Pan Cytokeratin (CK-PAN)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CK-PAN used to identify the epithelial origin of carcinoma of unknown primary origin. Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of CK-PAN antigen densities (see below).

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

1. Appendix, 2. Liver, 3. Esophagus, 4. Tonsil, 5. Lung adenocarcinoma,
6. Lung squamous cell carcinoma, 7. Renal clear cell carcinoma (CCRCC),
8. Diffuse large B-cell lymphoma (DLBCL).
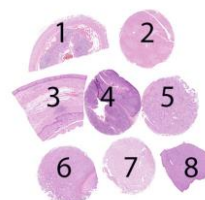
Criteria for assessing a CK-PAN staining as optimal were:

- 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 majority of hepatocytes.
- A strong, distinct cytoplasmic staining reaction of all squamous epithelial cells throughout all cell layers in the esophagus and tonsil.
- A strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma and squamous cell carcinoma.
- An at least weak to moderate, predominantly membranous staining reaction of the majority of neoplastic cells in the renal clear cell carcinoma.
- No staining in lymphocytes in tonsil and neoplastic cells in the DLBCL. Interstitial reticulum cells (CIRCs) with dendritic/reticular pattern was accepted and expected to show a weak to moderate cytoplasmic staining reaction due to expression of cytokeratin low mol. weight types 8/18.

All tissues were fixed in 10% neutral buffered formalin.

Participation

Number of laboratories registered for CK-PAN, run 58	342
Number of laboratories returning slides	326 (95%)

Results
326 laboratories participated in this assessment. 243 (75%) 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, especially mAb clone MNF116
- Less successful performance of the mAb clone cocktail AE1/AE3 on the Leica Bond platforms
- Technical issues.

Performance history
This was the tenth NordiQC assessment of CK-PAN. The overall pass rate increased significantly compared to the previous run 54, see Graph 1.

CK-PAN performance in NordiQC assesments 2003-2020
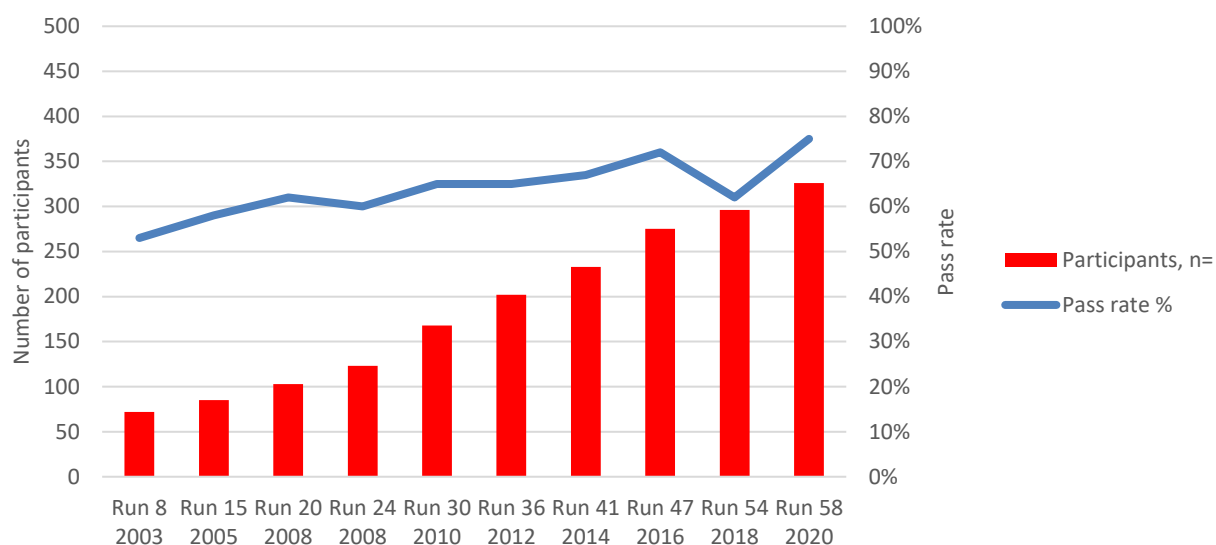
Graph 1. **Proportion of sufficient results for CK-PAN in the ten NordiQC run performed**

Conclusion
The mAb clone cocktails **AE1/AE3, AE1/AE3/PCK26** and mAb clone **BS5** can all be recommended for demonstration of CK-PAN. The mAb clone MNF116 should not be used due to a general poor performance. The epitope retrieval method must be specifically tailored to the clone/cocktail applied. The mAb clone cocktail AE1/AE3 showed an inferior performance on the Bond platform (Leica), and used within a laboratory developed assay, no optimal results could be obtained, whereas mAb clone BS5 was found to be more successful on the Bond platform. 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 tonsil/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 tonsil/esophagus throughout all cell layers must display a strong cytoplasmic staining reaction. Tonsil can also be used as negative tissue control, in which no staining reaction should be seen in lymphocytes. Dispersed interstitial reticulum cells with dendritic/reticular pattern can show a weak to moderate cytoplasmic staining reaction and must be accepted due to low level CK expression.

Table 1. **Antibodies and assessment marks for CK-PAN, run 58**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone cocktail **AE1/AE3**	73 2 10 10 2 1 1 1 1 1 1 1 1	Dako/Agilent Thermo/NeoMarkers Cell Marque Leica/Novocastra Biocare Medical Zytomed Diagnostic Biosystems Genemed Immunologic DCS Diagnostics Bio Sp Zytomed Zeta Corporation	51	26	18	9	74%	49%
mAb clone cocktail **AE1/AE3/5D3**	2 1 1	Biocare Medical Zytomed Abcam	-	3	-	1	-	-
mAb clone cocktail **PAN CK (Ab C2562)**	1	Sigma Aldrich	-	1	-	-	-	-
mAb clone **BS5**	4 10	Monosan Nordic Biosite	8	4	2	-	86%	57%
mAb clone **MNF116**	9	Dako/Agilent	-	-	-	9	0%	0%

							Suff.[1]	OR.[2]
mAb clone **OSCAR**	1	PhenoPath	-	-	1	-	-	-
mAb clone **KL1**	1	Zytomed	-	-	-	1	-	-
"Laboratory made" antibody cocktails							Suff.[1]	OR.[2]
Ab clone cocktail **AE1/AE3/8/18**	1	Leica/Novocastra	1	-	-	-	-	-
mAb clone cocktail **Unknown**	2		-	1	1	-	-	-
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%
mAb clone cocktail **AE1/AE3 GA053 (VRPS)**[3]	31	Dako/Agilent	27	1	2	1	90%	87%
mAb clone cocktail **AE1/AE3 GA053 (LMPS)**[4]	18	Dako/Agilent	17	1	-	-	100%	94
mAb clone cocktail **AE1/AE3 313M-XX**	2	Cell Marque	-	1	1	-	-	-
mAb clone cocktail **AE1/AE3 MAD 001000QD**	1	Master Diagnostica	-	1	-	-	-	-
mAb clone cocktail **AE1/AE3 PA0909**	2	Leica/Novocastra	-	1	1	-	-	-
mAb clone cocktail **AE1/AE3 PA0094**	5	Leica/Novocastra	1	3	1	-	80%	20%
mAb clone cocktail **AE1/AE3 PA0012**	3	Leica/Novocastra	-	3	-	-	-	-
mAb clone cocktail **AE1/AE3 PDM072**	2	Diagnostic Biosystems	-	-	2	-	-	-
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%
mAb clone cocktail **AE1/AE3/ VP011**	1	Biocare Medical	-	-	1	-	-	-
mAb clone **Lu-5 AM181-5M**	1	Biogenex	-	-	-	1	-	-
mAb clone cocktail **AE1/AE3/DC10 8309-C010**	1	Sakura Finetek	1	-	-	-	-	-
mAb clone **OSCAR Z-465-26-Y**	1	Zytomed Systems	-	-	1	-	-	-
Total	326		168	75	47	36	-	
Proportion			52%	23%	15%	11%	75%	

1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

Detailed analysis of CK-PAN, Run 58
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) (11/15)*, Cell Conditioning 1 (CC1, Ventana) (36/62) or Tris-EDTA/EGTA pH 9 (3/3) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 63 of 70 (90%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BS5**: Protocols with optimal results were based on HIER using TRS pH 9 (Dako) (1/3), Tris/EDTA pH 9 (1/1), CC1 (Ventana) (2/3), Bond Epitope Retrieval Solution 2 (BERS2, Leica) 3/6 and Bond Epitope Retrieval Solution 1 (BERS1, Leica) 1/1.The mAb was diluted in the range of 1:100-1:800 depending on the total sensitivity of the protocol employed. Using these settings 12/14 (86%) laboratories produced a sufficient staining result. One of the protocols that obtained an insufficient mark was due to technical issues.

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.

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 10-40 min. at 95-97°C), 15-30 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result. One laboratory did not produce a sufficient result with these settings due to technical issues.

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 20-30 min. at 97°C) and 10-20 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) as detection system. Using these protocol settings, 40 of 43 (93%) laboratories produced a sufficient staining result. Two of the protocols obtaining an insufficient mark was due to technical issues.

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 24-64 min. followed by enzymatic pre-treatment in Protease 3 (4 min.), 4-24 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, 42 of 48 (88%) laboratories produced a sufficient staining result. Four of the protocols obtaining an insufficient mark due to technical issues.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed 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 3. **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**	92% (12/13)	92% (12/13)	100% (9/9)	78% (7/9)
Dako Omnis mAb AE1/AE3 **GA053**	90% (28/31)	87% (27/31)	100% (16/16)	100% (16/16)
VMS Ultra/XT/GX mAb AE1/AE3/PCK26 **760-2135/2595**	76% (19/25)	44% (11/25)	70% (48/69)	42% (29/69)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and 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 participating laboratories were able to stain cytokeratins (CK) in the epithelial cells of bile ducts in liver and neoplastic cells of the lung adenocarcinoma, whereas demonstration of CK in hepatocytes and the neoplastic cells of the renal clear cell carcinoma 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 nine NordiQC CK-PAN assessments, is summarized in Table 4.

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%)

The data clearly stresses that the 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 9 successive NordiQC runs was 73%. Using HIER, a pass rate of 77% was obtained, significantly higher than the pass rate of 12% 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 74%, compared to 46% and 6% using either HIER or proteolysis as single retrieval method.

The mAb clone MNF116 has in these nine consecutive runs provided an inferior overall performance compared to the 3 other antibody cocktails listed in Table 4. No significant improvement of the performance could be identified by any of the different retrieval methods. Consequently, mAb clone MNF116 should be substituted by e.g. one of the mentioned Ab cocktails or the mAb clone BS5.

42% (136 of 326) of the participants used a laboratory developed (LD) assay and the mAb clone cocktail AE1/AE3 and the mAb clone BS5 could both be used to obtain an optimal staining result for CK-PAN (see Table 1). For both clones 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 and used as a concentrate, mAb clone cocktail AE1/AE1 gave an overall pass rate of 74% (77 of 104). As shown in Table 2, the performance of mAb clone cocktail AE1/AE3 seems to be influenced by the IHC stainer platform as optimal results could only be obtained on the platforms from Dako and Ventana, providing a careful calibration of the primary Ab and selection of appropriate protocol settings (Figs. 5-6). For yet unexplained reasons the mAb AE1/AE3 showed an overall inferior performance on the Bond platforms (Leica) where only 19% (3 of 16) were assessed as sufficient, none of which being optimal. No single parameter could be identified as root cause for the inferior performance

As mentioned in assessment report for run 54 (2018), 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 titer 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.

Although the number of participants using the mAb clone BS5 within a LD assay was low, this primary Ab seems robust and promising, as most protocols (12 of 14) were assessed as sufficient (see Table 1). This Ab might be an alternative to the more challenging Abs (e.g. MNF116 or AE1/AE3) on the Bond (Leica) platforms where 6/7 received a sufficient mark using mAb BS5. The mAb BS5 performed optimally using a dilution in the range of 1:100-1:800 carefully calibrated according to the overall sensitivity of the detection systems applied and using HIER in an alkaline buffer (e.g. BERS2, Leica) (Figs. 7a-b).

58% (190 of 326) 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 characteristics (expected reaction patterns). With this in focus, NordiQC has expanded the data analysis for the RTU systems from the main providers to assess the pass rates and proportion of optimal results when these systems are applied as "plug-and-play" or used with protocol modifications by the laboratories. The data can be seen in both Table 1 and Table 3.

In this assessment, the Dako RTU systems **IR053, IS053** and **GA053** based on the mAb clone cocktail AE1/AE3 provided the highest number of sufficient and optimal results. As shown in Table 3, and for laboratories using one of these systems, vendor recommended protocol settings gave a pass rate of 91% (40 of 44) of which 89% 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** was used by 94 participants and typically by laboratory modified protocol settings as shown in Table 1 and 3. When the RTU system was used by the vendor recommended protocol settings primarily based on a combined pre-treatment with HIER in CC1 and proteolysis in P3, a pass rate of 76% was observed, 44% being optimal. In general the pass rate and proportion of optimal results was reduced for laboratories modifying the protocol settings. Less successful modifications were especially related to e.g. substitution of P3 with P1 or P2, or use of proteolysis as single retrieval method. These specific modifications provided an overall inferior pass rate of 42%, 16% optimal. Typical staining patterns of these different pre-treatment procedures are illustrated in Figs. 1-4.

This was the tenth assessment of CK-PAN in NordiQC (see Graph 1). Although CK-PAN has been used for many years and is a central part of the primary panel for the identification and classification of carcinoma of unknown primary origin (together with S100, Vimentin and CD45), the marker is still technically challenging although the pass rate in this run 58 increased compared to the latest run 54, 2018. Several elements influenced the final outcome:
 1) Less successful performance on the Bond platform (Leica) which is one of the 4 main IHC platforms. 42 participants used this platform and 48% received a sufficient mark, only 14% with optimal results.
2) 94 participants used the RTU system 760-2135/2595 (Ventana) and only 27% of them followed the vendor recommendation with combined pretreatment with HIER in CC1 and proteolysis in P3. Laboratory based modifications substituting P3 with P1 or 2, or using single retrieval methods, caused a decrease of the pass-rate to 42%.
3) The use of the less successful clone MNF116.
Conclusive and of central importance, laboratories should apply an Ab that will work on their in-house IHC platform, calibrate the protocols correctly with focus on appropriate settings for the specific clone and verify the protocol according to the expected antigen level and pattern in the recommended tissue control materials (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. No staining should be seen in stromal cells in the liver. 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. Smooth muscle cells in vessels and in muscularis mucosae in esophagus will typically show a weak to moderate patchy cytoplasmic staining

reaction. As alternative to esophagus and concordant to the guidelines published by the International Ad Hoc Expert Committee[1] for positive tissue controls, tonsil can be used as positive tissue control but also as a negative tissue control. If used as negative tissue control, no staining reaction should be seen in lymphocytes, whereas dispersed interstitial reticulum cells with dendritic/reticular pattern can show a weak to moderate cytoplasmic staining reaction and must be accepted due to low level CK expression.

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.
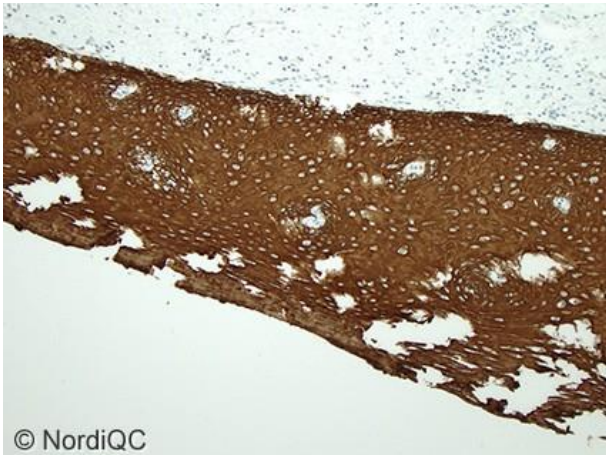
Fig. 1a (x100)
Optimal CK-PAN staining of the esophagus using the mAb clone cocktail AE1/AE3/PCK26 as RTU (Ventana), combined retrieval using HIER in CC1 (32 min.), proteolysis P3 (4 min.) and a 3-step multimer based detection system (OptiView).
All squamous epithelial cells show a strong and distinct cytoplasmic staining reaction.
Same protocol used in Figs. 2a-4a.
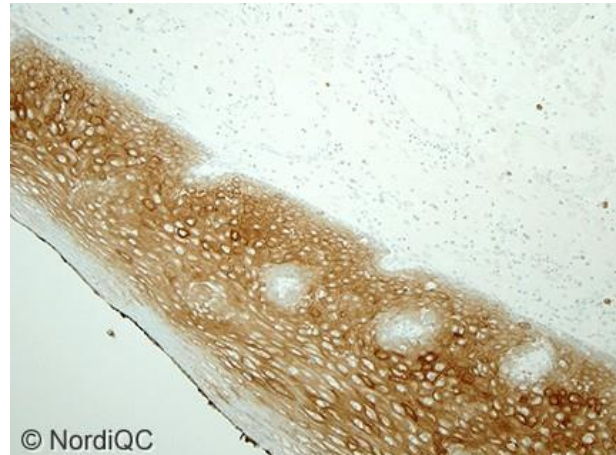
Fig. 1b (x100)
CK-PAN staining of the esophagus using an insufficient protocol with too low sensitivity based on the mAb clone cocktail AE1/AE3/PCK26 RTU (Ventana), but only with proteolysis in P1 as pre-treatment and UltraView as the detection system.
Same protocol used in Figs. 2b-4b.
The squamous epithelial cells show only a weak to moderate cytoplasmic staining reaction and are completely negative in the basal and upper layers of the squamous epithelium - compare to Fig 1.a (same field).
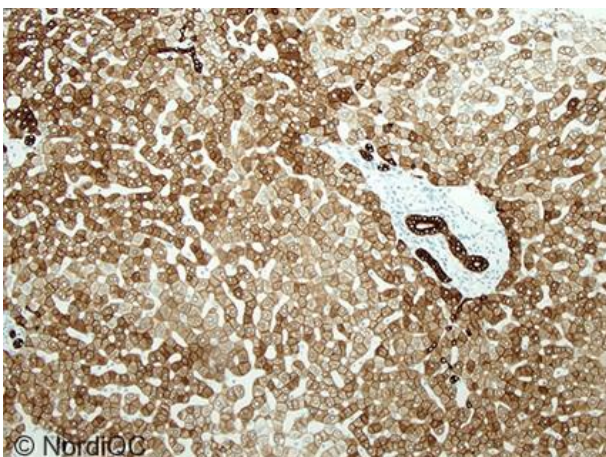

Fig. 2a (x100)
Optimal CK-PAN staining of the liver using same protocol as in Fig. 1a. The vast majority of hepatocytes show a moderate staining reaction (with membranous accentuation) while the columnar cells of the bile ducts display a strong cytoplasmic staining reaction.
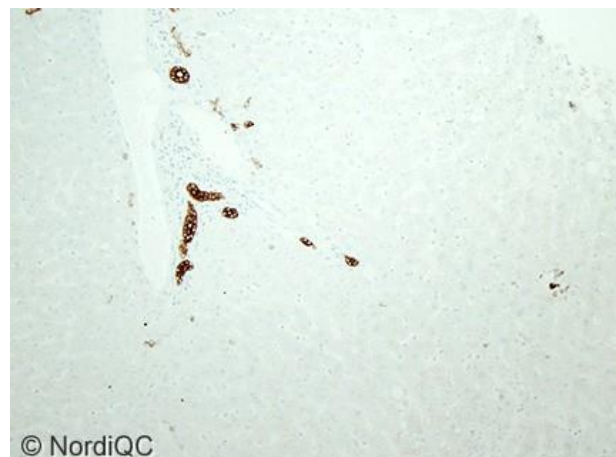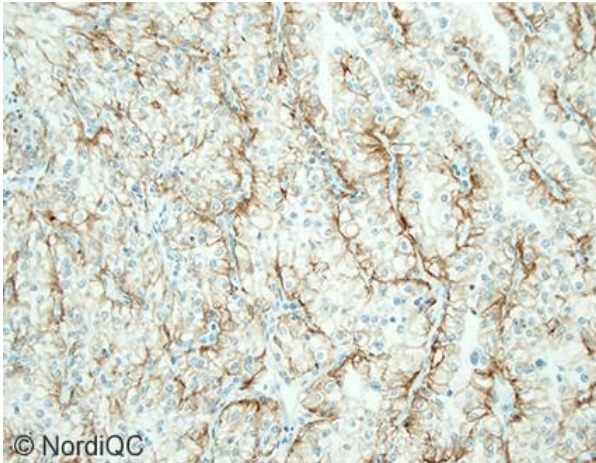

Fig. 2b (x100)
Insufficient CK-PAN staining of the liver using same protocol as in Fig. 1b.
Only epithelial cells of bile ducts are demonstrated due to high expression levels of CK-LMW (CK types 7, 8/18 and 19) whereas the hepatocytes are false negative (only express low antigen levels of CK 8/18).

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 weak to moderate, distinct cytoplasmic staining reaction with membranous accentuation.
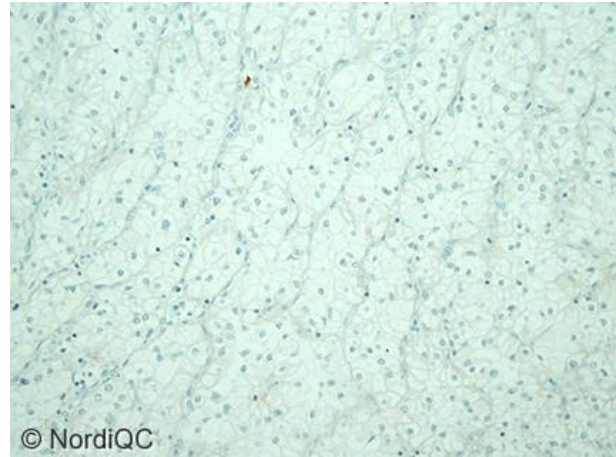
Fig. 3b (x200)
Insufficient CK-PAN staining of the CCRCC using same protocol as in Figs. 1b and 2b. The neoplastic cells are almost completely negative - same field as in Fig. 3a.
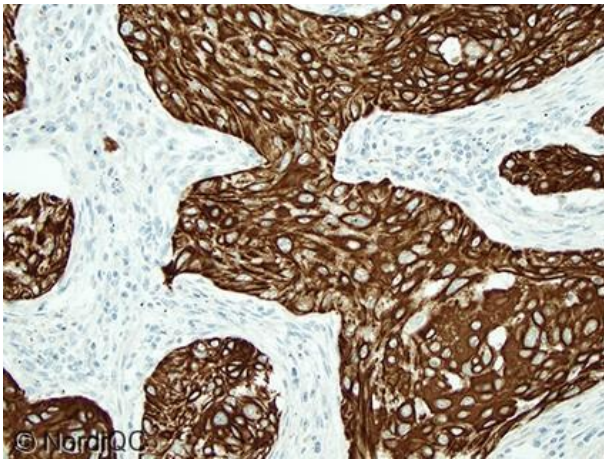
Fig. 4a (x200)
Optimal CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a moderate to strong and distinct cytoplasmic staining reaction.
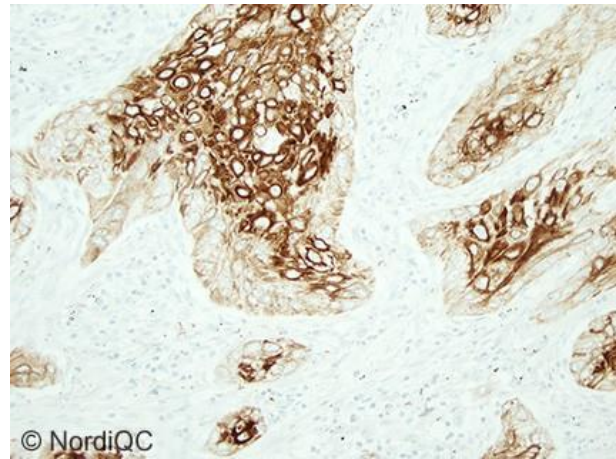
Fig. 4b (x200)
CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1b-3b – same field as in Fig. 4a. Virtually all squamous epithelial cells are demonstrated, but the intensity is reduced compared to the level expected (also compare with Fig. 3b, same protocol).
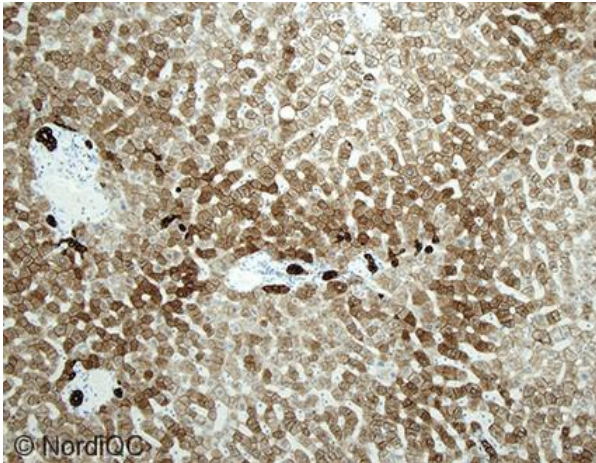
Fig. 5a (x100)
Optimal CK-PAN staining of the liver using the concentrated format of mAb clone cocktail AE1/AE3 (Dako/Agilent) on a Dako Autostainer, diluted 1:100 (20 min.), HIER in TRS High (10 min.) and a 3-step polymer based detection system (Envision Flex+, Dako). Same protocol for Fig. 6a.

Fig. 5b (x100)
Insufficient CK-PAN staining of the liver using the concentrated format of mAb clone cocktail AE1/AE3 (Dako/Agilent) on a Ventana BenchMark Ultra, diluted, 1:100 (32 min.), HIER in CC1 (36 min.) and a 2-step multimer based detection system (UltraView, Ventana). The hepatocytes are only weakly stained, even though the bile ducts show a strong staining reaction. The basic protocol settings are similar to the protocol used in Fig. 5-6a, but on a different platform. This shows the importance to carefully calibrate the individual antibody on different platforms and total level of the analytical sensitivity. Same protocol for Fig. 6.b.
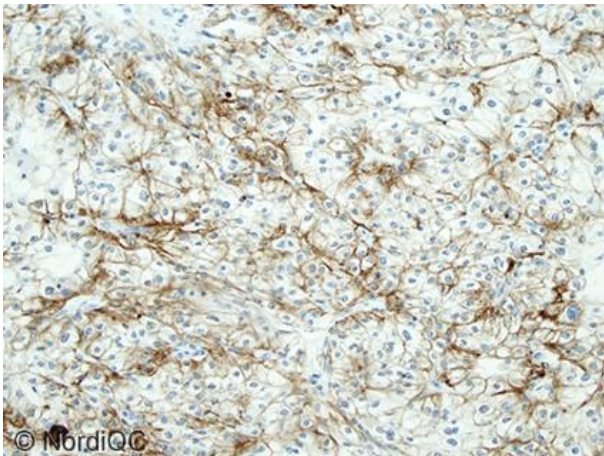

Fig. 6a (x200)
Optimal CK-PAN staining of the CCRCC using same protocol as Fig. 5a. This protocol provide a moderate, distinct cytoplasmic staining reaction with membranous accentuation of virtually all the neoplastic cells.
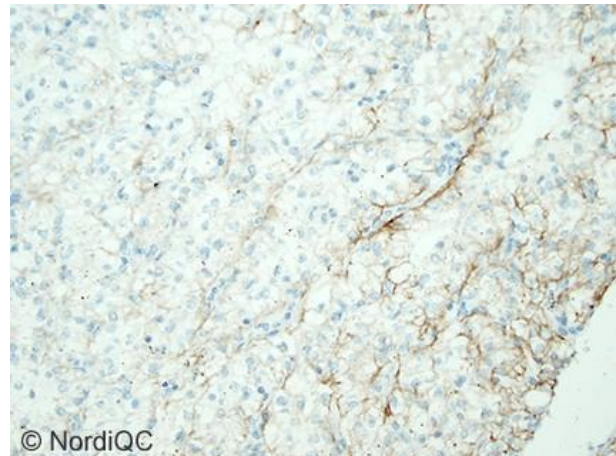

Fig. 6b (x200)
Insufficient CK-PAN staining of the CCRCC using the same protocol as Fig 5b. The vast majority of neoplastic cells are virtually all false negative and only dispersed cells at the edge of the tissue core show a weak to moderate membranous staining reaction.
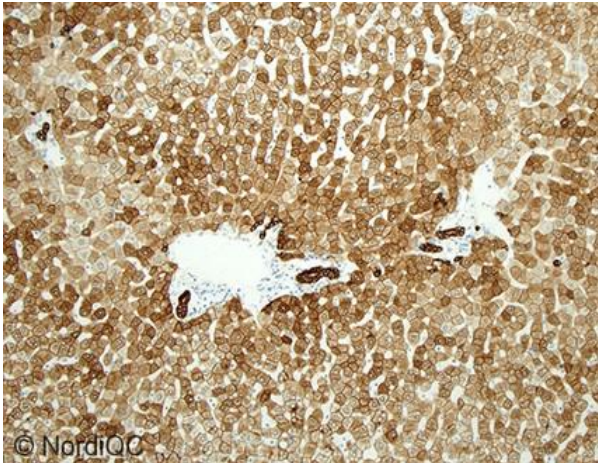
Fig. 7a (x100)
Optimal CK-PAN staining of the liver using the mAb clone BS5 (Nordic Biosite) on the Leica Bond platform. The antibody was diluted 1:200 (30 min.), HIER in BERS2 (Leica) for 20 min., and with a 3-layer polymer detection system (Bond Refine, Leica). Fig.´s 7a and 7b show the two most critical cores with liver and CCRCC, which are stained optimally.
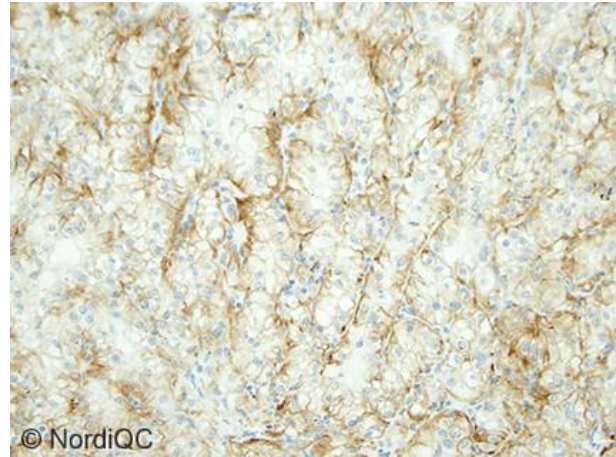In this assessment mAb clone BS5 was found to be superior to AE1/AE3 on the Leica Bond IHC platform.

Fig. 7b (x200)
Optimal CK-PAN staining of the CCRCC same protocol as Fig. 7a.
The neoplastic cells show the expected staining pattern.

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