Assessment Run 20 2007

Pan cytokeratin (CK-Pan)

The slide to be stained for CK-Pan comprised:
1. Appendix, 2. Liver, 3. Oesophagus, 4. Renal cell carcinoma, 5. Squamous cell carcinoma (uterine cervix), 6. Signet ring cell carcinoma (stomach).
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK-Pan staining as optimal included:

- A strong, distinct cytoplasmic reaction of virtually all the appendiceal enterocytes (including crypt basis) and the the bile ducts, and at least a moderate, predominantly membranous reaction of the large majority of hepatocytes.
- A strong, distinct cytoplasmic reaction of the squamous epithelial cells throughout all cell layers in the oesophagus (a negative reaction in the basal cells is accepted with mAb clone KL1).
- A strong, distinct cytoplasmic reaction in virtually all the neoplastic cells of the squamous cell carcinoma and the signet cell carcinoma.
- An at least moderate, distinct cytoplasmic in the majority of tumour cells of the renal cell carcinoma.

105 laboratories submitted stains. Of these 2 used an inappropriate antibody (CK7 and CK-HMW). Of the remaining 103 laboratories 28 achieved optimal marks (27 %), 36 good (35 %), 29 borderline (28 %) and 10 poor marks (10 %).

The following Abs were used:
mAb clone cocktail **AE1/AE3** (Dako, n=51; Zymed, n=4; BioGenex, n=1; Chemicon, n=1; Linaris, n=1; NeoMarkers, n=2)
mAb clone **MNF116** (Dako, n=12)
mAb clone **KL1** (Immmunotech, n=7; DPC, n=1; Serotec, n=1)
mAb clone cocktail **AE1/AE3/PCK26 (RTU)** (Ventana, n=9)
mAb clone cocktail **PAN-CK Ab-2** (NeoMarkers, n=4)
mAb clone cocktail **AE1/AE3 + 5D3** (BioCare Medical, n=3)
mAb clone cocktail **5D3, LP34** (Novocastra, n=2)
mAb clone **Lu-5** (NeoMarkers, n=2)
mAb clone cocktail **DC10 + 34βE12** (in-house, n=1)
pAb **Z0622** (Dako, n=1)

Optimal staining for **CK-Pan** in this assessment was obtained with the mAb clone cocktail **AE1/AE3** (19 out of 60), the mAb clone cocktail **AE1/AE3 + 5D3** (3 out of 3), the mAb clone cocktail **PAN-CK Cocktail Ab-2** (3 out of 4), the mAb clone **KL1** (1 out of 9) and the mAb clone **MNF116** (1 out of 12).

AE1/AE3: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) with either Tris-EDTA/EGTA pH 9, EDTA pH 9, Cell Conditioning1 (BenchMark, Ventana) or PT Module Buffer (LabVision) as the buffer. The mAb was typically diluted in the range of 1:50 – 1:150 depending on the total sensitivity of the protocol employed. With these settings 33 out of 39 (85 %) laboratories produced a sufficient staining (optimal or good).

AE1/AE3 + 5D3: The protocols giving an optimal result were all based on HIER in Tris-EDTA/EGTA pH 9. The Ab was diluted in the range of 1:200 - 400 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) antibody.

PAN-CK Ab2: The protocols giving an optimal result were either based on HIER with Tris-EDTA/EGTA pH 9 as the buffer, or enzymatic pre-treatment with Protease 1 (Ventana). The Ab was diluted in the range of 1:250 – 300, when HIER was used as pre-treatment, and 1:50, when enzymatic pre-treatment was used. With these settings all of 3 laboratories produced an optimal staining.

KL1: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9. The Ab was diluted 1:100. With these settings 2 out of 3 laboratories produced a sufficient staining.

MNF116: The protocol giving an optimal result was based on enzymatic pre-treatment with Proteinase K (Dako) and the Ab was diluted 1:200. Using these settings all of 4 laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were (often in combination):
- Inappropriate epitope retrieval (i.e., proteolysis for the mAb clone AE1/AE3; HIER for MNF116)
- Insufficient HIER
- Inappropriate choice of primary Ab
- Too low concentration of the primary antibody.

In this assessment (and in concordance with the CK-Pan assessment in run 15) the prevalent feature of an insufficient staining was a too weak or negative reaction of cells/structures supposed to be demonstrated. The majority of the laboratories were able to demonstrate CK in the benign epithelial cells of the appendix and the bile ducts as well as in the signet cell carcinoma. However, the demonstration of CK in the renal cell carcinoma was much more difficult and only seen for protocols with a high sensitivity, i.e. as obtained with mAb clone cocktails AE1/AE3 and AE1/AE3 + 5D3 as well as clone KL1 - all with HIER.
In accordance with previous assessments of CK-Pan, liver was a reliable positive control, as all laboratories that could demonstrate CK in the renal cell carcinoma also demonstrated CK in the hepatocytes.
In general the majority of the laboratories could demonstrate CK in the squamous epithelial cells of the oesophagus and the cervical squamous cell carcinoma. However, laboratories using the mAb clone KL1 in a too low concentration and/or insufficient HIER had a too weak staining reaction in these structures.

Table 1. **Most frequently used Abs and epitope retrieval methods used for CK-Pan in runs 15 and 20 related to the performance.**

	HIER		**Proteolysis**	
Clone	Protocols	Sufficient	Protocols	Sufficient
AE1/AE3	96	69 (72%)	12	2 (17%)
AE1/AE3 + PCK26 (RTU)	3	0 (0%)	12	0 (0%)
AE1/AE3 + 5D3	6	6 (100%)	0	-
MNF116	8	3 (38%)	12	11 (92 %)
KL1	19	13 (68%)	0	-
Ab2	4	4 (100%)	2	1 (50%)
5D3/LP34	4	0 (0%)	1	0 (0%)

As shown in Table 1, the results for the most used mAb clone cocktail AE1/AE3 are much better with HIER than with proteolytic pretreatment. The combination of AE1/AE3 + PCK26 gave insufficient results in all of 15 laboratories. When HIER was used, the sensitivity was high but at the same time an unwanted cross reaction with smooth muscle cells occurred.
In contrast to the AE1/AE3 combinations and KL1, MNF116 must be used with proteolytic pre-treatment to give a sufficient staining reaction.

Overall, the proportion of sufficient stains increased from 58% in run 15 to 62% in the current run. The main reason for the low improvement rate appears to be reluctance to change the protocols according to the recommendations from NordiQC (particularly inappropriate epitope retrieval and too low concentration of the Ab).

When CK-Pan was assessed in run 15 2005, 85 laboratories participated. Out of these 36 laboratories (42%) obtained an insufficient mark. Each was given a specific recommendation to improve their protocol. 30 submitted a new CK-Pan stain in run 20. 13 of these followed the recommendation, of which 9 improved to good or optimal (69 %). 17 laboratories did not follow the recommendation, and only 3 of these (18 %) obtained a sufficient staining in run 20.

Conclusion
The mAb clones **Ab-2**, **AE1/AE3**, **AE1/AE3 + 5D3**, and **KL1** were the most successful markers for CK-Pan when using HIER. The mAb clone **MNF116** could be for CK-Pan with proteolytic pre-treatment. Liver is appropriate control tissue: almost all liver cells should show a distinct cytoplasmic staining with enhancement along the cell membranes. Appendix can not be recommended (as this tissue not will necessarily identify an

insufficient protocol, due to the higher CK expression in the enterocytes).
The laboratories obtaining insufficient marks are strongly recommended to follow the NordiQC advice for improvement.

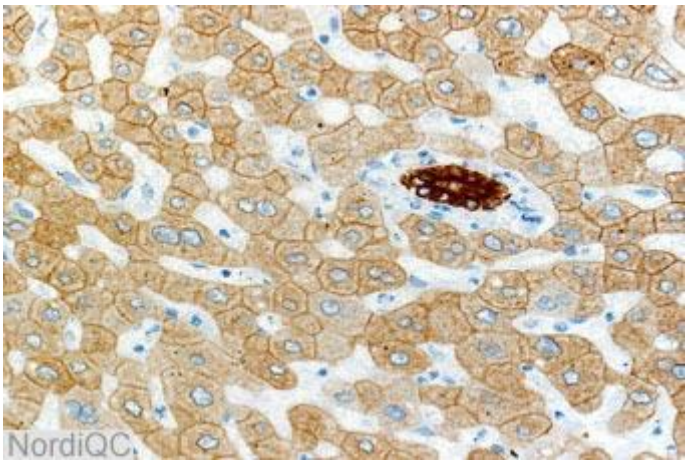

Fig. 1a
Optimal staining for CK-Pan of the liver. The majority of the hepatocytes show a distinct, moderate, predominantly membranous reaction. The bile duct is strongly stained.

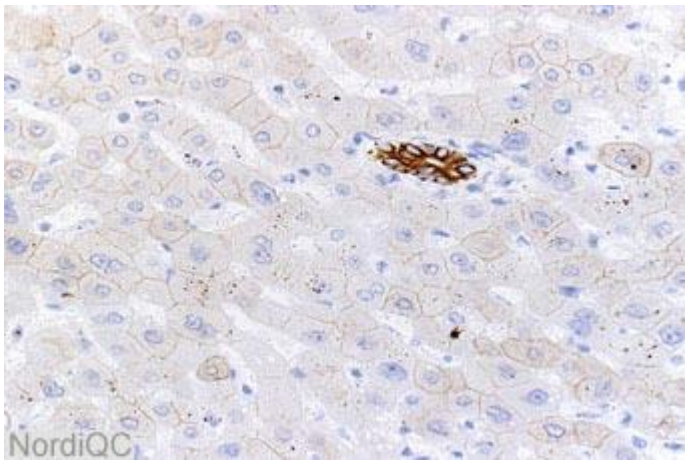

Fig. 1b
Staining for CK-Pan of the liver using an insufficient protocol (same field as in Fig. 1a.). Only the bile duct is stained, while the hepatocytes are virtually unstained. The antibody is too dilute.

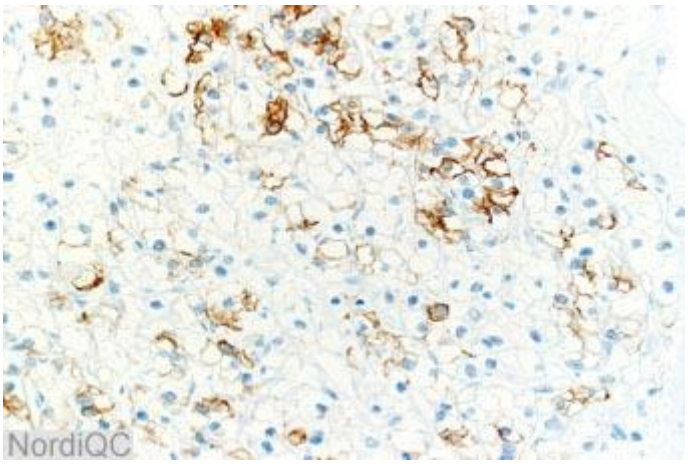

Fig. 2a
Optimal staining for CK-Pan of the renal cell carcinoma. About half of the neoplastic cells are moderately or strongly stained (same protocol as in Fig. 1a).

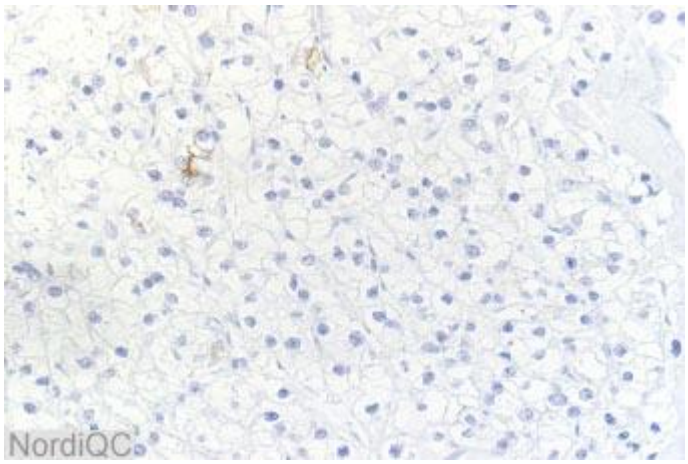

Fig. 2b
Insufficient staining for CK-Pan of the renal cell carcinoma (same field as in Fig 2a). Only scattered neoplastic cells are weakly positive while the majority are totally negative (same protocol as in Fig. 1b).

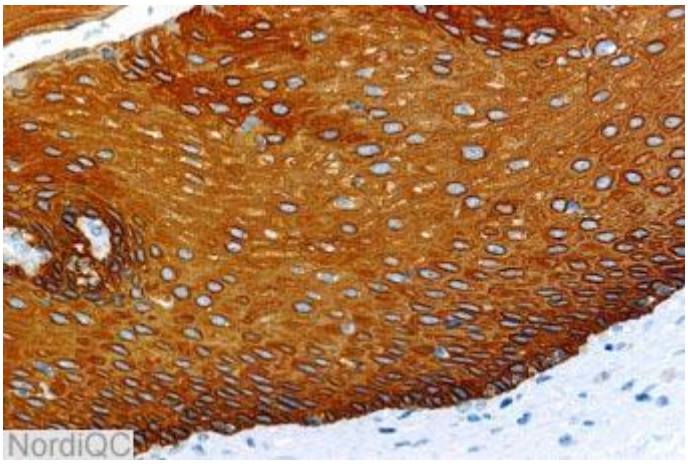

Fig. 3a
Optimal staining for CK-Pan in the oesophagus. All the squamous epithelial cells are stained (same protocol as in Fig. 1a).

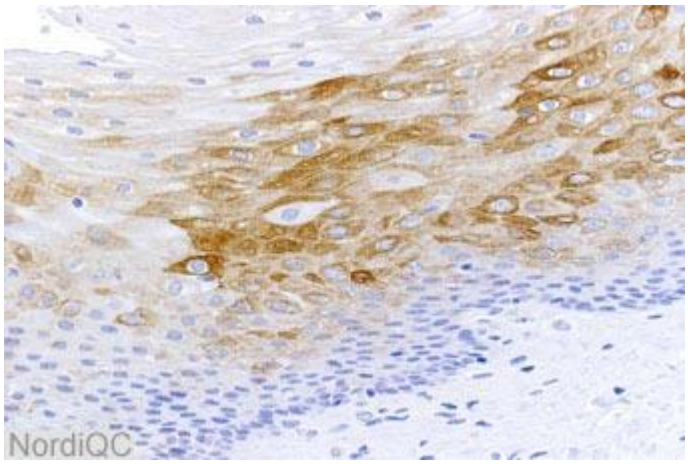

Fig. 3b
Staining for CK-Pan in the oesophagus using an insufficient protocol (same field as in Fig. 3a). The intermediate squamous epithelial cells are moderately stained while the others are unstained (same protocol as in Fig. 1b).

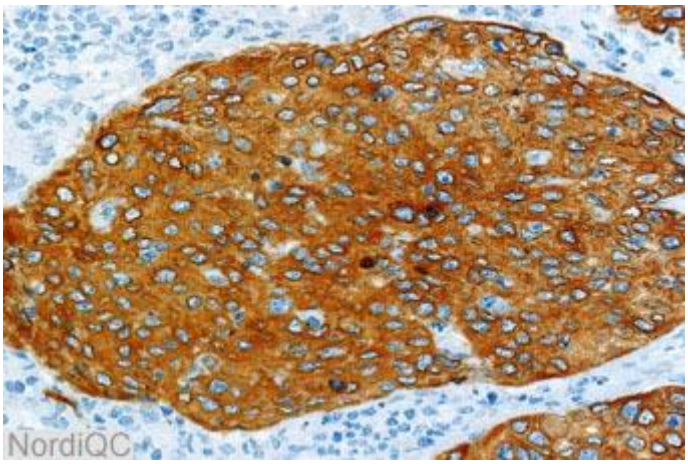

Fig. 4a
Optimal staining for CK-Pan of the squamous cell carcinoma. All the tumour cells show a strong cytoplasmic reaction (same protocol as in Fig. 1a).

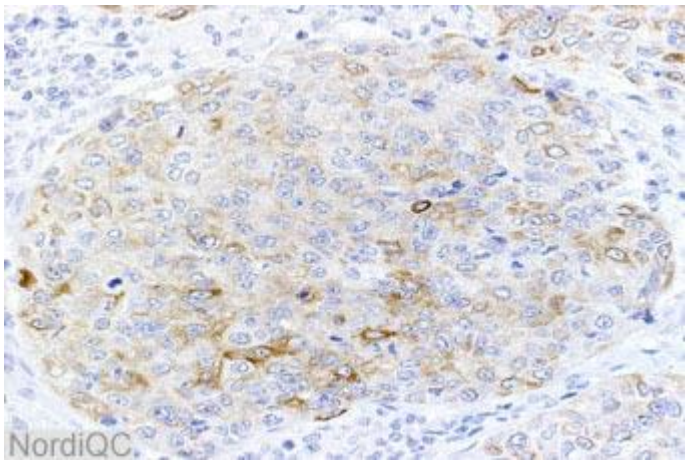

Fig. 4b
Insufficient staining for CK-Pan of the squamous cell carcinoma (same field as in Fig 4a). Scattered neoplastic cells are weakly positive while the majority are negative (same protocol as in Fig. 1b).

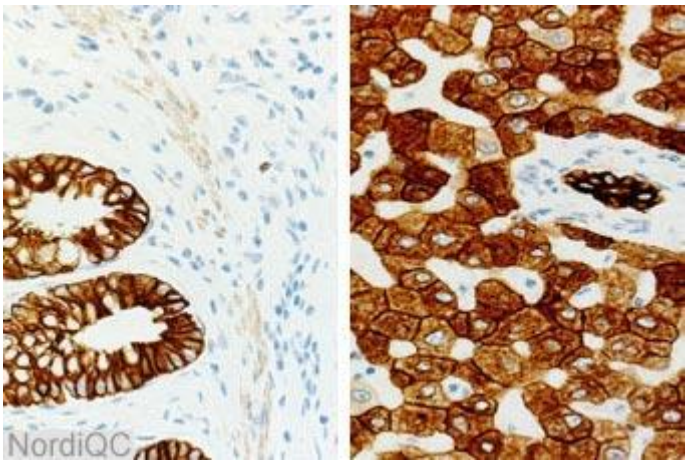

Fig. 5a
Insufficient staining for CK-Pan using the mAb clone cocktail AE1/AE3 + PCK26 (RTU product) using HIER.
Left: The appendiceal enterocytes show a strong distinct cytoplasmic reaction. However, also the smooth muscle cells are stained.
Right: The liver cells and bile duct are strongly stained.

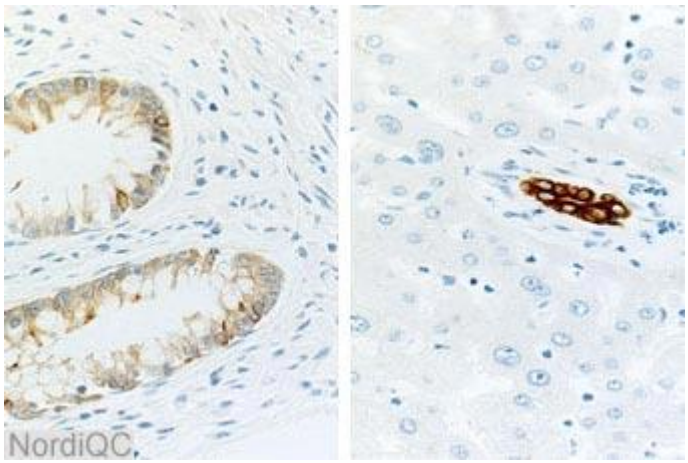

Fig. 5b
Insufficient staining for CK-Pan using the mAb clone cocktail AE1/AE3 + PCK26 (RTU product) using proteolytic pre-treatment but otherwise same protocol as in Fig. 5a.
Left: The appendiceal enterocytes only show a weak reaction, whereas the smooth muscle cells are negative (compare to Fig. 5a).
Right: Only the bile duct epithelial cells are demonstrated, while the hepatocytes are false negative.

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