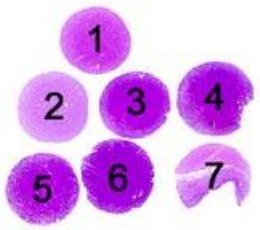
## Assessment Run 24 2008

# CK-Pan

The slide to be stained for CK-Pan comprised:
1. Liver, 2. Renal clear cell carcinoma, 3. Lung squamous cell carcinoma, 4. Lung small cell carcinoma, 5- 6. Colon adenocarcinoma, 7. Esophagus.
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 bile ductal epithelial cells, 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 esophagus (a negative reaction in the basal cells was accepted with mAb clone KL1).
- A strong, distinct cytoplasmic reaction in the majority of the neoplastic cells of the colon adenocarcinomas and the lung squamous cell carcinoma.
- An at least moderate, distinct cytoplasmic reaction in the majority of the neoplastic cells of the renal cell carcinoma and the lung small cell carcinoma.

123 laboratories submitted stains. At the assessment 45 achieved optimal marks (37%), 28 good (23%), 35 borderline (28 %) and 15 poor marks (12 %).

The following Abs were used:
mAb clone cocktail **AE1/AE3** (Dako, n=64; NeoMarkers/Thermo, n=8; BioGenex, n=3; Novocastra/Leica, n= 2; Zymed, n=2; Chemicon, n=1; Linaris, n=1)
mAb clone **MNF116** (Dako, n=19)
mAb clone **KL1** (Immmunotech, n=5; Serotec, n=1)
mAb clone cocktail **AE1/AE3 + 5D3** (BioCare Medical, n=5)
mAb clone cocktail **AE1/AE3/PCK26** (Ventana, n=5)
mAb clone **Lu-5** (NeoMarkers, n=2; BMA Biomedicals, n=1)
mAb clone cocktail **PAN-CK Ab-2** (NeoMarkers/Thermo, n=2)
mAb clone cocktail **5D3 and LP34** (Novocastra/Leica, n=1)
mAb clone cocktail **OSCAR** (Signet Laboratories, n=1)

Optimal staining for **CK-Pan** in this assessment was obtained with the mAb clone cocktail **AE1/AE3** (34 out of 81), the mAb clone cocktail **AE1/AE3 + 5D3** (5 out of 5), the mAb clone cocktail **AE1/AE3/PCK26 (RTU)** (3 out of 5), the mAb clone **KL1** (1 out of 6) and the mAb clone **MNF116** (3 out of 19).

**AE1/AE3:** The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) with either Tris-EDTA/EGTA pH 9.0 (16/33)*, Cell Conditioning1 (BenchMark, Ventana) (8/12), Target Retrieval Buffer pH 9, (Dako) (5/11), EDTA/EGTA pH 8 (2/2), PT Module Buffer (LabVision) (1/1), Target Retrieval Solution pH 6.1 (Dako) (1/1), Citrate pH 6.0 (1/12) as HIER buffer. The mAb was typically diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab. With these settings 53 out of 68 (78 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

**AE1/AE3 + 5D3:** The protocols giving an optimal result were all based on HIER with either Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/2), Target Retrieval Buffer pH 9, (Dako) (2/2) or Citrate pH 6.0 (1/1) as HIER buffer. The Ab was diluted in the range of 1:75 - 800 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab. All 5 laboratories using this mAb cocktail obtained the mark optimal.

**AE1/AE3/PCK26 (RTU):** The protocols giving an optimal result were based on a combined enzymatic pre-treatment using Protease 3 (Ventana) and HIER in Cell Conditioning1 (BenchMark, Ventana) (3/3). The Ab was used as a Ready-To-Use Ab. With these settings all 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 1:25. With these settings 1 out of 1 laboratory produced a sufficient

staining marked as optimal.

**MNF116:** The two protocols giving an optimal result were either based on enzymatic pre-treatment with Proteinase K (Dako) and a dilution of 1:200 of the primary Ab or based on heat induced epitope retrieval (HIER) using Citrate pH 6.0 and a Ready-To-Use Ab. With these settings 2 out of 2 laboratories produced a sufficient staining marked as optimal.

The most frequent causes of insufficient stains were (often in combination):
- Inappropriate epitope retrieval (e.g., proteolysis for the mAb clone AE1/AE3)
- Too low concentration of the primary antibody
- Less successful primary Ab
- Insufficient HIER (Citrate pH 6.0 for AE1/AE3)

In this assessment and in concordance with the previous CK-Pan assessments in run 15 and 20 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 two colon adenocarcinomas, the lung small cell carcinoma and the squamous cell carcinoma as well as in the bile ductal cells. However, the demonstration of CK in the renal cell carcinoma was more difficult and only seen for protocols with a high sensitivity and appropriate protocol settings, e.g. as obtained with a correct titre of the mAb clone cocktails AE1/AE3 and AE1/AE3 + 5D3 combined with efficient HIER.
In accordance with previous assessments of CK-Pan, liver was a reliable positive control, as all laboratories that could demonstrate the membranous CK in the hepatocytes also could demonstrate CK in the renal cell carcinoma. Thus, the demonstration of the primary CK-LMW subtypes 8 & 18 in the normal and and neoplastic simple epithelial was most challenging, whereas the demonstration of the primary CK-HMW subtypes 5 & 14 in the squamous epithelial cells was less problematic.

The choice of retrieval method has to be specifically linked to the choice of the Ab clone for CK-Pan, as the Ab cocktail AE1/AE3 shows a superior performance when used with HIER, while proteolytic pre-treatment should be used for the mAb clone MNF116. This is illustrated in table 1 where the cumulated data for the most widely used clones is listed relating the pass rate for the clone to the epitope retrieval method. E.g. the pass rate for AE1/AE3 was 71% when HIER was applied and 13% when protease was used.

Table 1. **Cumulated data for selected CK-Pan clones**

| | **Pass rate for run 15, 20 & 24** | | | | | |
|---|---|---|---|---|---|---|
| | **Total** | | **HIER** | | **Prot. pre-treatm.** | |
| | **Protocols** | **Sufficient** | **Protocols** | **Sufficient** | **Protocols** | **Sufficient** |
| mAb clone AE1/AE3 | 189 | 125 (66%) | 174 | 123 (71%) | 15 | 2 (13%) |
| mAb clone MNF116 | 39 | 21 (54%) | 15 | 5 (33%) | 24 | 17 (71%) |
| mAb clone KL1 | 25 | 15 (60%) | 25 | 15 (60%) | 0 | 0 |
| mAb clone AE1/AE3 + PCK26 | 20 | 3* (15%) | 3 | 0 | 14 | 0 |
| mAb clone AE1/AE3 + 5D3 | 11 | 11 (100%) | 11 | 11 (100%) | 0 | 0 |
| mAb clone Ab2 | 8 | 6 (75%) | 4 | 4 (100%) | 4 | 2 (50%) |
| mAb clone 5D3/LP34 | 6 | 1 (17%) | 5 | 1 (17%) | 1 | 0 |

*3 using a sequential retrieval, HIER followed by enzymatic pre-treatment - all 3 were assessed as optimal.

This was the fourth assessment of CK-Pan. The overall pass rate has been almost constant in the 4 runs as shown in table 2:

Table 2. **Pass rate for CK-Pan in four runs**

| | Run 8 2003 | Run 15 2005 | Run 20 2007 | Run 24 2008 |
|---|---|---|---|---|
| Participants, n= | 72 | 85 | 103 | 123 |
| Sufficient results | 53% | 58% | 62% | 60% |

In the last assessment of CK-Pan run 20, 103 laboratories participated out of which 39 (38%) obtained an insufficient mark. Each was given a specific recommendation to improve their protocol. 30 of them submitted a new CK-Pan stain in run 24. 13 followed the recommendation, of which 9 improved to good or optimal (69 %). 12 laboratories did not follow the recommendation, and 2 of these (17 %) obtained a sufficient staining in run 24. 5 laboratories changed their entire IHC system and 2 of these obtained a sufficient staining.

In total 109 laboratories obtaining an insufficient result in run 8, 15 and run 20 have been given specific recommendations how to improve their protocol. 89 laboratories submitted a new CK-Pan stain in the subsequent run. 44 followed the recommendation, of which 31 improved to good or optimal (71 %). 40 laboratories did not follow the recommendation, and 6 of these (15 %) obtained a sufficient staining in the subsequent run.

**Conclusion**

The mAb clones **AE1/AE3**, **AE1/AE3 + 5D3**, **AE1/AE3 + PCK26**, **KL1** and **MNF116** all can be used to obtain an optimal staining for CK-Pan. The epitope retrieval and protocol settings have to be specifically tailored to each of the clones/cocktails. Liver is an appropriate control tissue irrespective of the clone/cocktail applied: Almost all hepatocytes must show a distinct cytoplasmic staining with enhancement along the cell membranes.

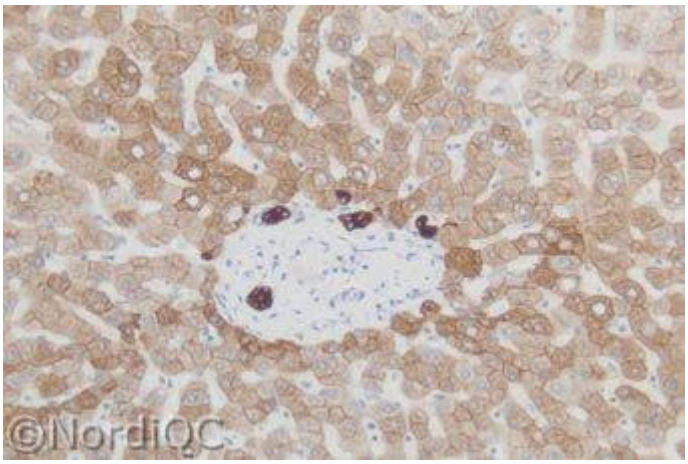



Fig. 1a
Optimal staining for CK-PAN of the liver using the mAb clone cocktail AE1/AE3+PCK26 with a combined retrieval of proteolysis and HIER. The majority of the hepatocytes show a distinct, moderate to strong, predominantly membranous staining.

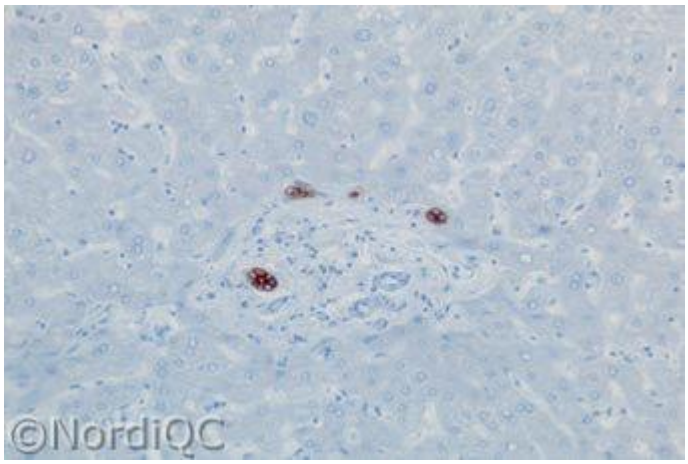



Fig. 1b
Insufficient Staining for CK-PAN of the liver using the mAb clone cocktail AE1/AE3+PCK26 with proteolysis (same field as in Fig. 1a.). Only the bile duct epithelial cells are demonstrated, while the hepatocytes only expressing limited CK are unstained.

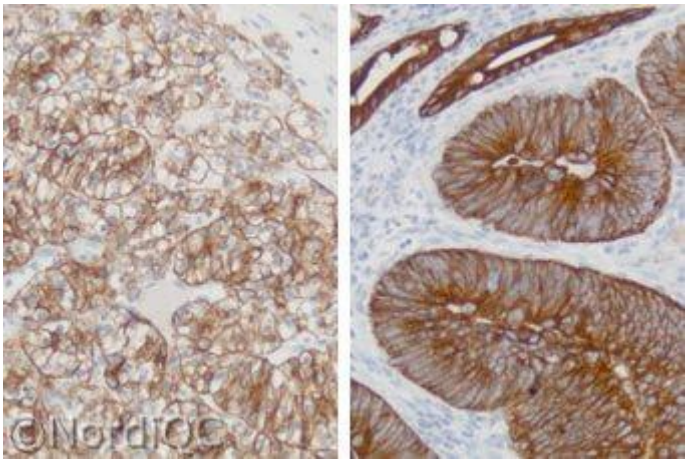



Fig. 2a
Optimal staining for CK-PAN using same protocol as in Fig. 1a.
Left: Renal cell carcinoma. A large proportion of the neoplastic cells show a moderate to strong staining.
Right: Colon adenocarcinoma. Virtually all the neoplastic cells show a strong cytoplasmic staining.

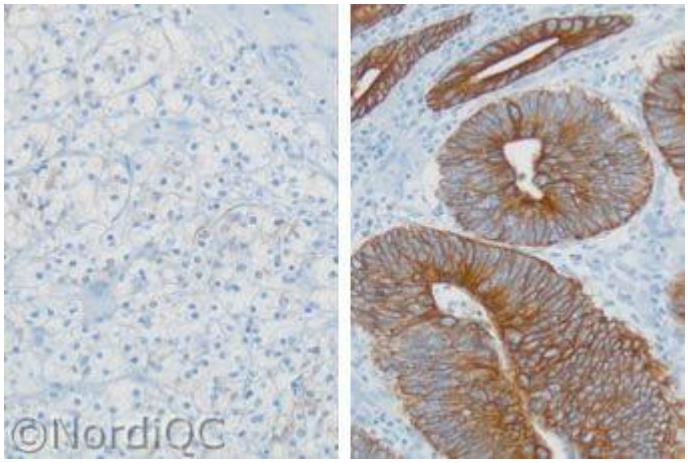



Fig. 2b
Insufficient staining for CK-PAN using same protocol as in Fig. 1b. Illustrating the false negative reaction in low antigen expressing tumours as RCC. Same fields as in Fig. 2a.
Left: Renal cell carcinoma. Only scattered neoplastic cells are weakly positive, while the majority is totally negative
Right: Colon adenocarcinoma. Virtually all the neoplastic cells show a moderate cytoplasmic staining.

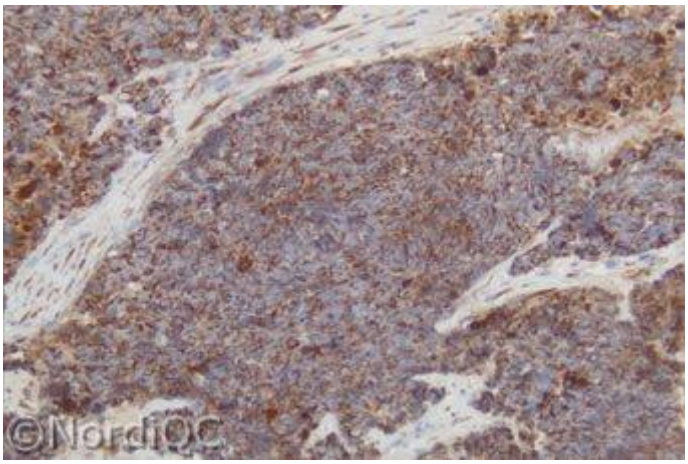



Fig. 3a
Optimal staining for CK-PAN using same protocol as in Fig. 1a. The majority of the neoplastic cells of the small cell lung carcinoma show a moderate cytoplasmic staining. Also myofibroblasts in the stroma show a weak cytoplasmic staining.

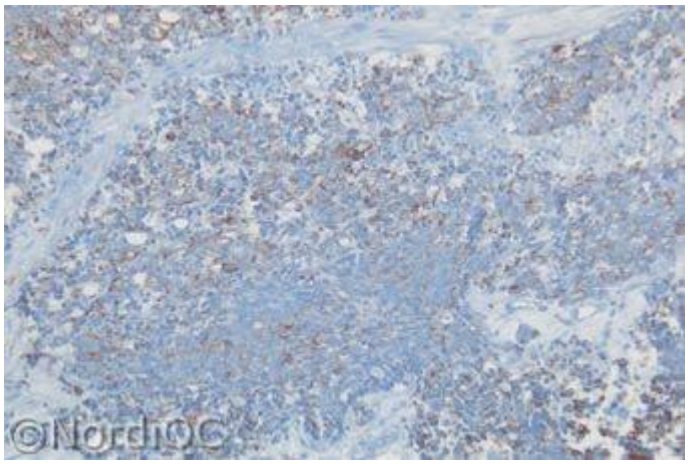



Fig. 3b
Insufficient staining for CK-PAN using same protocol as in Fig. 1b. – same field as in Fig. 3a.
Only scattered neoplastic cells are weakly positive, while the majority is totally negative.

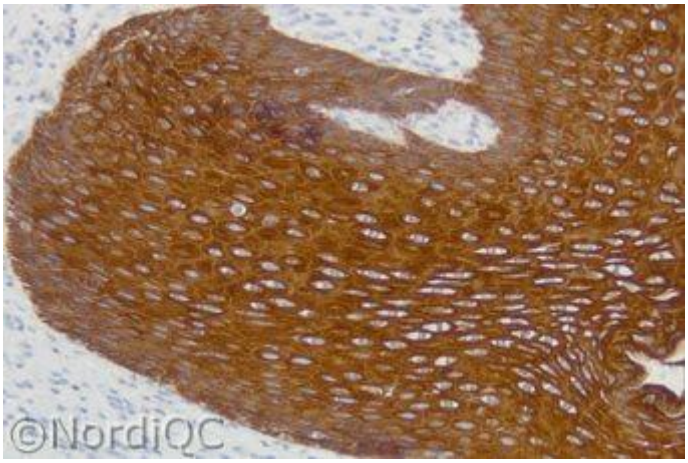



Fig. 4a
Optimal staining for CK-PAN in the esophagus using same protocol as in Fig. 1a. All the squamous epithelial cells throughout the entire epithelial layer are stained.

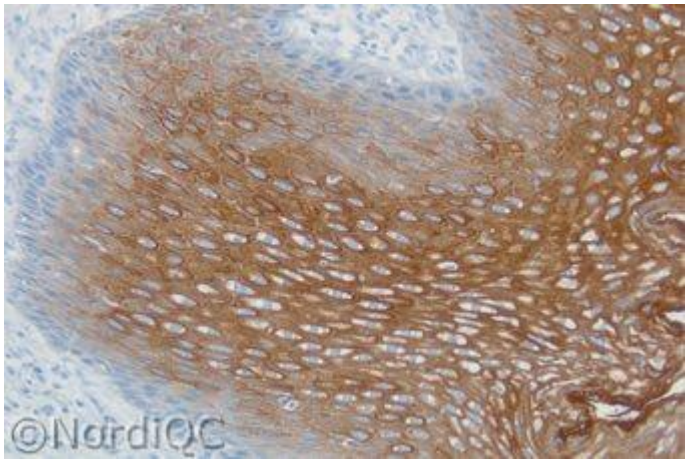



Fig. 4b
Staining for CK-PAN in the esophagus same insufficient protocol as in Fig. 1b - same field as in Fig. 4a. Only the intermediate and superficial squamous epithelial cells are stained, while the basal cells are negative.

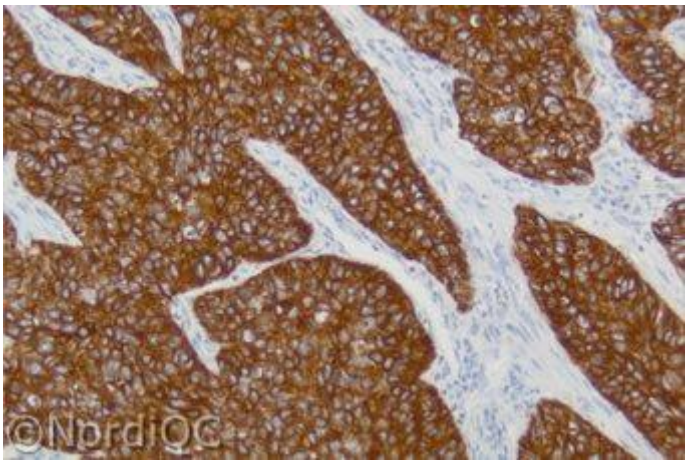



Fig. 5a
Optimal staining for CK-PAN of the squamous cell carcinoma using same protocol used in Fig. 1a. All the neoplastic cells show a strong cytoplasmic staining.

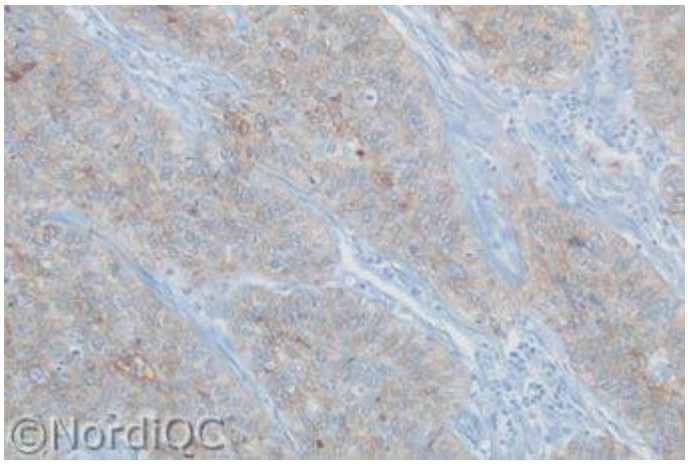



Fig. 5b
Insufficient staining for CK-PAN of the squamous cell carcinoma using same protocol as in Fig. 1b. (same field as in Fig 5a). The neoplastic cells only show a diffuse and weak staining.

SN/HN/MV/LE 8-12-2008