Assessment Run 16 2006

Cytokeratin, low molecular weight (CK-LMW)

The slide to be stained for low molecular weight cytokeratin (CK-LMW) comprised:
1. Tonsil, 2. Liver, 3. Appendix, 4. Esophagus, 5. Neuroendocrine carcinoma.
All specimens were fixed in 10 % NBF.

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

- A strong, distinct staining reaction of the appendiceal enterocytes and the bile ducts.
- A moderate staining of the large majority of hepatocytes, with an enhancement along the cell membranes.
- A weak to moderate cytoplasmic staining reaction of the tonsillar squamous epithelium.
- A negative staining reaction of the esophageal squamous epithelial cells, except for a basal cell staining if using an Ab reacting with CK type 19.
- A strong, distinct staining of the neuroendocrine carcinoma.

79 laboratories submitted stains. Of these 13 used a CK-LMW antibody considered inappropriate (se below). Assessment of the remaining 66 gave the following results: 10 achieved optimal marks (15 %), 20 good (30 %), 18 borderline (27 %) and 18 (27 %) poor marks.

The following CK-LMW Abs were used:

mAb	Reactivity	Producer and number
35βH11	CK8	Dako, n=5; Ventana, n= 2; BioTrend, n=1; Cell Marque, n=1
5D3	CK8,18	Novocastra, n=3; BioGenex, n=1; Cymbus, n=1; Ventana, n=1
C51	CK8	Zymed, n=4; MedProbe, n=1; NeoMarkers, n=1
CAM 5.2	CK8,7(19)	Becton Dickinson, n=27
DC10	CK18	Dako, n=12; Novocastra, n=3; NeoMarkers, n=1
TS1	CK8	NeoMarkers, n=1

The mAbs clones **AE1** (reacting with CK 10, 13, 14, 15, 16 & 19), **Ks20.8** (reacting with CK 20), **MNF116** (reacting with CK 5, 6, 8, 17 & 19) and **OV-TL 12/30** (reacting with CK 7) were all considered inappropriate because they either also detected CK of high molecular weight types or did not detect CK8/18.

In this assessment an optimal staining was achieved with mAbs clone **DC10** (8 out of 15 were optimal) and the clone **C51** (2 out of 6 were optimal).

Using the mAb clone **DC10** all protocols resulting in an optimal stain were based on heat induced epitope retrieval (HIER) using either a Tris-EDTA/EGTA buffer pH 9 (7 out of 13 laboratories using this obtained optimal marks) or Citrate pH 6 (1 out of 1 using this obtained optimal marks). In the protocols giving optimal staining, the mAb typically was used in the range of 1:50 – 1:100 depending on the total sensitivity of the protocol employed. The combination of the mAb clone **DC10** diluted in the range of 1:50 – 1:100 and HIER in either Tris-EDTA/EGTA pH 9 or citrate pH 6 resulted in an optimal staining in 8 out of 11 laboratories (73 %).

Using the mAb clone **C51** the protocols resulting in an optimal staining were based on HIER using a Tris-EDTA/EGTA buffer pH 9 (2 out of 4 laboratories using this obtained an optimal mark). In the optimal protocols the mAb typically was used in the range of 1:50 – 200 depending on the total sensitivity of the protocol employed. The combination of the mAb clone **C51** diluted in the range of 1:50 – 1:200 and HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining in 2 out of 4 laboratories (50 %).

The most frequent causes of insufficient staining were:
- Less successful primary Ab's
- Too low conc. of the primary Ab.

In this assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the hepatocytes. In general the majority of the laboratories were able to detect CK-LMW in the columnar epithelial cells of the bile ducts and in the appendix. However for a diagnostic purpose (and demonstrated in previous NordiQC assessments of both CK-LMW (run 9) and Pan CK (run 15) it can be of utmost importance to be capable to demonstrate the relative low CK-LMW in hepatocytes to be sure to demonstrate CK in i.e. seminoma, small cell lung cancer and equivalent tumours which traditionally only express limited amounts of CK.

It should also be notified that the most frequent cause for insufficient staining in this assessment seemed to be related to the choice of the primary Ab. 17 out of 27 laboratories using the mAb clone **CAM5.2** (63 %) and 10 out of 12 using the clone **35βH11** (83 %) were marked as insufficient.

Conclusion
- The mAb clones **DC10** and **C51** seems to be the most sensitive and reproducible Abs for CK-LMW
- HIER (preferably in an alkaline buffer) is highly recommended for optimal performance
- Hepatocytes (or equivalent cells) expressing low amounts of CK-LMW should be used as control.

CK-LMW was also assessed in Run 9, in which 54 laboratories participated. Out of these, 23 laboratories (43%), which had an insufficient staining, were given specific recommendations to improve their protocol. 16 of them submitted a new CK-LMW stain in run 16. Seven followed the recommendation and five of them (71 %) improved from insufficient to sufficient. 9 laboratories did not follow the recommendations and none improved.

The overall proportion of insufficient staining in this run increased from 43 % in run 9 to 54 % in run 16. This can be due to more factors and is not necessary an indicator of a decline in the laboratory performance. However, it is noteworthy that many laboratories seem to use less successful primary Abs. Most likely they could improve their results simply by using either clone **DC10** or **C51** with HIER. Among the laboratories using the clones **DC10** or **C51** only 3 out of 22 (14 %) had an insufficient staining whereas among the laboratories using i.e. the clones **CAM5.2** or **35βH11** 27 out of 39 (69 %) had an insufficient staining.

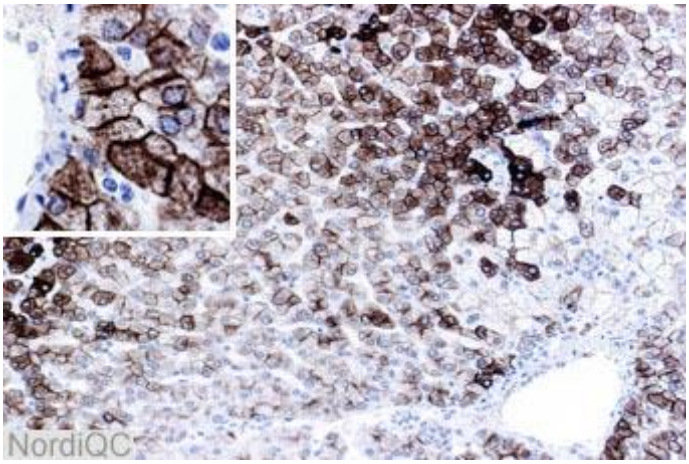

Fig. 1a
Optimal staining for CK-LMW of the liver. The majority of the hepatocytes show a distinct, moderate to strong, predominantly membranous reaction.

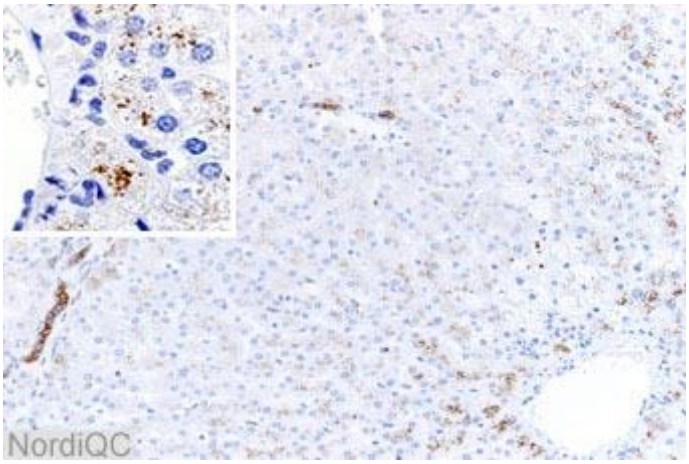

Fig. 1b
Staining for CK-LMW of the liver using an insufficient protocol (same field as in Fig. 1a.). Only the bile duct epithelial cells are demonstrated while the hepatocytes are unstained or only weakly positive.

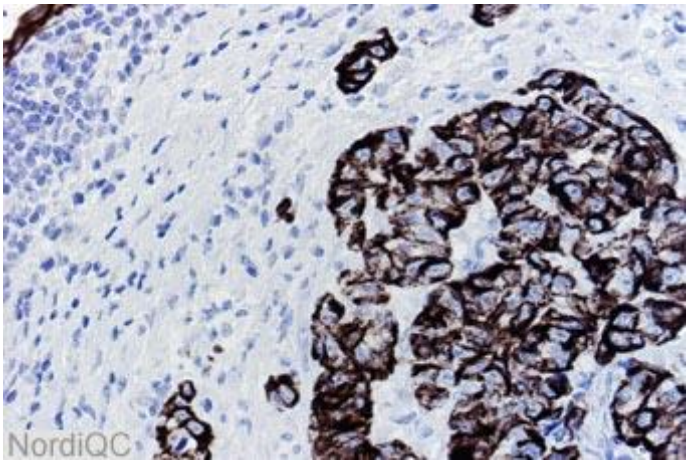

Fig. 2a
Optimal staining for CK-LMW of the neuroendocrine carcinoma. Virtually all neoplastic cells show a strong cytoplasmic reaction (same protocol used in Fig. 1a).

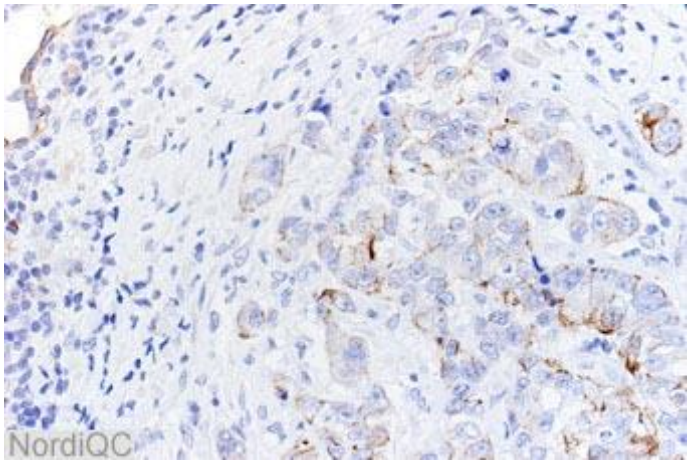

Fig. 2b
Insufficient staining for CK-LMW of the neuroendocrine carcinoma (same field as in Fig 2a). The neoplastic cells are only weakly positive or totally negative (same protocol used in Fig. 1b).

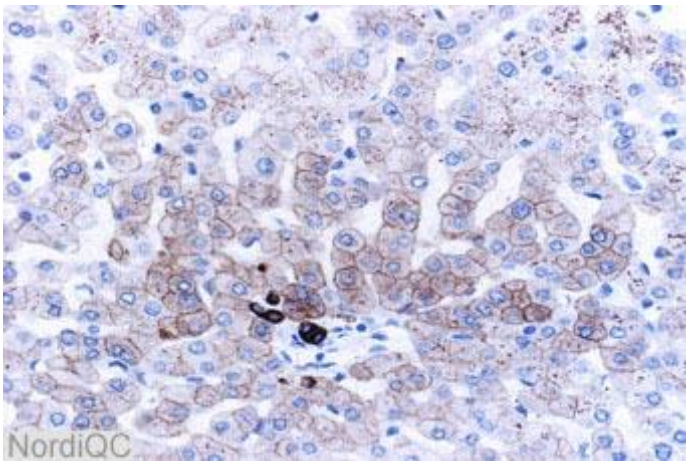

Fig. 3a
Staining for CK-LMW of the liver using an inappropriate Ab (AE1). The majority of the hepatocytes are stained. However compare with Fig. 3b.

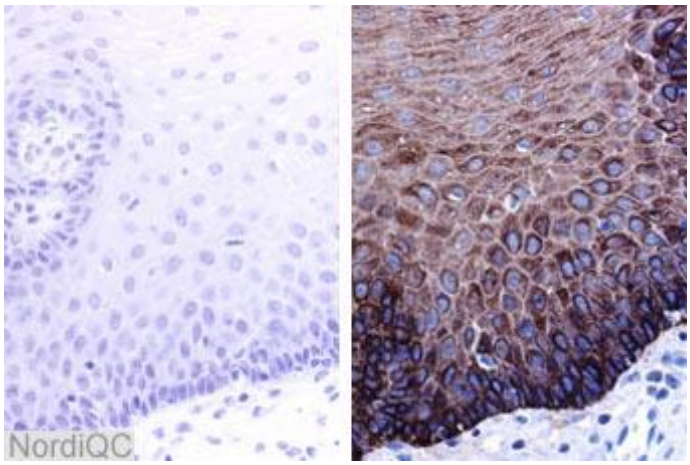

Fig. 3b
Left. Optimal staining for CK-LMW of the esophagus using the mAb clone DC10. The squamous epithelial cells are negative. **Right**. Staining for CK-LMW using an inappropriate Ab (AE1). All the squamous epithelial cells are positive.

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