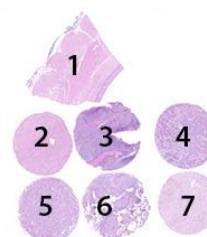


Material

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.

All tissues were fixed in 10% neutral buffered formalin.

Participation

Number of laboratories registered for CK-PAN, run 54	315
Number of laboratories returning slides	296 (94%)

Results

296 laboratories participated in this assessment. 184 (62%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Insufficient HIER – too short efficient heating time and/or use of non-alkaline HIER buffers
- Inappropriate epitope retrieval
- Less successful primary antibodies
- Less successful performance of the mAb clone cocktail AE1/AE3 on the Leica Bond platforms
- Technical issues.

Performance history

This was the ninth NordiQC assessment of CK-PAN. The overall pass rate decreased significantly compared to the previous run 47, see table 2.

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%

Conclusion

The mAb clone cocktails **AE1/AE3**, **AE1/AE3/5D3**, **AE1/AE3/PCK26** and mAb clone **BS5** can all be recommended for demonstration of CK-PAN. The epitope retrieval method must be specifically tailored to the clone/cocktail applied. Applying the mAb clone cocktail AE1/AE3 on the Bond platform (Leica) is

challenging, and used within a laboratory developed assay, no optimal results could be obtained. The mAb clone MNF116 should not be used due to poor performance. The Ready-To-Use (RTU) systems from Dako based on mAb clone cocktail AE1/AE3 were in this assessment the most successful and provided high proportions of sufficient and optimal results.

Liver and esophagus in combination are recommendable as positive and negative tissue controls. The vast majority of hepatocytes must show a distinct cytoplasmic staining reaction with membrane accentuation, while virtually all squamous epithelial cells of the esophagus throughout all cell layers must display a strong cytoplasmic staining reaction. No staining reaction should be seen in the stromal cells except for a focal reaction of smooth muscle cells of muscularis propria in the esophagus (weak to moderate intensity).

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	-	-
"Laboratory made" antibody cocktails								
mAb clone cocktail AE1/AE3/5D3	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone cocktail Unknown	2		1	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone cocktail AE1/AE3 IR053	24	Dako/Agilent	18	6	0	0	100%	100%
mAb clone cocktail AE1/AE3 IR053³	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 GA053³	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	-	-

PA0012									
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&mAb 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%		

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.

Detailed analysis of CK-PAN, Run 54

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone cocktail **AE1/AE3**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (6/16)*, Cell Conditioning 1 (CC1; Ventana) (21/60) or Tris-EDTA/EGTA pH 9 (3/5) as retrieval buffer. The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 43 of 58 (74%) laboratories produced a sufficient staining result (optimal or good).

* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone cocktail **AE1/AE3/5D3**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1), CC1 or Tris-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed.

mAb clone cocktail **PAN CK (C2562; Sigma Aldrich)**: One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (BERS2, Leica) as retrieval buffer. The mAb was diluted 1:1,000 and Bond Refine (Leica) was used as the detection system.

mAb clone **BS5**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1), CC1, Tris-EDTA/EGTA pH 9 or BERS2 as retrieval buffer. The mAb was diluted in the range of 1:100-1:2,500 depending on the total sensitivity of the protocol employed.

Table 3. 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	4/9**	-	0/2	-	15/41 (37%)	-	0/8	0/5

* 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.

Ready-To-Use antibodies and corresponding systems

mAb clone cocktail **AE1/AE3**, product no. **IR053**, Dako, Autostainer+/Autostainer Link:

Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000)

as detection system. Using these protocol settings, 20 of 20 (100%) laboratories produced a sufficient staining result.

mAb clone cocktail **AE1/AE3**, product no. **GA053**, Dako, OMNIS: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 24-30 min. at 97°C) and 12.5-30 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) as detection system. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient staining result. The one protocol obtaining an insufficient mark used no pre-treatment at all.

mAb clone cocktail **AE1/AE3/PCK26**, product no. **760-2135/2595**, Ventana, BenchMark GX/XT/Ultra: Protocols with optimal results were typically based on a combined pre-treatment using HIER in CC1 for 24-36 min. followed by enzymatic pre-treatment in Protease 3 (4 min.), 8-32 min. incubation of the primary Ab and UltraView with or without amplification (760-500+760-080) or OptiView (760-700) as detection system. Using these protocol settings, 20 of 24 (83%) laboratories produced a sufficient staining result. Although applying optimal protocol settings, 4 of 4 protocols were assessed as insufficient due to technical issues on the Ventana Benchmark platform.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 assessed protocols). The performance was evaluated both as “true” plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

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.

Comments

In concordance with the previous NordiQC assessments for CK-PAN, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. Virtually all the participating laboratories were able to stain cytokeratins (CK) in bile ducts liver and neoplastic cells of the lung adenocarcinoma, whereas demonstration of CK in neoplastic cells of the SCLC and CCRCC was more difficult, and was only obtained by protocols with appropriate protocol settings. The pass rate was highly influenced by the choice of Ab and retrieval method applied, which underlines the necessity for individual optimization for each clone/clone cocktail used for the demonstration of CK. This correlation, observed in the last seven NordiQC CK-PAN assessments, is summarized in Table 5.

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%)

The data clearly support that choice of epitope retrieval has significant impact on the staining result. For the most widely used Ab clone cocktail AE1/AE3, the overall pass rate in these 8 successive NordiQC runs was 72%. Using HIER, a pass rate of 76% was obtained, significantly higher than the pass rate of 11% when proteolytic pre-treatment was applied for AE1/AE3. For the second most commonly used Ab clone cocktail, AE1/AE3/PCK26, combined epitope retrieval using HIER in CC1 (Ventana) followed by proteolysis, provided a pass rate of 73%, compared to 43% and 5% using either HIER or proteolysis as single retrieval method.

The mAb clone MNF116 has in these eight consecutive runs provided an inferior overall performance compared to the 3 other antibody cocktails listed in Table 5. No significant improvement of the performance could be identified by any of the different retrieval methods. Consequently, mAb clone MNF116 should be substituted by one of the mentioned Ab cocktails.

Used within a laboratory developed (LD) assay, the mAb clone cocktails AE1/AE3, AE1/AE3/5D3, PAN CK (Ab C2562) and the mAb clone BS5 could all be used to obtain an optimal staining result for CK-PAN (see Table 1). Irrespective of the clone used, HIER in an alkaline buffer was mandatory for optimal performance.

The mAb clone cocktail AE1/AE3 was the most widely used antibody for demonstration of CK-PAN. Used as a concentrate, mAb clone cocktail AE1/AE1 gave an overall pass rate of 58% (61 of 106). As shown in Table 3, the mAb clone cocktail AE1/AE3 is technically challenging and optimal results could only be obtained on IHC platforms from Dako and Ventana. No single parameters could be identified explaining the overall poor performance on the Bond platforms (Leica) where only 15% (2 of 13) were assessed as sufficient, none of which was optimal.

As mentioned in assessment report for run 47 (2016), too weak or false negative staining result was the main feature of an insufficient result and was typically caused by protocols with too low sensitivity. The titre of the primary Ab must be carefully calibrated to provide an IHC protocol, which is "fit-for-the-purpose", i.e. a protocol able to demonstrate CK-PAN in structures with both low-level and high-level CK expression, which is the range seen in carcinomas. Using protocol settings based on the concentrated format of the mAb clone cocktail AE1/AE3, HIER performed in an alkaline buffer and applying a standard 2- or 3-step polymer/multimer based detection system, the Mean Dilution Value (MDV) of the primary Ab for optimal results was 1:112 (range 1:40-1:300, n=29). In comparison a MDV of 1:186 (range 1:50-1:800, n=35) was seen for protocols with insufficient results (borderline or poor). There was no significant difference in performance between 2-step and 3-step multimer/polymer detection systems.

Although the number of participants using the mAb clone BS5 within a LD assay was low, this primary Ab seems robust and promising, as all protocols (5 of 5) were assessed as optimal (see Table 1). This Ab might be an alternative to the more challenging Abs (e.g. MNF116 or AE1/AE3 on specific platforms), performing optimally using a wide spectrum of dilutions (1:100-1:2,500) as long as HIER was performed in alkaline buffer (e.g. BERS2, Leica) and the concentration of the primary Ab was calibrated according to the overall sensitivity of the detection systems applied.

55% (163 of 296) of the laboratories used a Ready-To-Use (RTU) format for detection of CK-PAN. The number of assays based on these RTU formats is consistently increasing (compare with previous runs for CK-PAN on the NordiQC webpage). Ideally, a RTU format of a primary Ab should be used within a system that has been thoroughly validated, providing precise information on vendor recommended protocol settings, equipment, reagents and performance characteristic (expected reaction patterns).

In this assessment the Dako RTU systems **IR053** and **GA053** based on the mAb clone cocktail AE1/AE3 provided the highest number of sufficient and optimal results. As shown in Table 4, and for laboratories using one of these systems, vendor recommended protocol settings gave a pass rate of 100% (36 of 36) of which 67% were assessed as optimal. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) also provided high proportion of sufficient and optimal results.

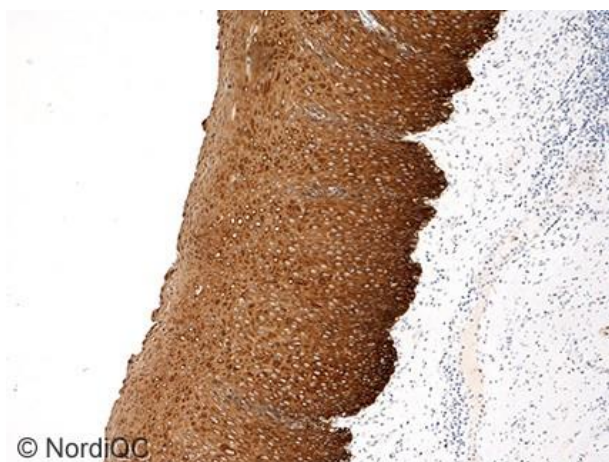
The Ventana RTU system **760-2135/2595** based on the mAb clone cocktail AE1/AE3/PCK26 gave an overall pass rate of 51% with 30% optimal. However, using optimal protocol settings a pass rate of 83% was obtained, which was significantly lower compared to 96% in the previous run 47. As mentioned above, a considerable proportion of laboratories were challenged by technical issues on the Ventana Benchmark platform which could account for the overall lower performance seen in this run. Optimal results could be obtained using both vendor recommended and laboratory modified protocol settings (see Table 4). Applying combined pre-treatment, HIER in CC1 and enzymatic pre-treatment in protease 3 (P3) (all protocol settings), the overall pass rate was 79% (37 of 47) of which 40% (19 of 47) were assessed as optimal. In comparison, and using HIER in CC1 or combined pre-treatment with HIER in CC1 and proteolysis in either Protease 1 or Protease 2 (all protocol settings), the proportion of sufficient result was 27% (6 of 22) and only 14% (3 of 22) were optimal. Two laboratories were able to obtain an optimal

result using HIER in CC1 (24-32 min.) as single retrieval method applying OptiView as the detection system. Using proteolysis as single retrieval method, the pass rate was 15% (2 of 13) and only one protocol was assessed as optimal. Typical staining patterns of the different pre-treatment procedures are illustrated in Figs. 5a-c.

This was the ninth assessment of CK-PAN in NordiQC (see Table 2). Although CK-PAN has been used for many years and is a part of the primary panel (together with S100, Vimentin and CD45), the marker is still technically challenging and the pass rate in this run 54 decreased compared to the latest run 47, 2016. Several elements influenced the final outcome: 1) The pass rate for new participants was only 48% (30 of 62) compared to 66% (154 of 234) for laboratories participating more than once in a CK-PAN assessment, 2) The use of proteolytic pre-treatment either as single retrieval method or in combination with HIER (e.g. the RTU system 760-2135/2595, Ventana) is difficult to control in the laboratory, impacting proportion of sufficient results. In total, 28% (82 of 296) of the protocols were based on some kind of enzymatic pre-treatment providing a pass rate of 48% (40 of 82) of which 26% (21 of 82) were assessed as optimal, 3) The use of the less successful clone MNF116 and the use of the clone AE1/AE3 on the Bond platform (Leica) also influenced the overall increase of insufficient results. Importantly, laboratories should apply an Ab that will work on the in-house IHC platform, calibrate the protocols correctly and stain according to the expected antigen level of the recommended control material (see below).

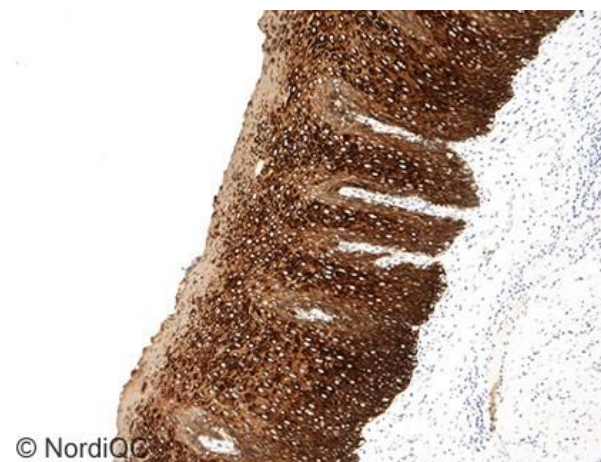
Controls

As seen in the previous NordiQC assessments, liver and esophagus in combination are recommendable as positive tissue controls for CK-PAN. It is crucial that the vast majority of hepatocytes (expressing only a limited amount of the primary LMW-CK types 8 and 18) show an at least moderate, distinct cytoplasmic and membranous staining reaction. In esophagus, virtually all squamous epithelial cells throughout all cell layers must show a strong distinct cytoplasmic staining reaction due to expression of HMW-CK types 5 and 14. No staining should be seen in stromal cells in the liver. Smooth muscle cells in vessels and in muscularis mucosae in esophagus will typically show a weak to moderate patchy cytoplasmic staining reaction.



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Fig. 1a (x100)
Optimal CK-PAN staining of the esophagus using the mAb clone BS5 carefully calibrated, HIER in BERS2 (Leica) (20 min.) and a 3-step polymer based detection system (Bond Refine; Leica) as the detection system. All squamous epithelial cells show a strong and distinct cytoplasmic staining reaction. Same protocol used in Figs. 2a-4a.



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Fig. 1b (x100)
CK-PAN staining of the esophagus using an insufficient protocol with too low sensitivity based on the mAb clone AE1/AE3 carefully calibrated, but with HIER in BERS1 (Leica) and Bond Refine (Leica) as the detection system. Same protocol used in Figs. 2b-4b. Although the staining pattern is similar to the optimal protocol seen in Fig. 1a (same field), the assay provided too weak staining reaction in critical tissue specimens – compare Fig. 2a-2b, 3a-3b and 4a-4b.

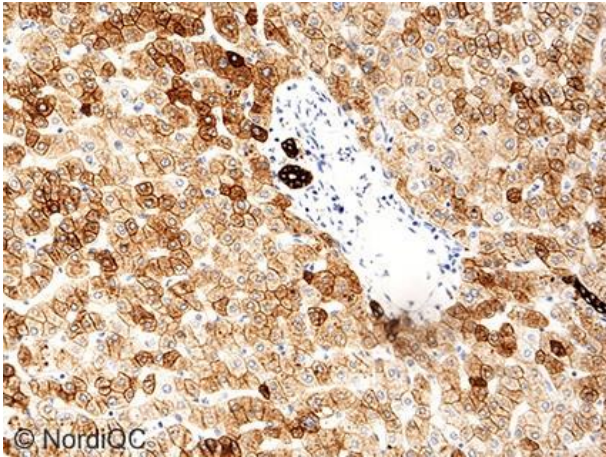


Fig. 2a (x200)
Optimal CK-PAN staining of the liver using same protocol as in Fig. 1a. The vast majority of hepatocytes show a moderate to strong staining reaction (membranous accentuation) while the columnar cells of the bile ducts display strong cytoplasmic staining reaction.

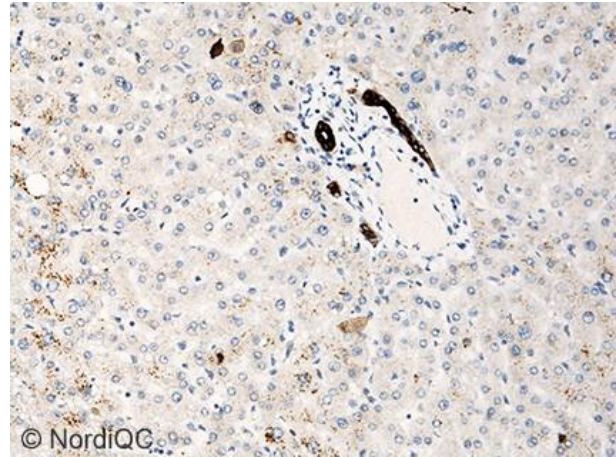


Fig. 2b (x200)
Insufficient CK-PAN staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a. Only bile ducts are demonstrated due to high expression of CK-LMW (CK types 7, 8/18 and 19) whereas the hepatocytes are false negative (only express low antigen levels of CK 8/18). In addition, CK-PAN clone AE1/AE3 only detects CK8.

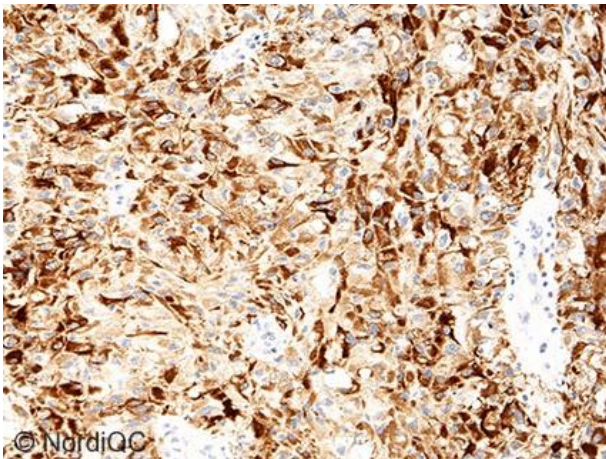


Fig. 3a (x200)
Optimal CK-PAN staining of the CCRCC using same protocol as in Figs. 1a and 2a. The vast majority of neoplastic cells display a moderate to strong, distinct cytoplasmic staining reaction with membranous accentuation.

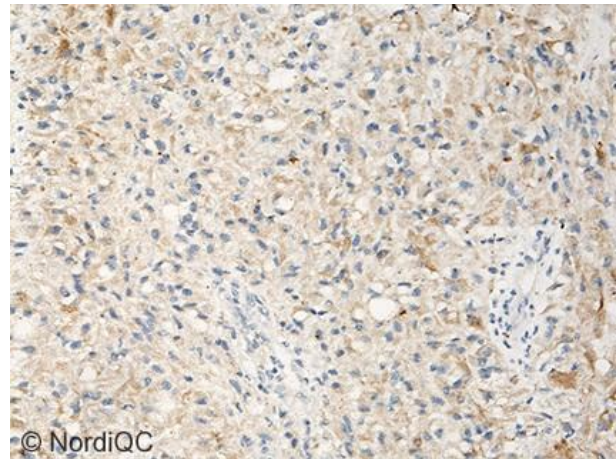


Fig. 3b (x200)
Insufficient CK-PAN staining of the CCRCC using same protocol as in Figs. 1b and 2b. The neoplastic cells only display faint staining intensity or is false negative - same field as in Fig. 3a.

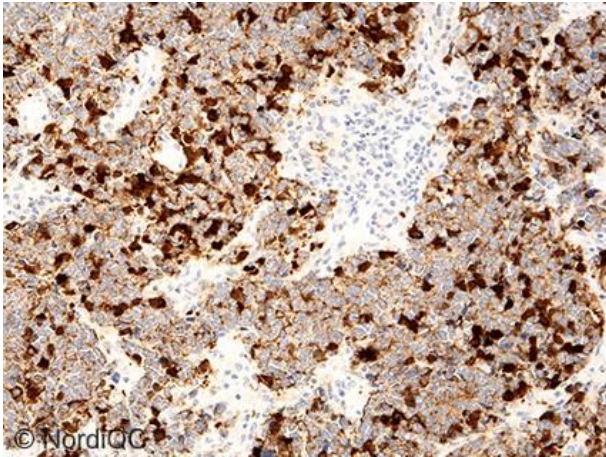


Fig. 4a (x200)
Optimal CK-PAN staining of the SCLC using same protocol as in Figs. 1a-3a. The vast majority of the neoplastic cells show a weak to strong but distinct cytoplasmic and dot-like staining reaction.

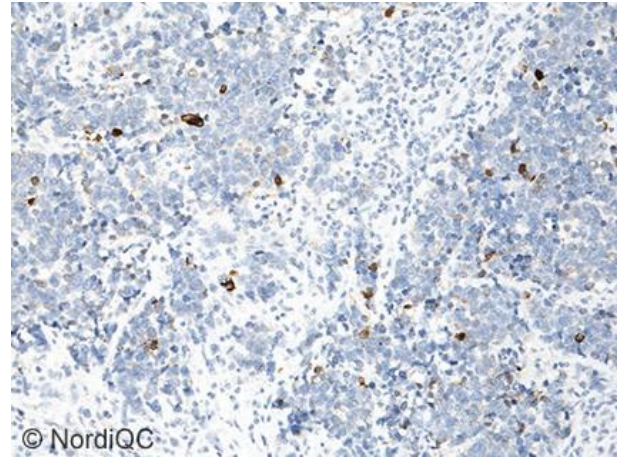


Fig. 4b (x200)
Insufficient CK-PAN staining of the SCLC using same protocol as in Figs. 1b-3b. The proportion of positive cells is significantly reduced. Only scattered neoplastic cells display a dot like staining - same field as in Fig. 4a.

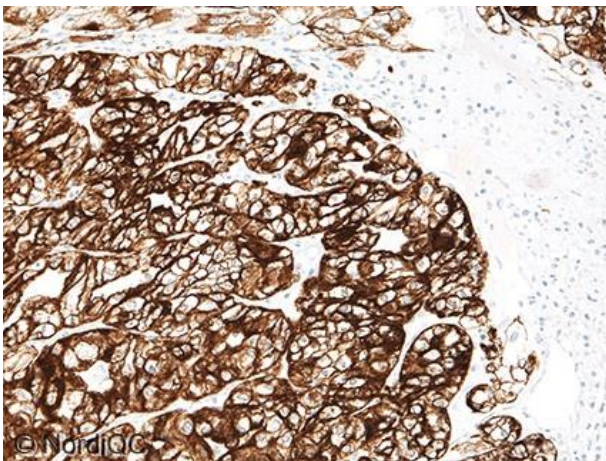


Fig. 5a (x200)
Optimal CK-PAN staining of the CCRCC using the RTU product 790-2135/2595 (Ventana) based on the mAb cocktail AE1/AE3/PCK26, HIER in CC1 (32 min.) followed by proteolysis (P3, 4 min.) and a 3-step multimer based detection system (Optiview, Ventana). All the neoplastic cells display a strong cytoplasmic staining reaction with membranous accentuation. Hepatocytes of the liver and squamous epithelium in the esophagus displayed the expected reaction pattern (data not shown - see Figs. for optimal performance above).

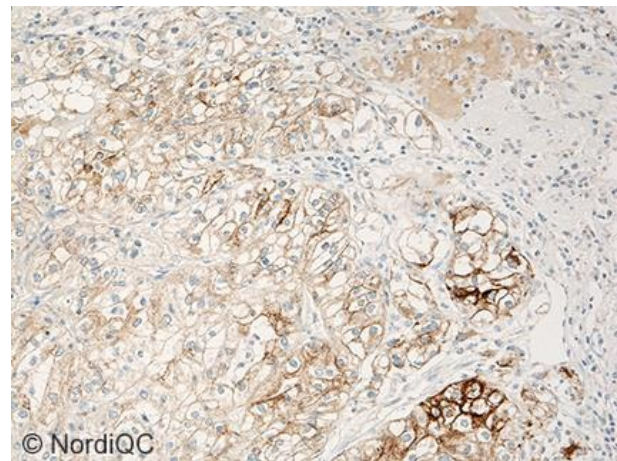


Fig. 5b (x200)
Insufficient CK-PAN staining of the CCRCC using the RTU product 790-2135/2595 (Ventana) based on the mAb cocktail AE1/AE3/PCK26, HIER in CC1 (8 min.) followed by proteolysis (P1, 4 min.) and a 3-step multimer based detection system (Optiview, Ventana). The neoplastic cells show too weak staining intensity. Prolonging HIER time will impair morphology due to the use of the enzymatic digestion with P1 (more efficient than P3). Performing combined antigen retrieval is difficult and in this case, the vendor recommendation to pre-treatment should have been followed to give the expected reaction pattern in critical tissue controls (esophagus and liver).

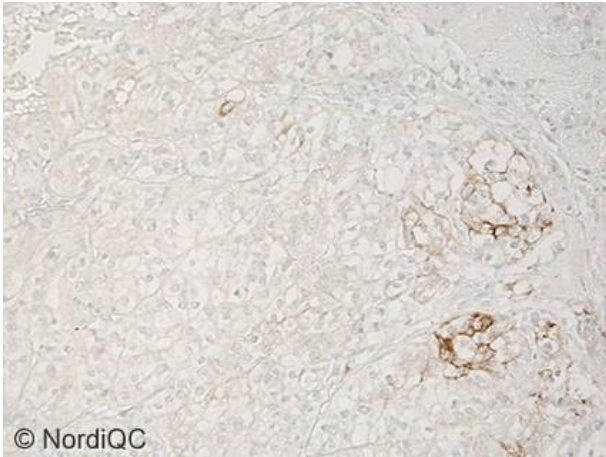


Fig. 5c (x200)

Insufficient CK-PAN staining of the CCRCC using the RTU product 790-2135/2595 (Ventana) based on the mAb cocktail AE1/AE3/PCK26, proteolysis (P1, 8 min.) as single retrieval method and a 3-step multimer based detection system (Optiview, Ventana). The vast majority of neoplastic cells are false negative. Enzymatic pre-treatment without HIER in general provide poor results.

MB/LE/MV/RR 27.11.2018