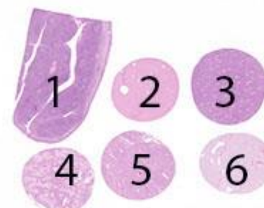
NordiQC

Assessment Run 71 2024
Pan Cytokeratin (CK-PAN)

Updated 19.07.24

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. Tonsil, 2. Liver, 3. Testicular diffuse large B-cell lymphoma (DLBCL), 4. Lung squamous cell carcinoma, 5. Lung adenocarcinoma, 6. Clear cell renal carcinoma (ccRCC).

All tissues were fixed in 10% neutral buffered formalin.

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 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 ccRCC.
- 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.

KEY POINTS FOR CK-PAN IMMUNOASSAYS
- The mAb clone **BS5** is recommendable on all main fully automated platforms.
- The choice of epitope retrieval must be tailored to the clone/cocktail for optimal performance.
- The mAb clones **MNF116** cannot be recommended due to inferior performance.
- The mAb clone cocktail **AE1/AE3** from Leica Biosystems both as concentrate and RTUs gave an inferior performance.

Participation

Number of laboratories registered for CK-PAN, run 71	453
Number of laboratories returning slides	412 (91%)

Results
412 laboratories participated in this assessment. Two laboratories used an inappropriate antibody and were not included in the following analysis. 287 (70%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3-4).

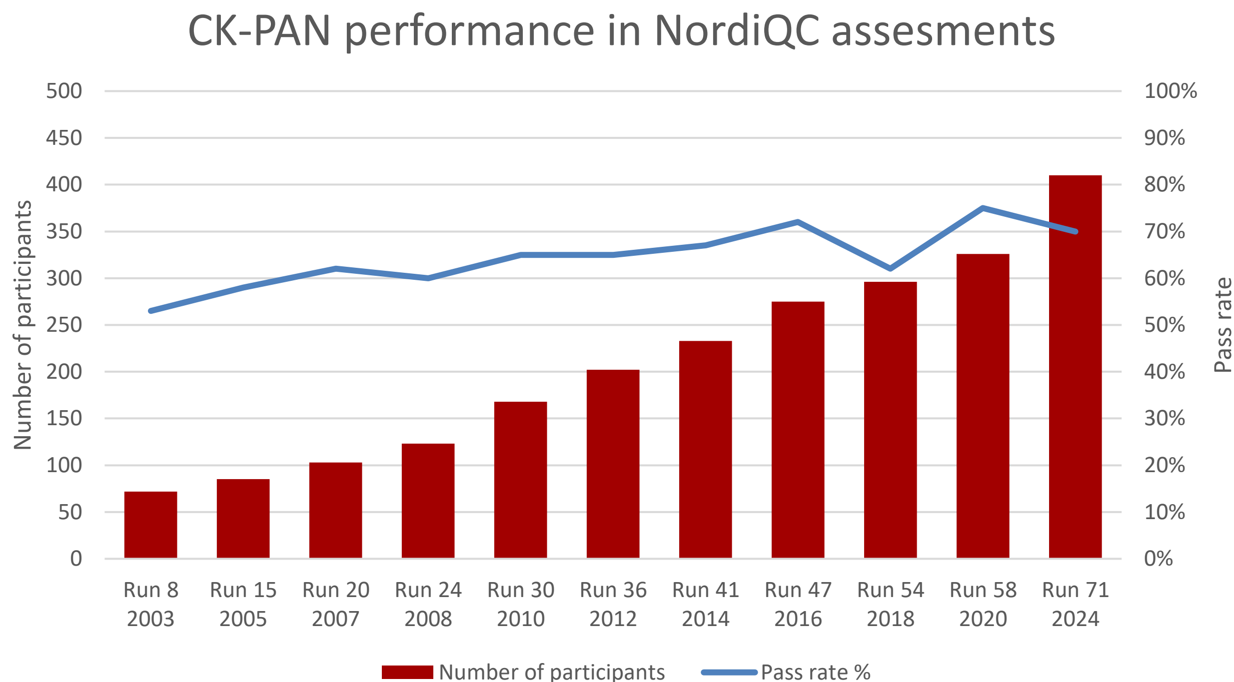
The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Inefficient HIER – too short efficient heating time and/or use of non-alkaline HIER buffers
- Inappropriate choice of epitope retrieval method
- Less successful primary antibodies, especially mAb clone MNF116
- Less successful performance of the mAb clone cocktail AE1/AE3 on the Leica Bond platforms

Accredited by DANAK under registration number 616 to proficiency testing

Performance history
This was the eleventh NordiQC assessment of CK-PAN. The overall pass rate decreased compared to the previous run 58, see Graph 1. The number of participants has increased significantly in this run, and first-time-participants obtained a pass rate of 55%, 35% optimal, compared to 78%, 58% optimal, obtained by laboratories also participating in previous run 58, being on par to the level seen in run 58.

Graph 1. **Proportion of sufficient results for CK-PAN in the eleven NordiQC run performed.**



Controls
Liver and tonsil 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. Concordant to the guidelines published by the International Ad Hoc Expert Committee[1] for positive tissue controls, tonsil can be used both as a positive and negative tissue control, as all squamous epithelial cells must show a moderate to strong cytoplasmic staining reaction, and 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.

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 performance of mAb clone cocktail AE1/AE3 was highly impacted on the supplier and IHC platform applied. In this assessment both the concentrated format and corresponding Ready-To-Use (RTU) systems from Leica Biosystems gave an inferior pass rate. The mAb clone BS5 was found to be more successful on the Bond platform. The RTU systems from Dako/Agilent based on mAb clone cocktail AE1/AE3 were in this assessment most successful and provided high proportions of sufficient and optimal results.

[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.

Table 1a. **Overall results for CK-PAN, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	124	58	23	25	18	65%	47%
Ready-To-Use antibodies	286	147	59	44	36	72%	51%
Total	410	205	82	69	54		
Proportion		50%	20%	17%	13%	70%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CK-PAN, run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone cocktail **AE1/AE3**	4	Cell Marque	1	-	1	2		
	63	Dako/Agilent	37	11	8	7	76%	59%
	1	DCS Diagnostics	-	1	-	-		
	1	Diagnostic Biosystems	-	-	-	1		
	2	Epredia	-	1	1	-		
	1	GenomeMe	-	1	-	-		
	9	Leica Biosystems	-	1	5	3	11%	0%
	1	Zeta Corporation	-	-	-	1		
	3	Zytomed Systems	-	1	2	-		
mAb clone cocktail **AE1/AE3/5D3**	1	Abcam	-	-	1	-	-	-
	1	Biocare Medical	-	-	1	-	-	-
	4	Zytomed Systems	1	1	-	2	-	-
mAb clone cocktail **PAN CK (Ab C2562)**	1	Sigma Aldrich	1	-	-	-	-	-
mAb clone **BS5**	2	Monosan	1	1	-	-	-	-
	17	Nordic Biosite	13	4	-	-	100%	76%
mAb clone **Lu-5**	1	BMA Biomedicals	-	-	1	-	-	-
mAb clone **MNF116**	6	Dako/Agilent	1	-	3	2		
mAb clone **OSCAR**	1	Cell Marque	1	-	-	-	-	-
rmAb clone **QR124**	1	Quartett	1	-	-	-	-	-
"Laboratory made" antibody cocktails							Suff.[1]	OR.[2]
mAb clone cocktail **AE1/AE3/5D3**	2	Leica Biosystems	-	1	1	-	-	-
Ab clone cocktail **AE1/AE3/CAM5.2**	1	Unknown	-	-	1	-	-	-
mAb clone cocktail **AE1/AE3/BS5**	1	Leica Biostems/Monosan	1	-	-	-	-	-
Total	124		58	23	25	18		
Proportion			47%	18%	20%	14%	65%	

1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).

Table 1c. **Ready-To.Use antibodies and assessment marks for CK-PAN, run 71**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
mAb clone cocktail **AE1/AE3 IR/IS053 (VRPS)[3]**	12	Dako/Agilent	10	2	-	-	100%	83%
mAb clone cocktail **AE1/AE3 IR/IS053 (LMPS)[4]**	15	Dako/Agilent	5	4	4	2	60%	33%
mAb clone cocktail **AE1/AE3 GA053 (VRPS)[3]**	47	Dako/Agilent	40	5	2	-	96%	85%
mAb clone cocktail **AE1/AE3 GA053 (LMPS)[4]**	31	Dako/Agilent	27	2	2	-	94%	87%
mAb clone cocktail **AE1/AE3 313M-XX**	3	Cell Marque	-	1	-	2	-	-
mAb clone cocktail **AE1/AE3 MAD 001000QD**	2	Master Diagnostica	-	-	2	-	-	-
mAb clone cocktail **AE1/AE3 PA0909 (LMPS)[4]**	7	Leica Biosystems	-	-	-	7	0%	0%
mAb clone cocktail **AE1/AE3 PA0094 (VRPS)[3]**	9	Leica Biosystems	-	3	6	-	33%	0%
mAb clone cocktail **AE1/AE3 PA0094 (LMPS)[4]**	7	Leica Biosystems	2	3	1	1	71%	29%
mAb clone cocktail **AE1/AE3 PDM072**	3	Diagnostic Biosystems	-	-	-	3	-	-
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (VRPS)[3]**	25	Ventana/Roche	5	17	2	1	88%	20%
mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (LMPS)[4]**	114	Ventana/Roche	52	21	22	19	64%	46%
mAb clone cocktail **AE1/AE3 GM351502**	1	Gene Tech	1	-	-	-	-	-
mAb clone cocktail **AE1/AE3 AM071-XXM**	1	Biogenex	-	-	1	-	-	-
mAb clone cocktail **AE1/AE3/DC10 8309-C010**	3	Sakura Finetek	1	1	1	-	-	-
mAb clone cocktail **AE1/AE3/5D3 PM162AA/H**	1	Biocare Medical	-	-	1	-	-	-
mAb clone **DA040 MMB1A088**	1	Dartmon	1	-	-	-	-	-
mAb clone **MX005 MAB-0671**	1	Fuzhou Maixin	1	-	-	-	-	-
m&rmAb clone cocktail **B22.1/B23.1 EP24/EP67 MAD-000680QD**	1	Master Diagnostica	-	-	-	1	-	-
rmAb clone cocktail **BP6051/BP6058 BX50143**	1	Biolynx	1	-	-	-	-	-
Ab clone **830F6E7 PA125**	1	Abcarta	1	-	-	-	-	-
Total	286		147	59	44	36		
Proportion			51%	21%	15%	13%	72%	

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

Detailed analysis of CK-PAN, Run 71
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/Agilent) (9/10)*, TRS pH 6,1 (3-in-1) (Dako/Agilent) (1/1) Cell Conditioning 1 (CC1, Ventana/Roche) (26/52) or Tris-EDTA/EGTA pH 9 (1/3) as retrieval buffer. The mAb was diluted in the range of 1:40-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 51 of 68 (75%) 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/Agilent) (1/3), Tris/EDTA pH 9 (4/4), CC1 (Ventana/Roche) (4/5), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (4/8) and Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (2/2).The mAb was diluted in the range of 1:100-1:800 depending on the total sensitivity of the protocol employed. Using these settings 18/18 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CK-PAN for the most commonly used antibody concentrates on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	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**	3/1**	1/1	6/6 (100%)	0/1	26/46 (57%)	-	1/6 (17%)	0/1
mAb clone **BS5**	-	-	4/4	-	4/5 (80%)	-	4/8 (50%)	2/2

* 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.
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra plus
3) Bond III, Max

Ready-To-Use antibodies and corresponding systems
mAb clone cocktail **AE1/AE3**, product no. **IR/IS053**, Dako/Agilent, Autostainer/Autostainer Link 48+:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20-40 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings, 19 of 20 (95%) laboratories produced a sufficient staining result.

mAb clone cocktail **AE1/AE3**, product no. **GA053**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C) and 10-30 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) or EnVision Flex+ (GV800/GV823/GV821) as detection system. Using these protocol settings, 71 of 73 (97%) laboratories produced a sufficient staining result.

mAb clone cocktail **AE1/AE3/PCK26,** product no. **760-2135/2595**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra plus:
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.), 4-32 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, 79 of 85 (93%) laboratories produced a sufficient staining result. 19 laboratories used HIER as single pretreatment, 14 (74%) produced a sufficient staining result.

mAb clone cocktail **AE1/AE3**, product no, **PA0094**, Leica Biosystems, Bond III:
Two protocols with optimal results were based on HIER using BERS2 for 15-20 min. and 15 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Only the two laboratories used these protocol settings.

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 Autostainer mAb AE1/AE3 **IR/IS053**	100% (12/12)	83% (10/12)	78% (7/9)	33% (3/9)
Dako Omnis mAb AE1/AE3 **GA053**	96% (45/47)	85% (40/47)	93% (27/29)	86% (25/29)
Leica Bond mAb AE1/AE3 **PA0094**	33% (3/9)	0% (0/9)	67% (4/6)	33% (2/6)
Ventana BenchMark mAb AE1/AE3/PCK26 **760-2135/2595**	88% (22/25)	20% (5/25)	64% (73/114)	46% (52/114)

* 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, being observed in 91% (112/123) of the insufficient staining results. 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 ccRCC was more difficult and was only obtained by protocols with appropriate protocol settings. The remaining insufficient staining results were caused by e.g. false positive staining reaction, poor signal-to-noise ratio or impaired morphology.
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-PAN This correlation, observed in NordiQC CK-PAN assessments, is summarized in Table 4 for the latest three assessment runs.

Table 4. **Pass rates for antibody cocktails combined with epitope retrieval methods in the last three NordiQC runs**

Pass rate for compiled data from run 54, 58 and 71								
	Total		**HIER**		**Proteolysis**		**HIER + proteolysis**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb AE1/AE3	609	449 (74%)	579	442 (76%)	12	1 (8%)	9	1 (11%)
mAb AE1/AE3/5D3	21	12 (57%)	21	12 (57%)	-	-	-	-
mAb AE1/AE3/PCK26	323	208 (64%)	39	22 (56%)	28	3 (11%)	254	181 (71%)
mAb MNF116	27	2 (7%)	18	0 (0%)	7	2 (29%)	1	0
mAb BS5	38	36 (95%)	38	36 (95%)	-	-	-	-

The data clearly stresses that the choice of clone and epitope retrieval has significant impact on the staining result. For the most widely used Ab clone cocktail **AE1/AE3**, the overall pass rate in the 3 successive NordiQC runs was 74%. Using HIER, a pass rate of 76% was obtained, significantly higher than the pass rate of 8% 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/Roche) followed by proteolysis, provided a pass rate of 71%, compared to 56% and 11% using either HIER or proteolysis, respectively, as single retrieval method.
The mAb clone **MNF116** has in these consecutive runs provided an inferior overall performance compared to the 4 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 giving the highest pass rate of 95% in the last three runs.

30% (124 of 410) of the participants used a laboratory developed (LD) assay and the mAb clone cocktails **AE1/AE3** and **AE1/AE3/5D3** and the mAb clone **BS5** could be used to obtain an optimal staining result for CK-PAN (see Table 1b).
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/AE3** gave an overall pass rate of 64% (54 of 85). As shown in Table 2, optimal results could be obtained on all main IHC platforms. However, an inferior performance was observed when applied on the Leica Bond platform compared to performance on the other platforms, despite similar protocol settings applied. In addition to the impact of performance of the mAb clone cocktail **AE1/AE3** on different IHC platforms, it was also observed that the origin of the mAb cocktail influenced the pass rate. E.g. if the IHC assays were based on the concentrated format of **AE1/AE3** from Dako/Agilent a pass rate of 76% was observed, compared to 11% when using the product from Leica Biosystems. No definitive explanation for the difference in performance has been identified but might be related to the relative mixture or concentration of the two clones AE1 and AE3 in the final commercial products from the different suppliers.

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 (19 of 19) were assessed as sufficient (see Table 1b). This Ab might be an alternative to the more challenging Abs (e.g. **MNF116** or **AE1/AE3**) on the Leica Bond platforms where 6/10 IHC assays based on mAb clone BS5 provided an optimal result.

70% (286 of 410) of the laboratories used a Ready-To-Use (RTU) format for detection of CK-PAN.
In this assessment, the Dako/Agilent RTU systems **IR/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, vendor recommended protocol settings **IR/IS053** gave a pass rate of 100% of which 83% were assessed as optimal. If using the Omnis **GA053**, a pass rate of 96% was obtained, 85% optimal. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) also provided high proportion of sufficient and optimal results on the Omnis platform.

Fifteen laboratories used the RTU **PA0094** from Leica Biosystems, based on the mAb clone cocktail AE1/AE3, giving an inferior pass rate of 33% if using vendor recommended protocol settings, based on HIER in BERS1. If using HIER in an alkaline buffer as BERS2, a pass rate of 60% (3 of 5) was seen.
Six laboratories used **PA0909** also based on the mAb clone cocktail AE1/AE3. All participants modified the protocol settings, however all results were assessed as poor, despite applying similar protocol settings giving sufficient results with PA0094.
As for the corresponding concentrated Ab cocktail from Leica Biosystems, the detection of CK-LMW type 8 being recognized by mAb clone AE3 seems to be challenging for the Leica Biosystems mAb clone cocktail AE1/AE3 compared to analogous products from e.g. Dako/Agilent. The mAb clone cocktail AE1/AE1 only detects CK8 (AE3), whereas the other main primary CK LMW type 18 is not detected by either AE1 or AE3.

The Ventana RTU system **760-2135/2595** was the most widely used IHC assay, being used by 139 participants and typically by laboratory modified protocol settings as shown in Table 1c 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 subsequent proteolysis in P3, a pass rate of 88% was observed, 20% being optimal. In general, the pass rate and proportion of optimal results was reduced for laboratories modifying the protocol settings being 64% and 46% respectively. Less successful modifications were especially related to e.g. substitution of P3 with P1 or P2, or use of proteolysis as single retrieval method.

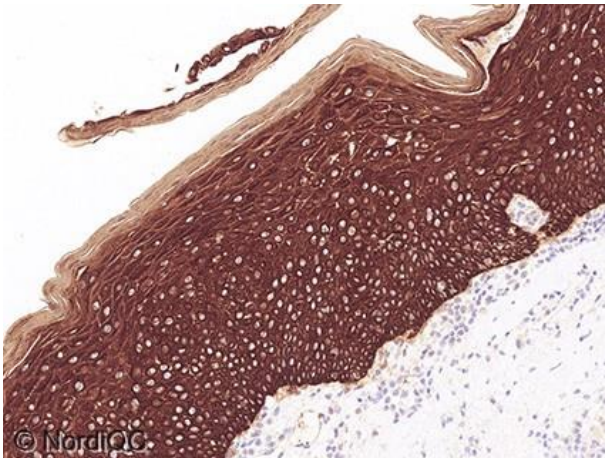


Fig. 1a
Optimal CK-PAN staining of the tonsil using the mAb clone cocktail AE1/AE3 as RTU (Dako/Agilent), using HIER in TRS High pH (30 min.) and a 2-step detection system (EnVision Flex).
All squamous epithelial cells show a strong and distinct cytoplasmic staining reaction.
Same protocol used in Figs. 2a-6a.

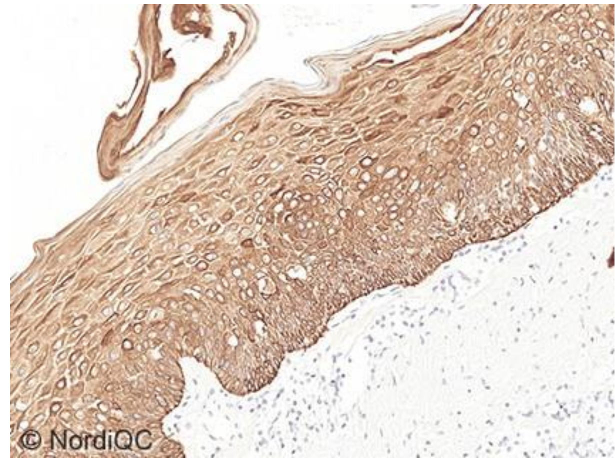


Fig. 1b

CK-PAN staining of the tonsil using an insufficient protocol with too low sensitivity based on the mAb clone cocktail AE1/AE3/PCK26 RTU (Ventana) using proteolysis in P1 as pre-treatment and UltraView as the detection system. Same protocol used in Figs. 2b-6b.
The intensity of the staining reaction in the squamous epithelial cells is significantly reduced and only show a weak to moderate cytoplasmic staining reaction - compare with Fig 1.a (same field) and Figs. 2b-6b, same protocol.

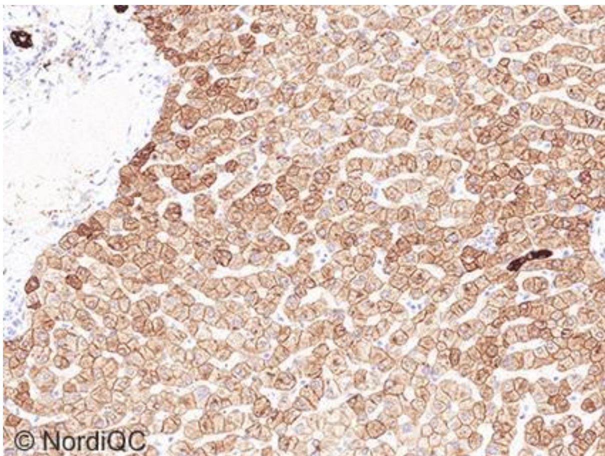


Fig. 2a
Optimal CK-PAN staining of the liver using same protocol as in Fig. 1a.
The vast majority of hepatocytes show a moderate staining reaction (with membranous accentuation) while the columnar cells of the bile ducts display a strong cytoplasmic staining reaction.

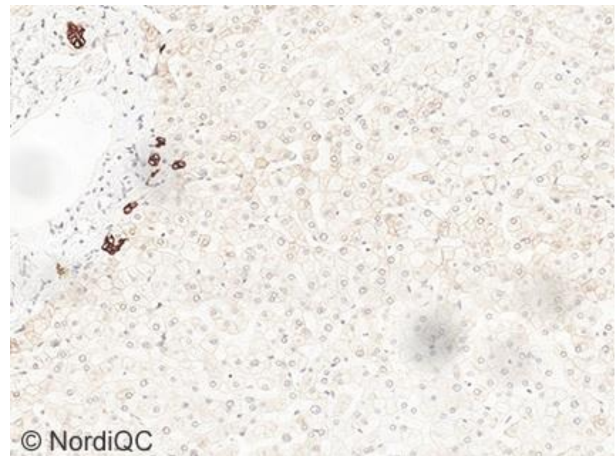


Fig. 2b
Insufficient CK-PAN staining of the liver using same protocol as in Fig. 1b.
Only epithelial cells of bile ducts are demonstrated due to high expression levels of CK-LMW (CK types 7, 8/18 and 19) whereas the hepatocytes are false negative (only express low antigen levels of CK 8/18).

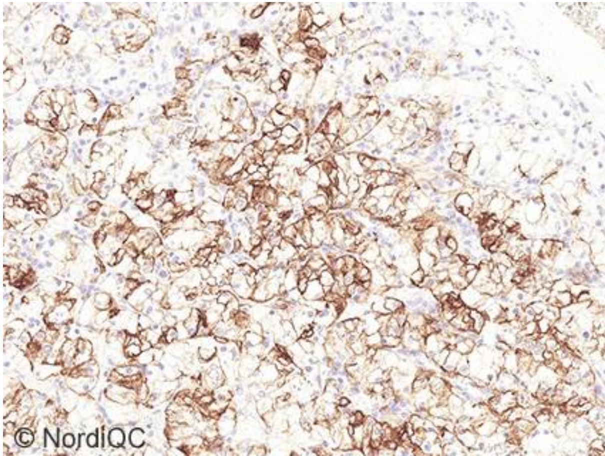


Fig. 3a
Optimal CK-PAN staining of the ccRCC using same protocol as in Figs. 1a and 2a. The vast majority of neoplastic cells display a weak to moderate, distinct cytoplasmic staining reaction with membranous accentuation.

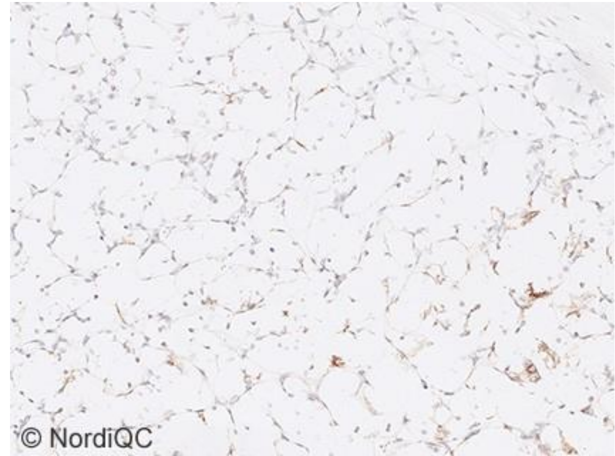


Fig. 3b
Insufficient CK-PAN staining of the ccRCC using same protocol as in Figs. 1b and 2b. The neoplastic cells are almost completely negative. Also, the morphology is impaired due to the proteolytic pretreatment - same field as in Fig. 3a.

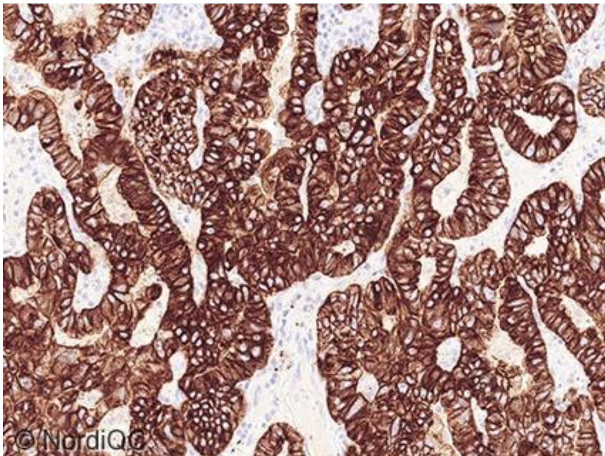


Fig. 4a
Optimal CK-PAN staining of the lung adenocarcinoma using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a moderate to strong and distinct cytoplasmic staining reaction.

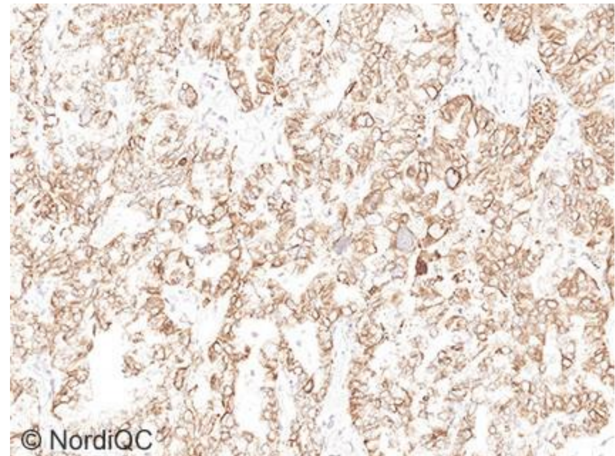


Fig. 4b
CK-PAN staining of the lung adenocarcinoma using same protocol as in Figs. 1b-3b – same field as in Fig. 4a. Virtually all neoplastic cells are demonstrated, but the intensity is reduced compared to the level expected (also compare with Fig. 3b, same protocol).

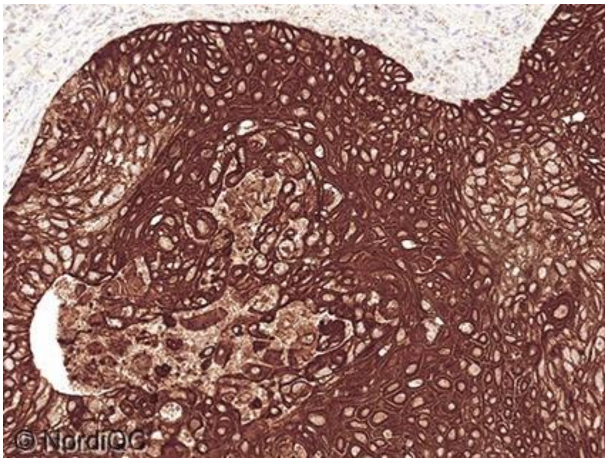


Fig. 5a
Optimal CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1a-4a. Virtually all neoplastic cells show a moderate to strong and distinct cytoplasmic staining reaction.

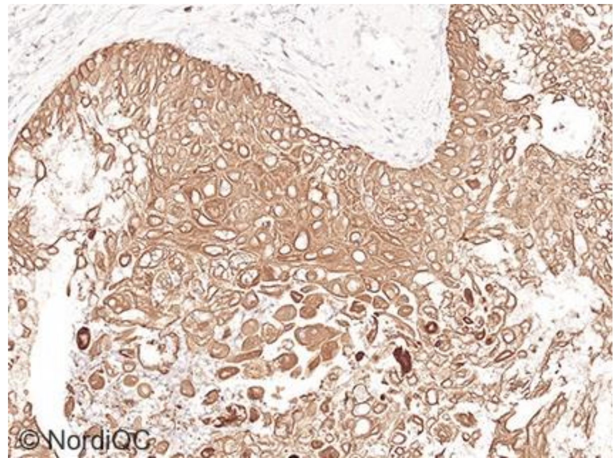


Fig. 5b
CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1b-4b – same field as in Fig. 5a. Virtually all neoplastic cells are demonstrated, but the intensity is reduced compared to the level expected (also compare with Fig. 3b, same protocol).

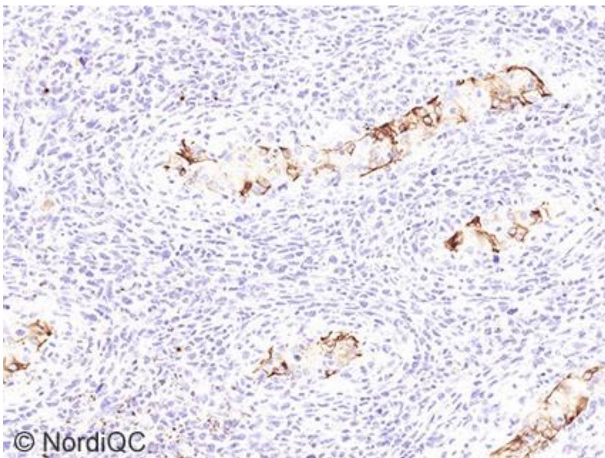


Fig. 6a
Optimal CK-PAN staining of the testicular DLBCL using same protocol as Fig. 1a-5a. The neoplastic cells are negative, whereas the remnants of normal germ cells are positive, serving as an internal positive control.

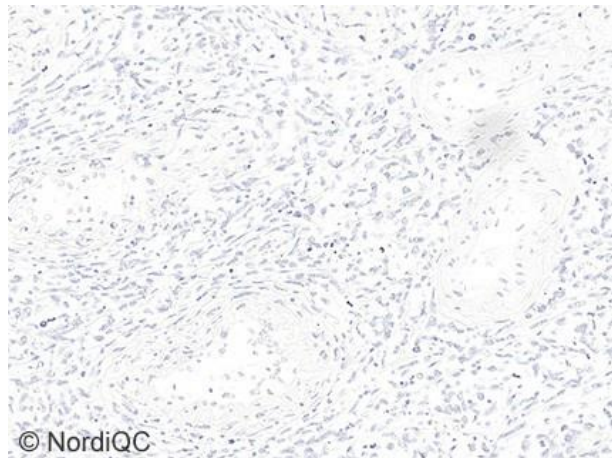


Fig. 6b
Insufficient CK-PAN staining of the testicular DLBCL using the same protocol as Fig 1b-5b. Both neoplastic and normal cells are virtually all negative.

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Version	Description of change and reason	Date	Authorized by
2	Table 1a has been updated as wrong numbers was written in version 1.	19.07.24	HLK/SN