## Assessment Run 20 2007

# Cytokeratin low molecular weight (CK-LMW)

The slide to be stained for CK-LMW comprised:
1. Appendix, 2. Liver, 3. Esophagus, 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-LMW staining as optimal included:

- A strong, distinct staining reaction of the appendiceal enterocytes and the hepatic bile ducts.
- A moderate to strong, distinct staining of the large majority of hepatocytes, with an enhancement along the cell membranes.
- A moderate to strong, distinct staining of the majority of the neoplastic cells of the renal cell carcinoma and the signet ring cell carcinoma.
- No staining of the esophageal squamous epithelial cells, except for a staining of the basal cells if using an Ab reacting with CK type 19 (e.g., CAM 5.2).
- A weak to moderate cytoplasmic staining reaction of the neoplastic cells in the squamous cells carcinoma and, if using an Ab reacting with CK type 19 (e.g., CAM 5.2), a strong staining.

89 laboratories submitted stains. Of these 15 used a CK-LMW antibody considered inappropriate (see below). Assessment of the remaining 74 laboratories gave following results: 26 achieved optimal marks (36 %), 23 good (31 %), 21 borderline (28 %) and 4 poor marks (5 %).

Appropriate Abs (i.e., Abs reacting with least one primary/constitutive CK of simple epithelia, i.e., CK8 or CK18, but not with high molecular weight CKs) used are indicated in Table 1.

Table 1. **Appropriate CK-LMW Abs used in run 20**

| mAb | Reactivity | Producer and number |
|---|---|---|
| **DC10** | CK18 | Dako, n=18; NeoMarkers, n=2; Novocastra, n=1 |
| **Ks-B17.2** | CK18 | Sigma, n=1 |
| **35βH11** | CK8 | Dako, n=10; Ventana, n=4 |
| **CAM 5.2** | CK8,7,19 | Becton Dickinson, n=20 |
| **5D3** | CK8,18 | Novocastra, n=6; NeoMarkers, n=2; BioGenex, n=1 |
| **C51** | CK8 | Zymed, n=7; BioGenex, n=1 |
| **K8.8 + DC10** | CK8,18 | NeoMarkers, n=1 |

The following Abs were considered inappropriate: clone **AE1** (CK-Pan reacting with CK10, 13, 14, 15, 16 & 19), clone **AE1/AE3** (CK-pan reacting with CK1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16 & 19), clone **Ks20.8** (reacting with CK20 only), clone **RCK108** (reacting with CK19 only), **OV-TL 12/30** (reacting with CK7 only) and **MNF116** (CK-Pan reacting with CK 5, 6, 8, 17 & 19).

Optimal staining for **CK-LMW** in this assessment was obtained with the mAb clone **DC10** (13 out of 21, 62%), the mAb clone **CAM 5.2** (3 out of 20, 15%), the mAb clone **5D3** (3 out of 9, 33%), the mAb clone **C51** (6 out of 8, 75%) and the mAb clone **KS-B17.2** (1 out of 1).

**DC10**: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 or Cell Conditioning 1 (BenchMark, Ventana). The mAb was typically diluted in the range of 1:50 – 1:150 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) antibody. With these settings 17 out of 18 (94 %) laboratories produced a sufficient staining (optimal or good).

**CAM 5.2**: the protocols giving an optimal result were either based on HIER with Tris-EDTA/EGTA pH 9 or Citrate pH 6 or proteolytic pre-treatment using Protease 1 (Ventana). The Ab was either used as a Ready-To-Use antibody or diluted in the range of 1:5– 1:50 (of the Ready-To-Use format) depending on the total sensitivity of the protocol employed. Using these settings 6 out of 8 (75 %) laboratories produced a sufficient staining.

**5D3**: the protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 or Citrate pH 7 as buffer. The Ab was typically diluted in the range of 1:20 - 1:50 depending on the total sensitivity of the protocol employed. Using these settings 4 out of 5 (80 %) laboratories produced a sufficient staining.

**C51**: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 or 1mM EDTA pH 9 as buffer. The mAb was typically diluted in the range of 1:100 – 1:200 depending on the total sensitivity of the protocol employed. Using these settings 8 out of 8 laboratories (100 %) produced a sufficient staining.

**Ks-B17.2**: the protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9. The Ab was diluted 1:100.

Table 2. **Results in runs 16 and 20 with five CK-LMW mAbs used by at least 6 laboratories.**

| | **Run 16 2006** | | **Run 20 2007** | | **Total** | |
|---|---|---|---|---|---|---|
| **Clone** | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient |
| **CAM5.2** | 27 | 10 | 20 | 11 | 47 | 21 (45%) |
| **DC10** | 16 | 14 | 21 | 19 | 37 | 33 (89%) |
| **35βH11** | 12 | 2 | 14 | 4 | 26 | 6 (23%) |
| **5D3** | 6 | 4 | 9 | 5 | 15 | 9 (60%) |
| **C51** | 6 | 5 | 8 | 8 | 14 | 13 (93%) |

From Table 2 it appears that mAbs clone **DC10** and **C51** in both run 16 and run 20 had a markedly higher pass rate than clones **CAM5.2** and **35βH11**.

The most frequent causes of insufficient staining were:
- Less successful primary Abs
- Too low concentration of the primary Ab
- Insufficient HIER – use of citrate pH 6 and/or too short HIER time.

The prevalent feature of an insufficient staining was a too weak or false negative staining of the hepatocytes. Virtually all the laboratories were able to detect CK-LMW in the columnar epithelial cells of the bile ducts and the appendix. The majority of the laboratories could demonstrate CK-LMW in the signet cell carcinoma, whereas the demonstration of CK-LMW in the renal cell carcinoma was more challenging and required a sensitive protocol (in parallel with the demonstration of CK-LMW in the hepatocytes).

In run 16, 2006, 66 laboratories submitted stains for CK-LMW. 36 (54%) obtained an insufficient mark and was given a specific recommendation to improve their protocol. 27 of them submitted a new CK-LMW stain in run 20. 15 followed the recommendation, of which 12 improved their mark to good or optimal (80 %). 12 laboratories did not follow the recommendation, and only 2 of these (20 %) obtained a sufficient staining in run 20.

**Conclusion**
The mAb clones **DC10** and **C51** appears to be robust and sensitive Abs for CK-LMW and should replace the old Abs clone **CAM 5.2** and clone **35βH11**.
HIER (preferably in an alkaline buffer) is highly recommended for optimal performance of DC10 and C51.
Hepatocytes (expressing low amounts of CK-LMW) should be used as control: strong, distinct staining of the large majority of hepatocytes, with an enhancement along the cell membranes, should be obtained.
The proportion of sufficient results has been increased from 46% in run 16 to 67% in the current run. NordiQC recommendations for protocol optimization resulted in a marked improvement of the performance.

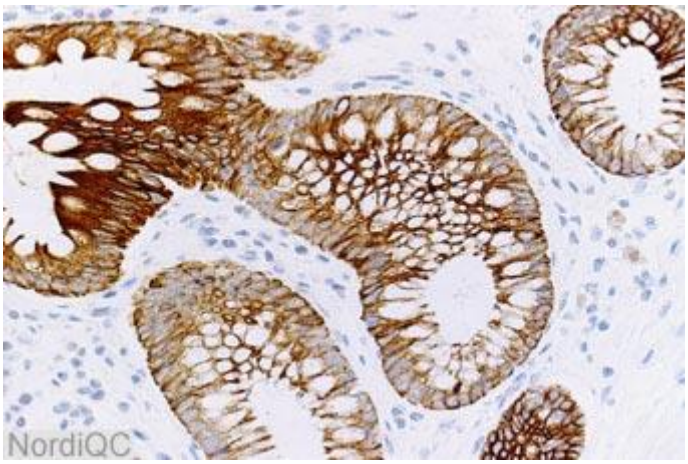


Fig. 1a
Optimal staining for CK-LMW of the appendix using clone DC10. All the appendiceal enterocytes are demonstrated – both luminal and basal cells show a distinct staining.

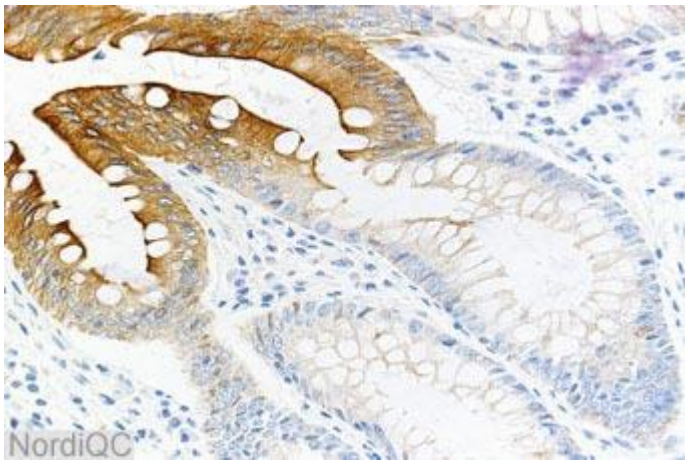


Fig. 1b
Insufficient staining for CK-LMW of the appendix using clone 35BH11 (same field as in Fig. 1a.). Only the luminal epithelial cells are demonstrated while the basal cells are unstained or only weakly positive.

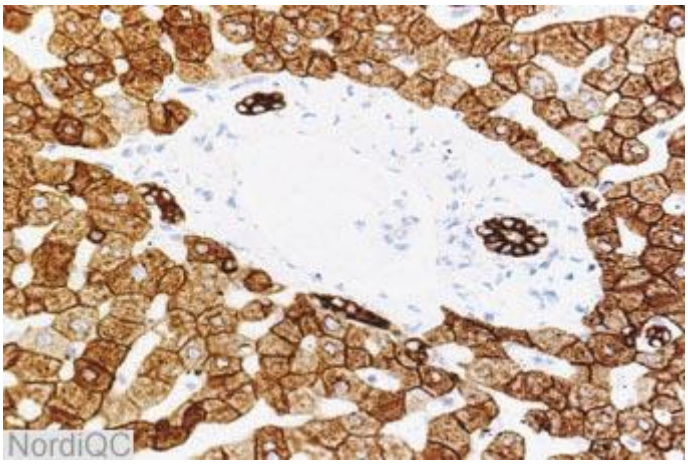


Fig. 2a
Optimal staining for CK-LMW of the liver using clone C51. The majority of the hepatocytes show a distinct, moderate to strong, predominantly membranous reaction.

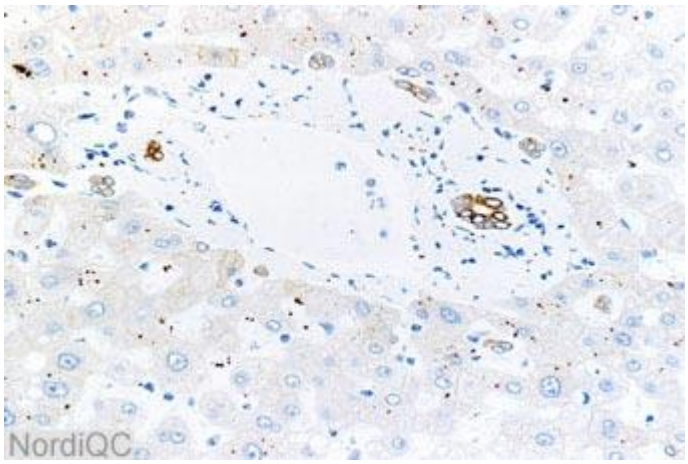


Fig. 2b
Insufficient staining for CK-LMW of the liver using clone 35 BH11 (same field as in Fig 2a). Only the bile duct epithelial cells are positive while the hepatocytes are entirely negative (same protocol as in Fig. 1b).

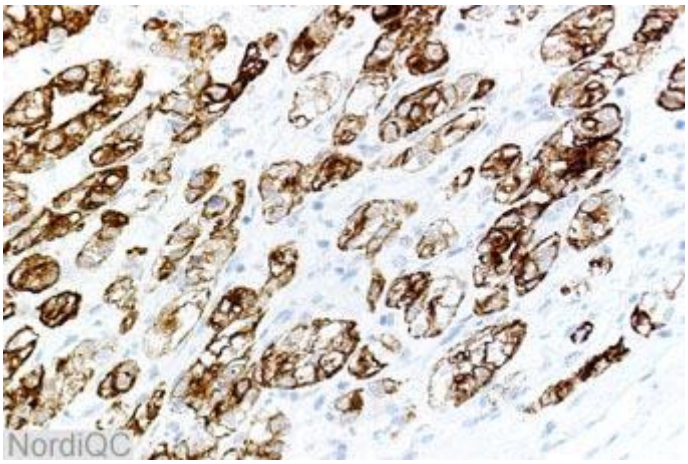


Fig. 3a
Optimal staining for CK-LMW of the renal cell carcinoma using clone C51. The majority of tumourcells show a strong reaction.

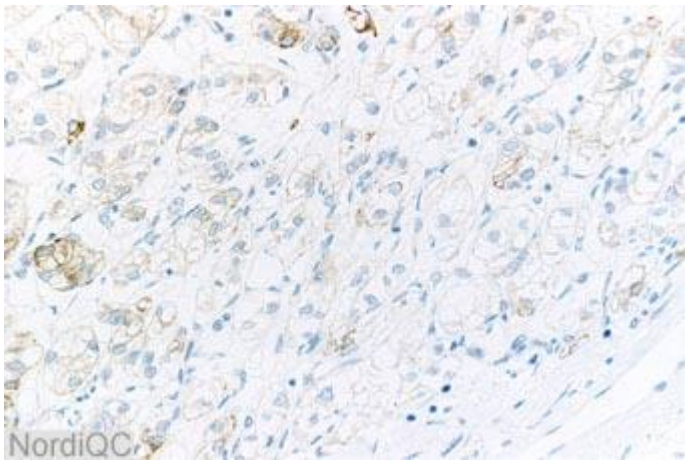


Fig. 3b
Insufficient staining for CK-LMW of the renal cell carcinoma using clone 35BH11 (same field as in Fig. 3a). Only few cells are stained (same protocol as in Fig. 1b).

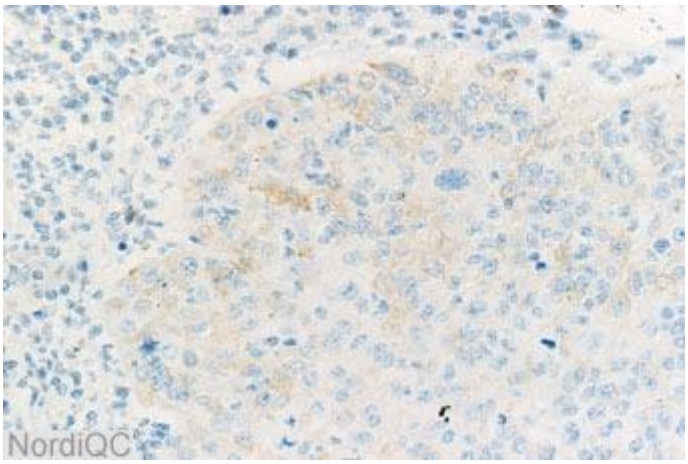


Fig. 4a
Optimal staining for CK-LMW of the squamous cell carcinoma using clone DC10. The tumour cells show a weak cytoplasmic reaction.

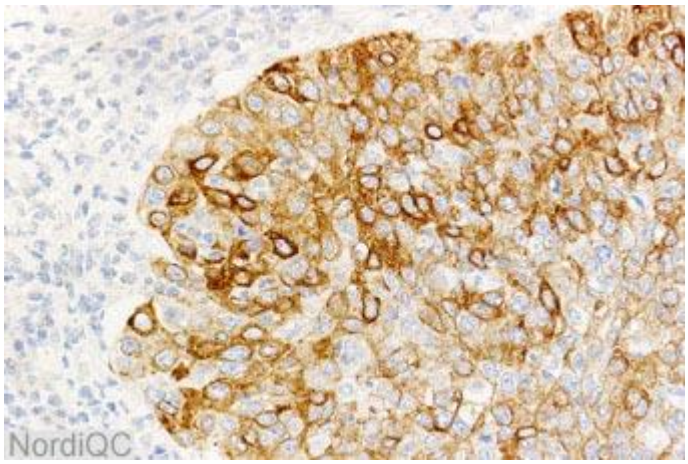


Fig. 4b
Optimal staining for CK-LMW of squamous cell carcinoma using clone CAM5.2 (same field as in Fig 4a). The majority of neoplastic cells show a strong reaction, as this antibody also detects CK19 that is often expressed in squamous cell carcinoma.

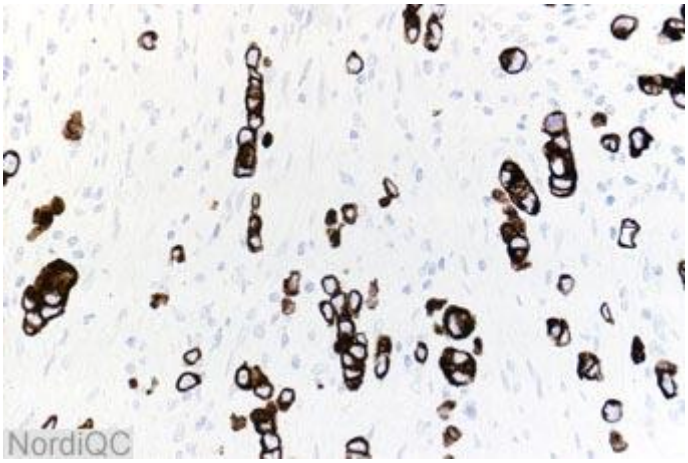


Fig. 5a
Optimal staining for CK-LMW of the signet ring cell carcinoma using clone DC10. Virtually all the tumour cells show a strong reaction.

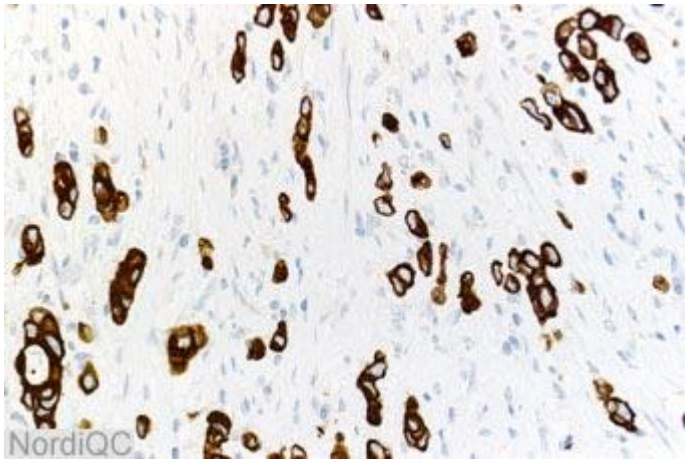


Fig. 5b
Optimal staining for CK-LMW of the signet ring cell carcinoma using same protocol as in Fig. 4. using clone CAM5.2. Virtually all the tumour cells show a strong reaction. However, this Ab is less apt to discriminate adenocarcinoma from squamous cell carcinoma (Fig. 4b).

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