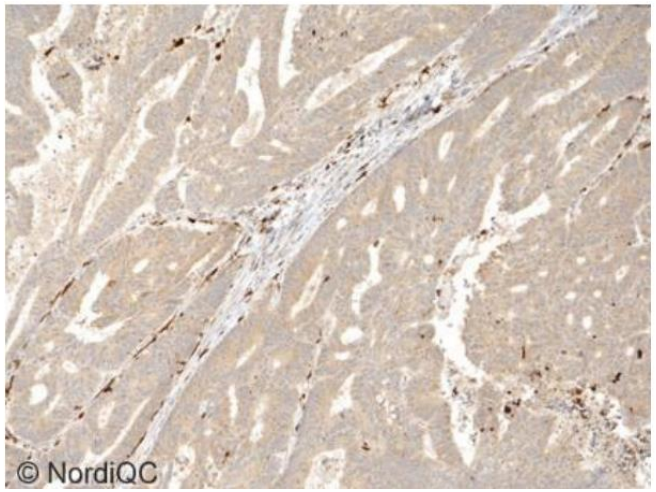
Insufficient
(weak)



Appendix



Colon adenocarcinoma



Insufficient
(false positive)

S100 - conclusions

- Antibody clone: pAb Z0311 provided the highest pass rate. pAbs had better performance than mAbs
- Antibody format: LDT using conc Abs outperformed RTU
- HIER: Mandatory and preferable in alkaline buffer

Table 3. **Proportion of optimal results for S100 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**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	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
pAb **Z311**	6/11** (55%)	0/1	3/4	-	30/46 (65%)	-	3/6 (50%)	0/3

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

Table 4. **Proportion of sufficient and optimal results for S100 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS pAb **IS/IR504**	80% (8/10)	0% (0/10)	88% (14/16)	0% (0/16)
Dako Omnis pAb **GA504**	100% (15/15)	7% (1/15)	83% (5/6)	0% (0/6)
Leica BOND MAX/III pAb **PA0900**	0% (0/0)	0% (0/0)	100% (6/6)	0% (0/6)
VMS Ultra/XT pAb **760-2523**	100% (6/6)	0% (0/6)	77% (17/22)	0% (0/22)
VMS Ultra/XT mAb 4C4.9 **790-2914**	33% (1/3)	0% (0/3)	58% (19/33)	0% (0/33)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Panels

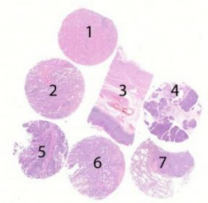
	Function	Last run	Pass rate
CK7	Simple epithelium, Adenocarcinomas of lung, breast, thyroid, upper GI, urothelial and renal	Run 40	84%
CK20	Adenocarcinomas of lower GI, merkel cell carcinoma, urothelial carcinomas	Run 47	92%
CK5	Basal cells, squamous cell carcinomas	Run 55	44%
CDX2	Carcinomas with intestinal differentiation	Run 48	80%

CK7

Assessment Run 40 2014

Cytokeratin 7 (CK7)

Material
The slide to be stained for CK7 comprised:



1. Kidney, 2. Lung, 3. Gastric corpus, 4. Pancreas, 5. Colon adenocarcinoma, 6-7. Lung adenocarcinomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK7 staining as optimal included:

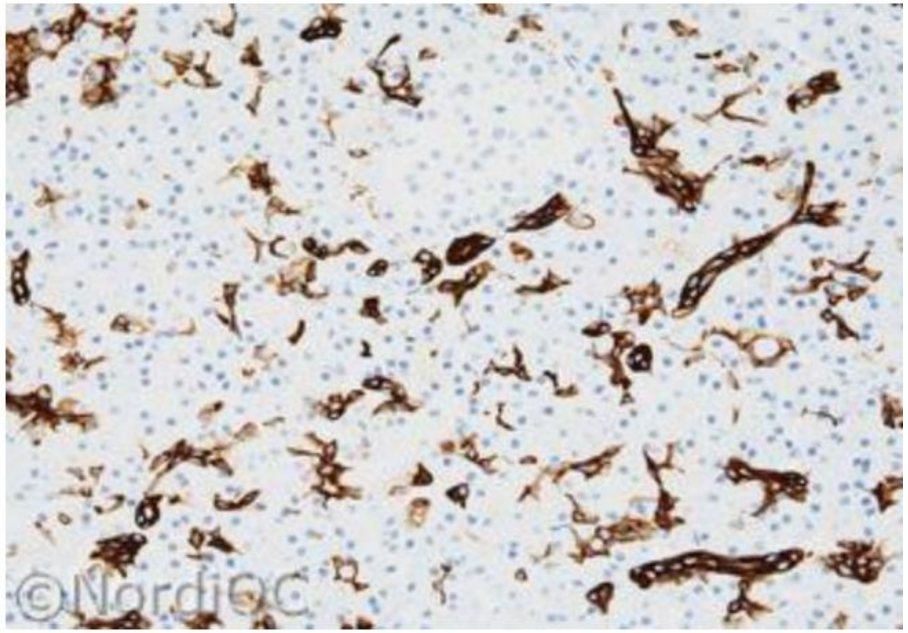
- A moderate to strong, distinct cytoplasmic staining reaction of virtually all epithelial cells of the renal collecting ducts and the scattered epithelial cells in the Bowman capsule.
- A strong, distinct cytoplasmic staining reaction of all alveolar epithelial cells in the lung tissue.
- An at least weak to moderate predominantly cytoplasmic staining reaction of the majority of luminal foveolar epithelial cells of the gastric corpus mucosa.
- A strong, distinct cytoplasmic staining reaction of virtually all epithelial cells of the large pancreatic ducts, while the majority of the epithelial cells of the intercalating ducts at least should show a weak to moderate cytoplasmic staining reaction.
- A strong, distinct cytoplasmic staining reaction of all neoplastic cells in the lung adenocarcinoma no. 6.
- An at least moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma no. 7.
- No staining reaction of neoplastic cells in the colon adenocarcinoma, epithelial cells of proximal tubules of the kidney or acinar cells of the pancreas.

Table 2. **Proportion of sufficient results for CK7 in three NordiQC runs performed**

	Run 8 2003	Run 25 2009	Run 40 2014
Participants, n=	71	130	246
Sufficient results	87%	86%	84%

<u>Control</u>

Normal pancreas. Epithelial cells of intercalating ducts show weak to moderate staining, while large ducts should be strongly positive.

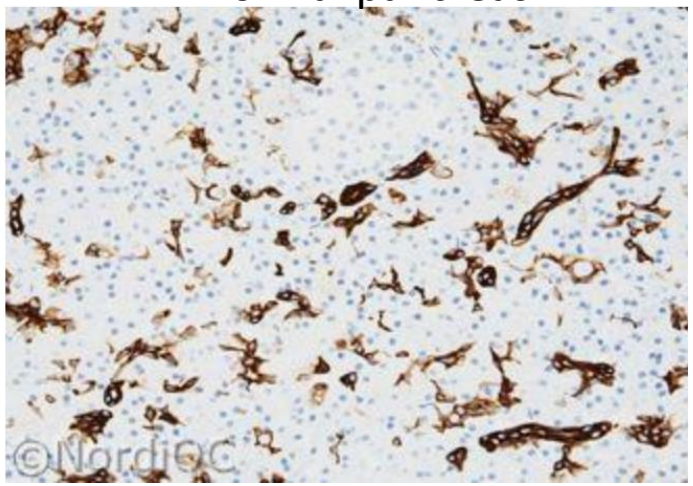


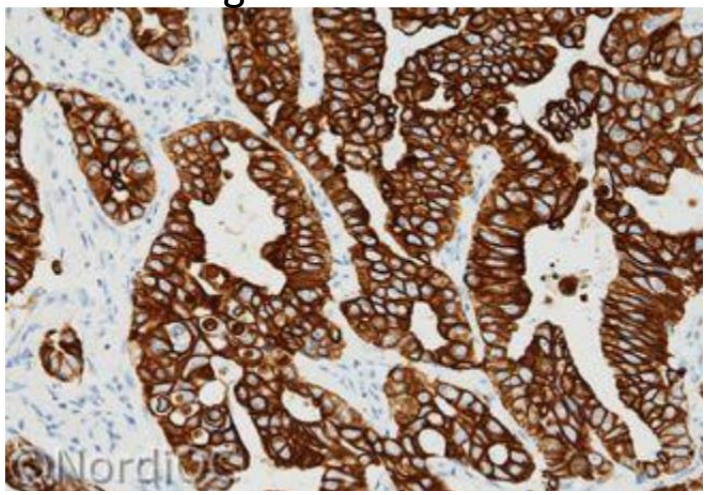
CK7 – insufficient results

Normal pancreas

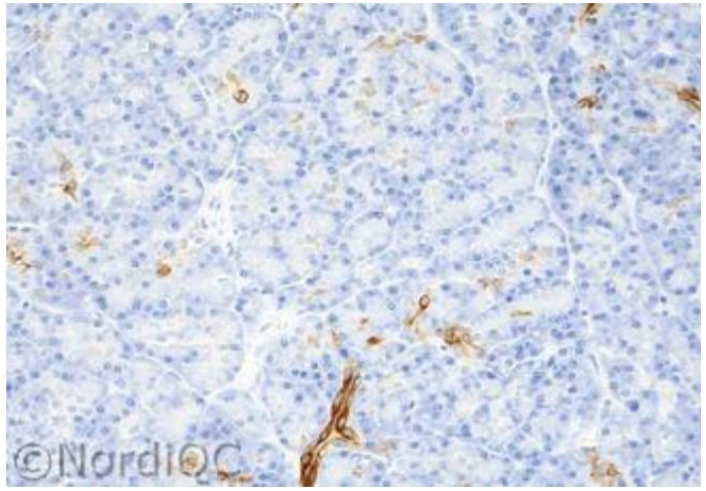
Lung adenocarcinoma

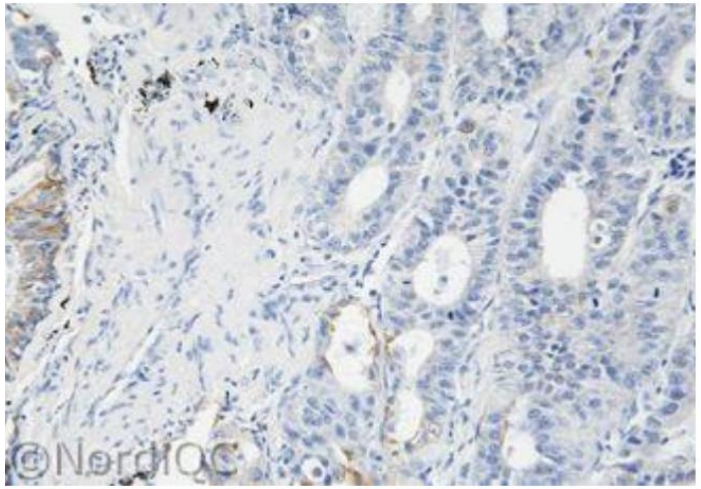
Optimal





Insufficient





CK7

Table 1. **Antibodies and assessment marks for CK7, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **OV-TL 12/30**	97 14 14 4 3 2 2 2 1 1	Dako Leica/Novocastra BioGenex Thermo S/ NeoMarkers Monosan Biocare Cell Marque Genemed ZytoMed Nordic Biosite	40	66	32	2	76%	94%
mAb clone **RN7**	3	Leica/Novocastra	1	2	-	-	-	-
rmAb clone **EPR1619Y**	1	Abcam	-	-	1	-	-	-
mAb clone **K72.7**	1	Thermo S/ NeoMarkers	-	1	-	-	-	-
Ready-To-Use antibodies:								
mAb clone **OV-TL 12/30, IR619**	41	Dako	36	5	0	0	100%	100%
mAb clone **OV-TL 12/30, MAD-001004QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **OV-TL 12/30, 307M-98**	1	Cell Marque	1	0	0	0	-	-
mAb clone **OV-TL 12/30, MON-RTU1074**	1	Monosan	1	0	0	0	-	-
mAb clone **OV-TL 12/30, PDM 097**	1	Diagnostic Biosystem	0	1	0	0	-	-
mAb clone **OV-TL 12/30, E061**	1	Linaris	0	1	0	0	-	-
rmAb clone **SP52, 790-4462**	45	Ventana	26	18	1	0	98%	98%
mAb clone **RN7, PA0942**	7	Leica/Novocastra	2	4	1	0	86%	100%
rmAb clone **BC1, PRM 339**	1	Biocare	0	0	1	0	-	-
Clone **unknown ZM-0071**	1	Zhongshan	1	0	0	0	-	-
Total	246		109	99	36	2	-	
Proportion			44%	40%	15%	1%	84%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Optimal clones

OV-TL 12/30:
- HIER in alkaline buffer
- 1:30-1:300
- 2 & 3 step detection systems

SP52:
- HIER in alkaline buffer

Insufficient results
- Too low conc. Of primary Ab
- Inappropriate epitope retrieval

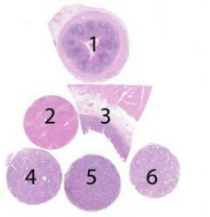
CK20

Assessment Run 47 2016

Cytokeratin 20 (CK20)

Material
The slide to be stained for CK20 comprised:

1. Appendix, 2. Liver, 3. Gastric corpus, 4. Colon adenocarcinoma, 5. Merkel cell carcinoma, 6. Urothelial carcinoma.



All tissues were fixed in 10% neutral buffered formalin.

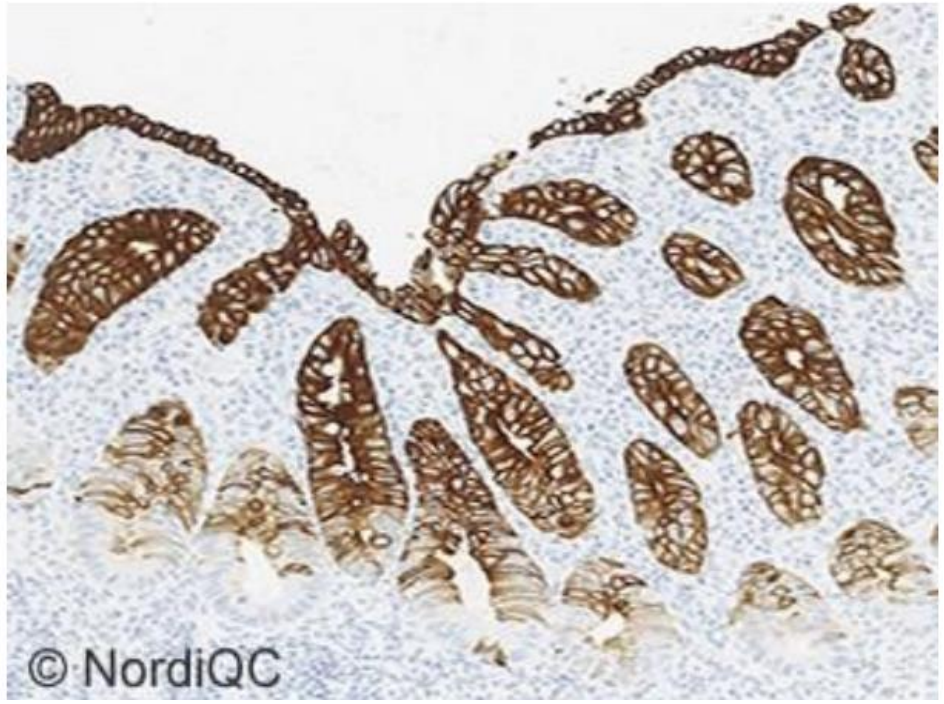
Criteria for assessing CK20 staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of all surface epithelial cells in the appendix and an at least weak to moderate staining reaction in most crypt cells.
- An at least moderate, distinct cytoplasmic staining reaction of the vast majority of foveolar epithelial cells in the gastric mucosa.
- A moderate to strong, distinct cytoplasmic and dot-like staining reaction of virtually all neoplastic cells in the Merkel cell carcinoma.
- A weak to strong, distinct cytoplasmic staining reaction of the vast majority of neoplastic cells in the colon adenocarcinoma.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the majority of neoplastic cells in the urothelial carcinoma.

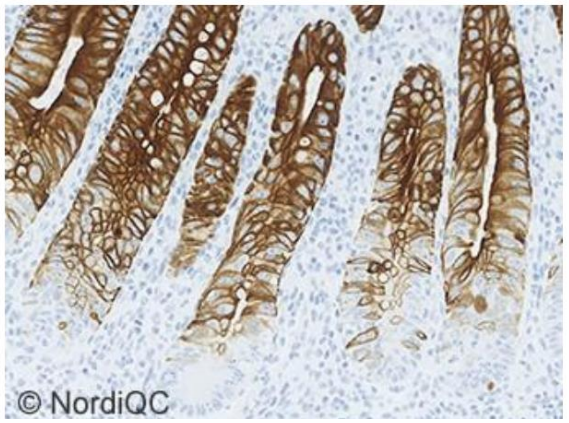
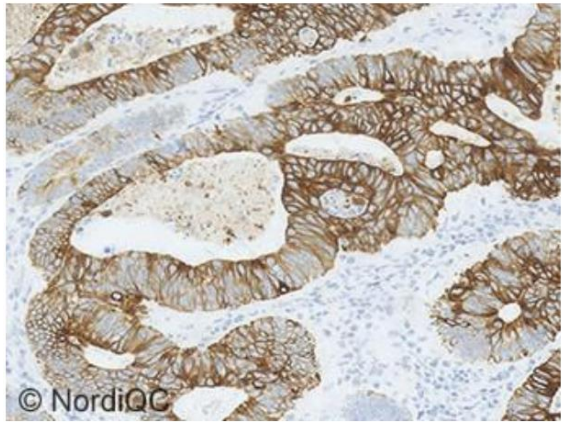
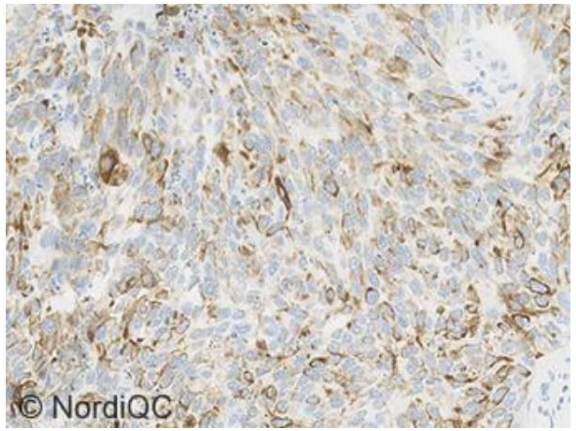
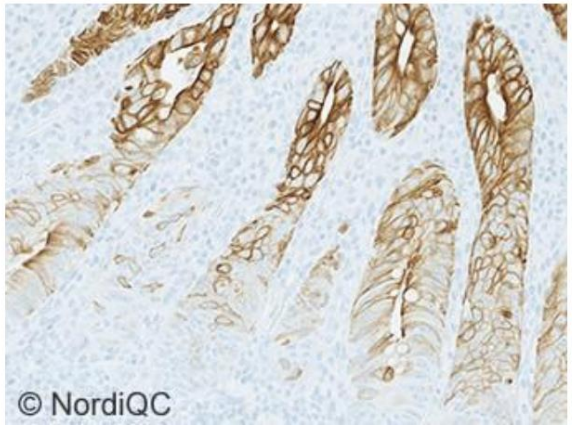
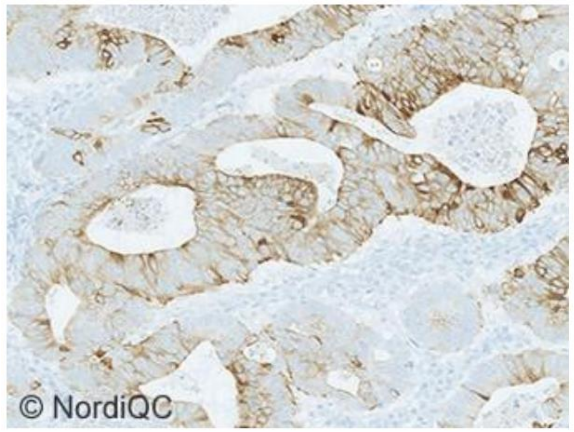
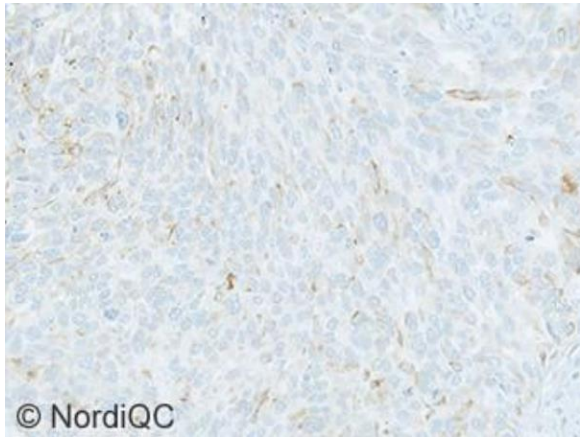
Table 2. **Proportion of sufficient results for CK20 in the four NordiQC runs performed**

	Run 8 2003	Run 25 2009	Run 35 2012	Run 47 2016
Participants, n=	71	130	195	284
Sufficient results	90%	64%	85%	92%

Control:
No optimal control. Best suggestion is normal colon or appendix. Majority of epithelial cells should be strongly positive, while basal cells should be at least weakly positive.



CK20 – insufficient results

	Appendix	Colon carcinoma	Urothelial carcinoma
Optimal			
Insufficient			

CK20

Table 1. **Antibodies and assessment marks for CK20, run 47**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BS101**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **Ks20.8**	97 11 5 5 2 2 1 1 1 1	Dako/Agilent Leica/Novocastra Cell Marque Thermo/Neomarkers EuroProxima Zeta Corporation Biocare DBS Euro Diagnostica PROGEN	55	58	13	0	90%	91%
rmAb clone **E19-1**	2	Immunologic	2	0	0	0	-	-
pAb **E16444**	2	Spring Bioscience	2	0	0	0	-	-
pAb **ILP 3202-C1**	1	Immunologic	1	0	0	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **Ks20.8 IR/IS777**	35	Dako/Agilent	31	4	0	0	100%	100%
mAb clone **Ks20.8 GA777**	19	Dako/Agilent	19	0	0	0	100%	100%
mAb clone **Ks20.8 PA0022**	10	Leica/Novocastra	6	3	1	0	90%	89%
mAb **Ks20.8 MAD-005105OD**	3	Master Diagnostica	2	0	1	0	-	-
mAb **Ks20.8 PM062**	1	Biocare	1	0	0	0	-	-
mAb clone **Ks20.8 E062**	1	Linaris	0	0	1	0	-	-
mAb clone **Ks20.8 Kit-0025**	1	Maixin	0	1	0	0	-	-
mAb clone **KS20.8 MON-RTU1083**	1	Monosan	0	0	1	0	-	-
mAb clone **PW31 PA0918**	1	Leica/Novocastra	0	1	0	0	-	-
rmAb clone **EPR1622Y**	1	Biogenex	1	0	0	0	-	-
rmAb clone **SP33 790-4431**	78	Ventana/Roche	53	20	3	2	94%	99%
Total	284		175	87	20	2	-	
Proportion			62%	30%	7%	1%	92%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
*discontinued products

Optimal clones
RTU > conc

Ks20.8
- Pass rate in RTU 100%
- HIER in alkaline buffer
- Titre 1:20-1:500

SP33
- Vendor recommended protocol gives optimal results
- HIER in alkaline buffer
- Ultraview/Optiview

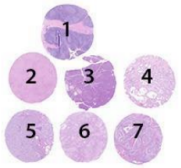
CK5

Assessment Run 55 2019

Cytokeratin 5 (CK5)

Material

The slide to be stained for cytokeratin 5 (CK5) comprised:



1. Tonsil, 2. Liver, 3. Pancreas, 4. Prostate hyperplasia, 5. Lung adenocarcinoma, 6-7. Lung squamous cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK5 staining as optimal included:

- A moderate to strong and distinct, cytoplasmic staining reaction in virtually all squamous epithelial cells in the tonsil.
- A weak to moderate, predominantly membranous staining reaction of scattered cuboidal epithelial cells in the pancreatic intercalated ducts .
- A strong and distinct cytoplasmic staining reaction in the majority of basal cells in the hyperplastic prostate glands.
- A moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung squamous cell carcinomas, tissue cores no. 6 and 7.
- No staining of neoplastic cells in the lung adenocarcinoma.
- No staining reaction in the liver.

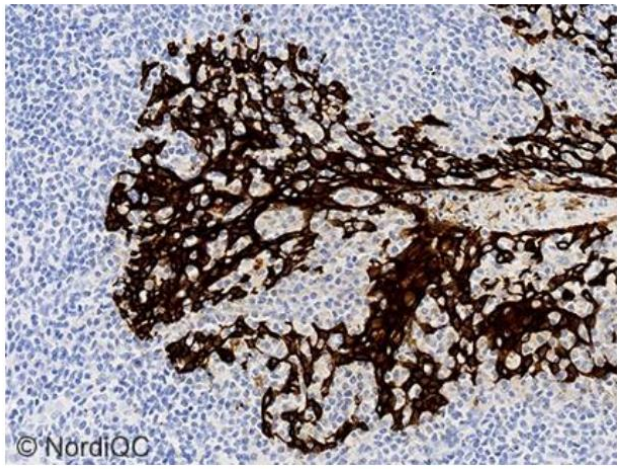
Table 2. **Proportion of sufficient results for CK5 in the three NordiQC runs performed**

	Run 12 2004	Run 46 2016	Run 55 2019
Participants, n=	74	266	263
Sufficient results	47%	68%	44%

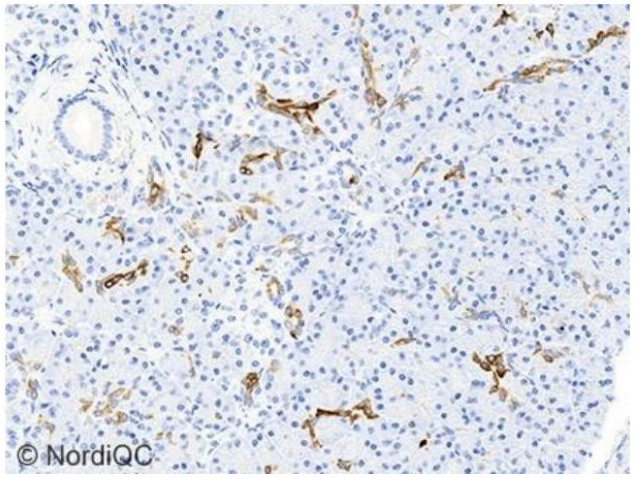
Controls

Tonsil:
All squamous epithelial cells should be strongly stained. No other staining should be seen.

Pancreas:
Scattered cuboidal cells of the intercalated ducts should display a weak to moderate staining reaction



Tonsil



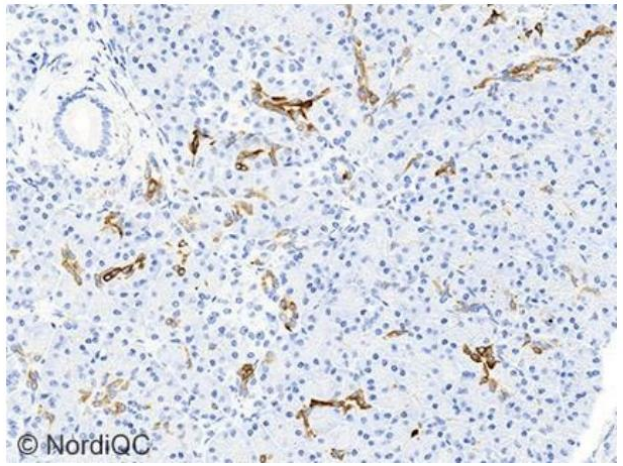
Pancreas

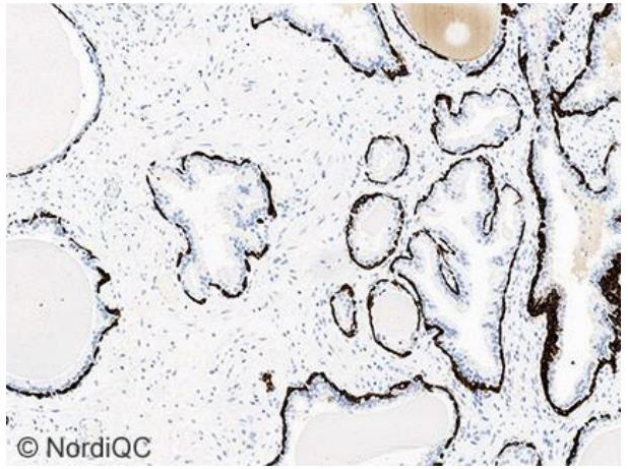
CK5 – insufficient results

Pancreas

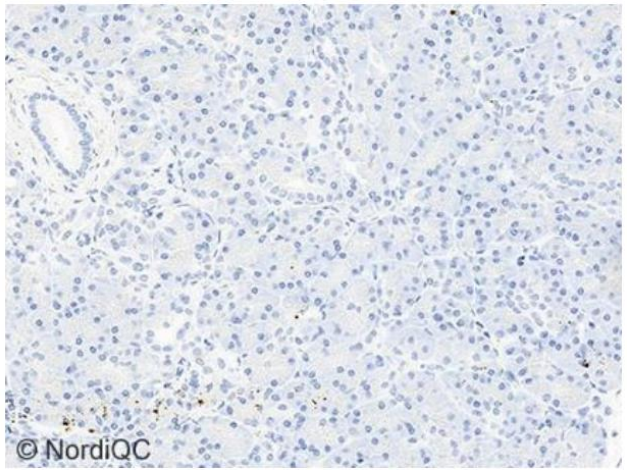
Prostate

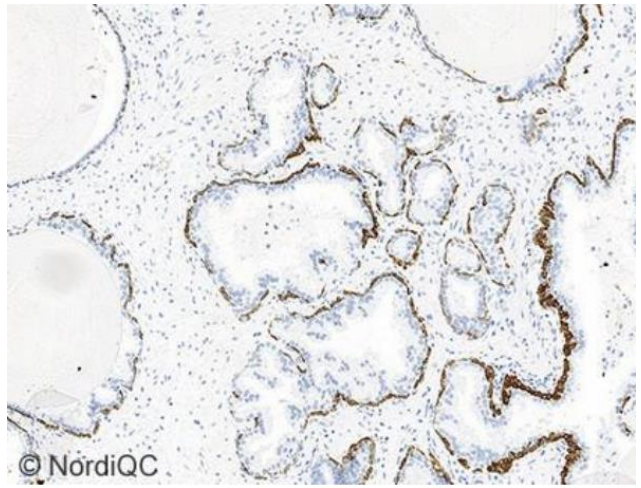
Optimal



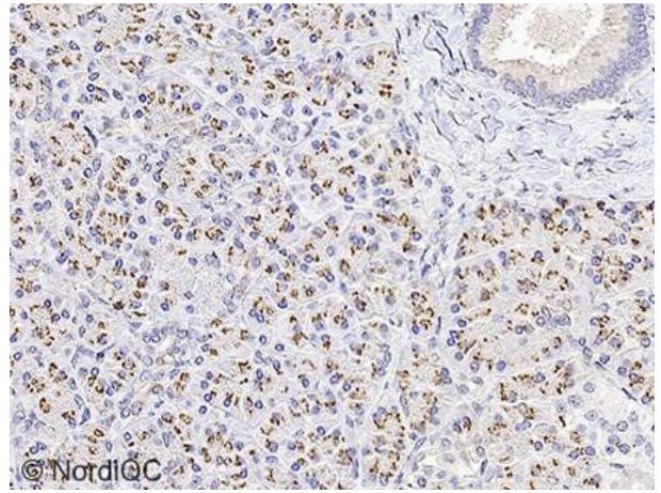


Insufficient





Weak staining reaction



False positive MAG-reaction using Clone D5/16 B4

CK5

Table 1. **Antibodies and assessment marks for CK5, run 55**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CK5/6.007**	1	Biocare	0	1	0	0	-	-
mAb clone **D5/16 B4**	45 7 1 1 1	Dako/Agilent Cell Marque Millipore Thermo Scientific Zytomed	4	10	31	10	25%	26%
mAb clone **XM26**	49 1 1 1 1	Leica/Novocastra Biocare Diagnostic BioSystems Histols Reagents Monosan	32	9	10	2	77%	81%
mAb clone **XM26/LL002**	1 1 1	Biocare Diagnostic BioSystems Zytomed	1	1	1	0	-	-
rmAb clone **BSR55**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EP1601Y**	5 1	Cell Marque Biocare	0	1	5	0	-	-
rmAb clone **EP24**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP27**	1	Immunologic	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **D5/16 B4 790-4554**	56	Ventana/Cell Marque	4	14	34	4	32%	82%
mAb **D5/16 B4 GA780**	21	Dako/Agilent	0	1	20	0	5%	-
mAb **D5/16 B4 GA780** [3]	1	Dako/Agilent	0	0	0	1	-	-
mAb clone **D5/16 B4 IR/IS780**	16	Dako/Agilent	0	0	12	4	0%	-
mAb clone **D5/16 B4 IR/IS780** [4]	9	Dako/Agilent	1	2	4	2	-	-
mAb clone **D5/16 B4 356M-10** [5]	2	Cell Marque	0	0	2	0	-	-
mAb clone **GM028 8294**	1	Sakura	0	0	1	0	-	-
mAb clone **XM26 PA0468**	7	Leica/Novocastra	4	2	1	0	-	-
mAb clone **XM26 PA0468** [6]	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **XM26 PM234**	1	Biocare	0	1	0	0	-	-
mAb clone **XM26/LL002 MSG106**	1	Zytomed	0	1	0	0	-	-
rmAb/mAb clone **EP1601Y/LL002 905H-8**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **EP1601Y**	4	Cell Marque	0	3	1	0	-	-
rmAb clone **EP24 RMA-0846**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP24/EP67 MAD-000651QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **SP27 760-4935**	18	Ventana /Cell Marque	15	3	0	0	100%	100%
Total	263		65	51	124	23	-	
Proportion			25%	19%	47%	9%	44%	

1) Proportion of sufficient stains (optimal or good),

SP27:
- Most successful clone
- HIER in alkaline buffer

XM26:
- HIER in alkaline buffer
- Ab titre 1:20-1:200

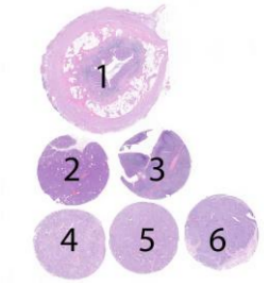
D5/16 B4:
Relative low sensitivity – and false negative

CDX2

Assessment Run 48 2016

CDX2

Material
The slide to be stained for CDX2 comprised:
1. Appendix, 2. Pancreas, 3. Tonsil, 4. Lung adenocarcinoma,
5-6. Colon adenocarcinoma.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CDX2 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all epithelial cells in the appendix
- An at least weak to moderate and distinct nuclear staining reaction of virtually all duct epithelial cells in the pancreas
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma, tissue core no. 6.
- An at least weak to moderate nuclear staining reaction of the majority of the neoplastic cells in the colon adenocarcinoma, tissue core no. 5
- No staining reaction in the lung adenocarcinoma and tonsil*.

A weak to moderate cytoplasmic reaction in cells with strong nuclear staining was accepted.

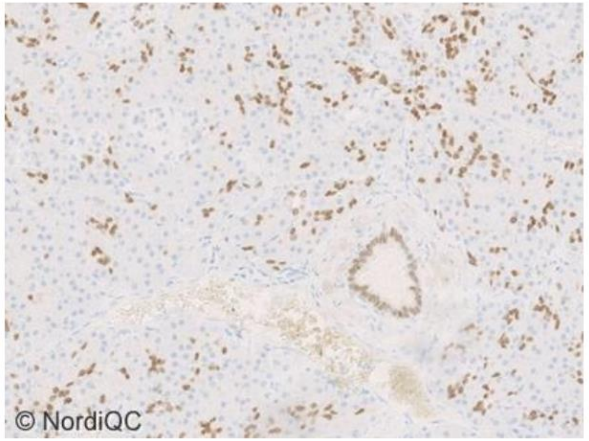
* In tonsil, few lymphocytes showed a weak nuclear staining reaction, which was accepted.

Table 2. **Proportion of sufficient results for CDX2 in the five NordiQC runs performed**

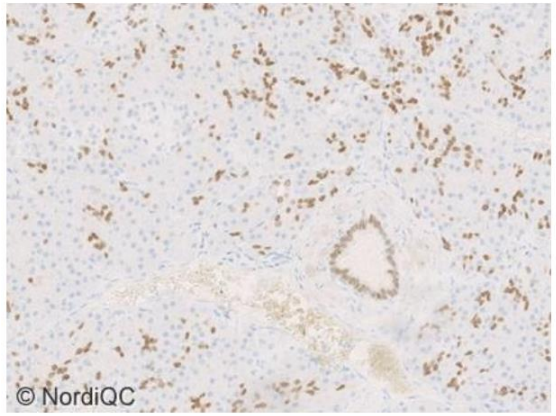
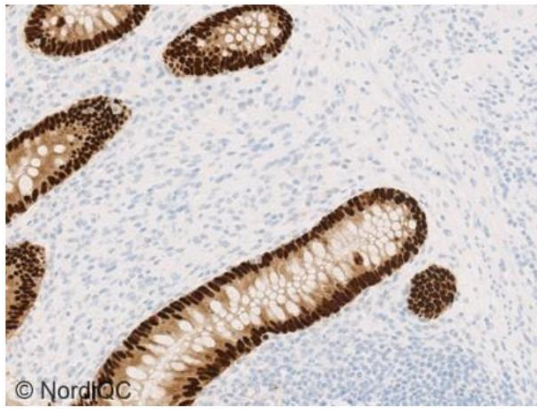
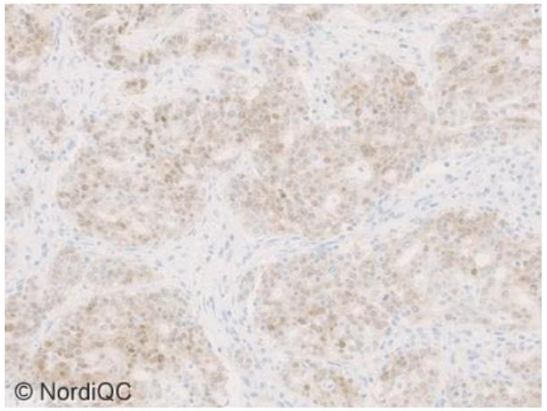
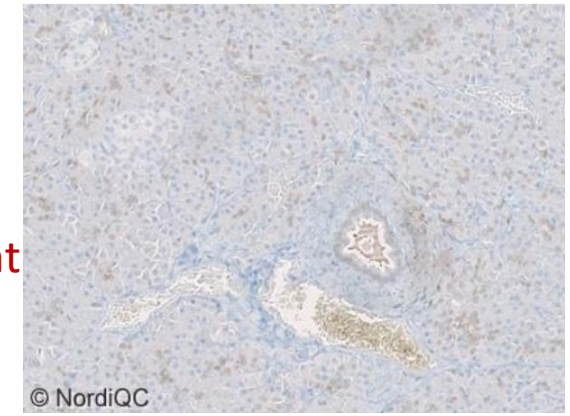
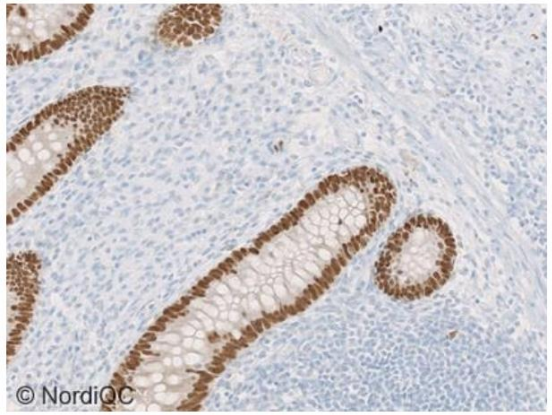
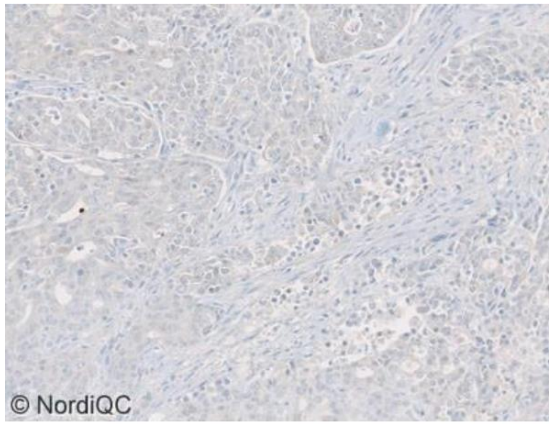
	Run 22 2008	Run 27 2009	Run 33 2011	Run 38 2013	Run 48 2016
Participants, n=	56	93	148	200	268
Sufficient results	64%	46%	51%	73%	80%

Control
Pancreas: moderate, nuclear staining in majority of duct epithelial cells.

Appendix and colon is not recommended, due to high level antigen expression



CDX2 – insufficient results

	Pancreas	Colon	Colon carcinoma
Optimal			
Insufficient			

CDX2

Table 1. **Antibodies and assessment marks for CDX2, run 48**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **AMT28**	2	Leica/Novocastra	0	0	0	2	-	-
mAb clone **CDX2-88**	2 2	Biocare Biogenex	0	0	1	3	-	-
mAb clone **DAK-CDX2**	31	Agilent/Dako	6	9	7	9	48%	57%
rmAb clone **EPR2764Y**	31 5 4 4 2 2 1 1 1 1	Cell Marque Thermo/Neomarkers Immunologic Zytomed Monosan Zeta Corporation A.Menarini Abcam Nordic Biosite Thermo/Pierce	28	14	7	3	81%	81%
Ready-To-Use antibodies								
mAb clone **BC39 API3184**	1	Biocare	0	0	0	1	-	-
mAb clone **CDX2-88 PM226**	1	Biocare	0	1	0	0	-	-
mAb clone **CDX2-88 AM392**	1	Biogenex	0	1	0	0	-	-
mAb **DAK-CDX2 IR080/IS080**	34	Agilent/Dako	18	10	5	1	82%	93%
mAb **DAK-CDX2 GA080**	26	Agilent/Dako	16	4	3	3	77%	100%
rmAb clone **EP25 RMPD059**	1	Diagnostic Biosystems	0	0	1	0	-	-
rmAb clone **EP25 PA0375**	7	Leica/Novocastra	4	3	0	0	100%	100%
rmAb clone **EP25 MAD-000645QD**	3	Master Diagnostica	0	3	0	0	-	-
rmAb clone **EPR2764Y RMA-0631**	1	Maixin	1	0	0	0	-	-
mAb clone **EPR2764Y RM-2116-R7**	1	Thermo/Neomarkers	0	0	1	0	-	-
rmAb clone **EPR2764Y 760-4380/ 235R***	103	Ventana/Cell Marque	81	15	5	2	93%	96%
Total	268		154	60	30	24	-	
Proportion			58%	22%	11%	9%	80%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Products merged due to imprecise antibody selection at the NordiQC homepage for protocol submission.

EPR2764Y:

- Most successful clone as concentrate
- Careful calibration of Ab titre
- 2- or 3-step detection system less important
- Fast deterioration at room temp.!

DAK-CDX2:

- Lower pass rate among LDT
- HIER in alkaline buffer
- 3 step detection system

The unknown primary tumour: Antivody selection, protocols and controls

Workshop in Diagnostic Immunohistochemistry 2-4. October 2019.

Rasmus Røge, MD, NordiQC scheme organizer