Assessment Run 9 2003

Low Molecular Weight Cytokeratin (CK-LMW)

The slide to be stained for Low Molecular Weight Cytokeratin (CK-LMW) comprised: 1: Colon, 2: Lung neuroendocrine carcinoma, 3: Tonsil, 4: Liver, 5: Pancreas, Esophagus, 7: Mantle cell lymphoma.

Criteria for assessing a CK-LMW staining as optimal included: A strong and distinct cytoplasmatic staining of the large majority of the normal epithelial cells in the colon, the pancreatic ducts and acini, and intrahepatic bile ducts, while most endocrine cells of the Langerhans' islets and hepatocytes should at least reveal a moderate staining. In the tonsil, squamous epithelium should display a moderate to strong staining and follicular dendritic cells a weak to moderate staining, while the squamous epithelium of the esophagus should be negative or only the basal cells stained. The lung neuroendocrine carcinoma should reveal a strong and distinct staining of the majority of cells, while the mantle cell lymphoma should be negative.

54 laboratories submitted stainings. At the assessment 14 achieved optimal staining (25 %), 17 good (32 %), 14 borderline (26 %) and 9 (17 %) poor staining.

The following CK mAbs were used:

mAb	Reactivity	Producer and number
35BH11	CK8	DakoCytomation, n=5, BioTrend, n=1, CellMarque, n=1
5D3	CK8,18	Novocastra, n=5, BioGenex, n=2, Ventana, n=1
AE1*	CK10,13,14,15,16,19	NeoMarkers, n=1
C51	CK8	Zymed, n=5, NeoMarkers, n=1, Zhongshan, n=1
CAM 5.2	CK8,7(?)	Becton Dickinson, n=21
DC10	CK18	DakoCytomation, n=8
Ksb17.2	CK18	Sigma, n=1
TS1	CK8	NeoMarkers, n=1

Optimal stainings in this assessment could be obtained with mAbs CAM 5.2 (Fig 1a), DC10 (giving a generally stronger staining than CAM 5.2; Fig. 1b, 2a, and 3a), 5D3, C51, Ksb17.2 and TS1 (which gave approximately the same staining reactions as DC10; not illustrated), while no optimal stainings were seen with mAb 35BH11 in this assessment.
*AE1 was considered as an inappropriate choice of Ab as it does not detect CK8 or CK18.

mAbs 5D3 and CAM 5.2 gave the best staining results with proteolytic pre-treatment (Pronase E, Proteinase K or Trypsin), while pre-treatment with HIER (with Tris/EDTA pH 9 or citrate pH 6 as the heating buffer) resulted in generally insufficient stainings (weak or negative reaction in many cells). Appropriate dilution of CAM 5.2 was very important. Thus, the laboratories obtaining an optimal result with CAM 5.2 used the mAb in the range of RTU-1:5, while laboratories with insufficient stainings used a too dilute mAb (up to 1:200 of the ready-to-use solution).
An optimal staining with mAbs DC10 and C51 could only be obtained using HIER with Tris/EDTA pH 9 as the heating buffer. Optimal dilution of mAb DC10 was 1:25 – 1:100, that of C51 1:50 – 1:400.

The most frequent causes of insufficient stainings (often in combination) were:
- Too low concentration of the primary mAb
- Insufficient HIER (too short efficient heating time)
- Inappropriate choice of the primary antibody
- Inappropriate pre-treatment for the mAb used (e.g., HIER with mAb CAM 5.2 and 5C3 instead of proteolytic pretreatment, or any other pretreatment with DC10 and C51 than HIER in an alkaline buffer).

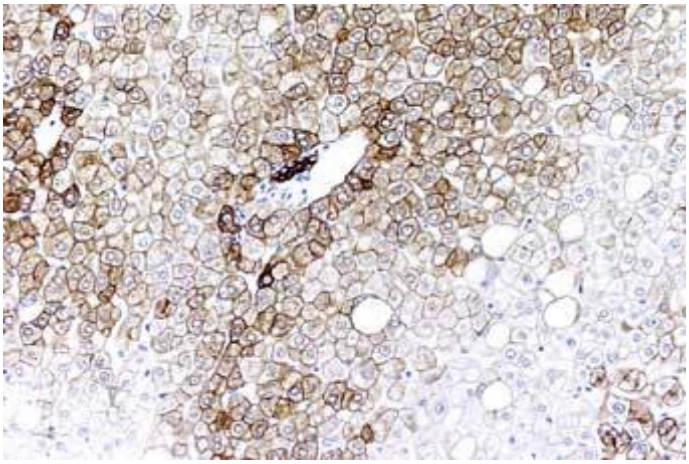

Fig. 1a
Optimal staining using mAb CAM5.2. Normal liver. The bile duct (centre) is strongly stained, while most liver cells show a moderate to strong staining with membranous enhancement.

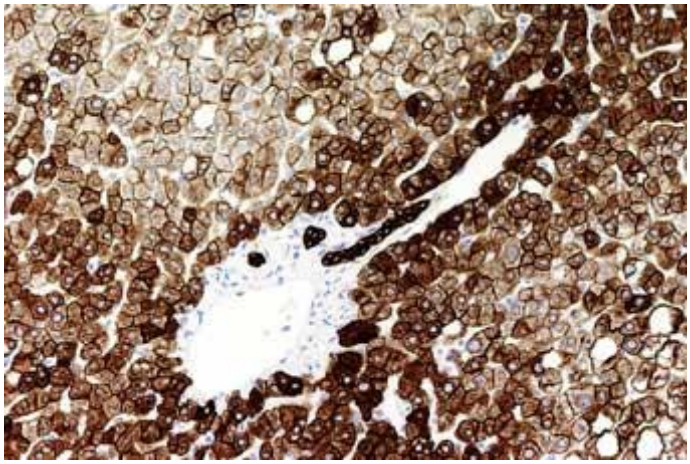

Fig. 1b
Optimal staining of normal liver (same field as in Fig. 1a) using mAb DC10. The bile ducts (centre) are strongly stained, while all liver cells show a strong staining with membranous enhancement.

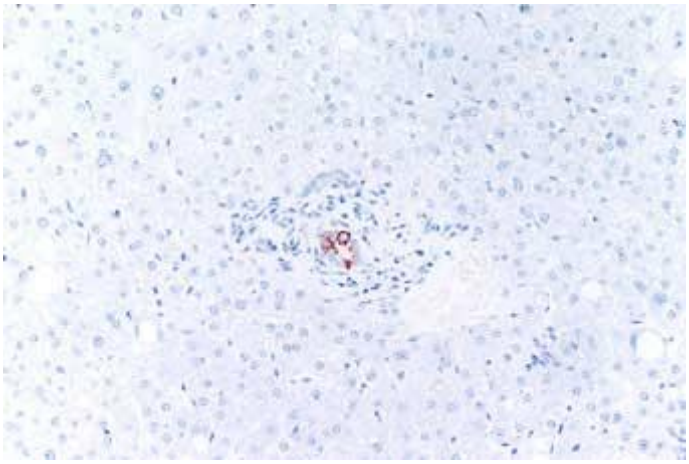

Fig. 1c
Insufficient staining of normal liver (same field as in Fig. 1a) using mAb CAM5.2. The bile duct (centre) is weakly stained, while the liver cells are negative.

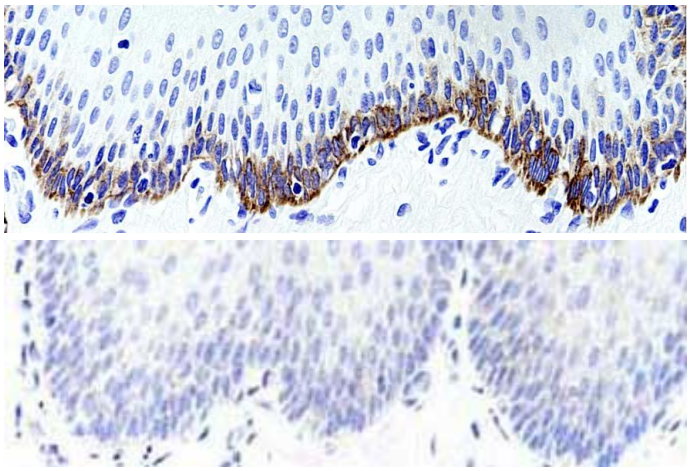

Fig. 2
Normal esophagus mucosa stained with mAb CAM5.2 (above) and DC10 (below). Only CAM5.2 stains basal cells suggesting a reactivity to CK19(!).

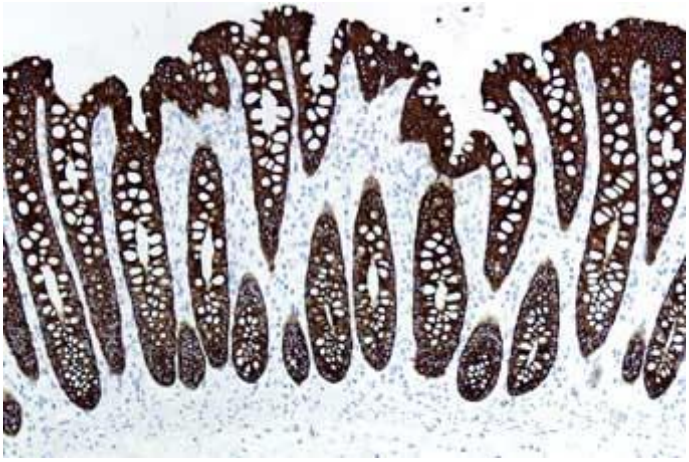

Fig. 3a
Optimal staining of normal colon mucosa using mAb DC10. Normal colon. Strong staining of all epithelial cells.

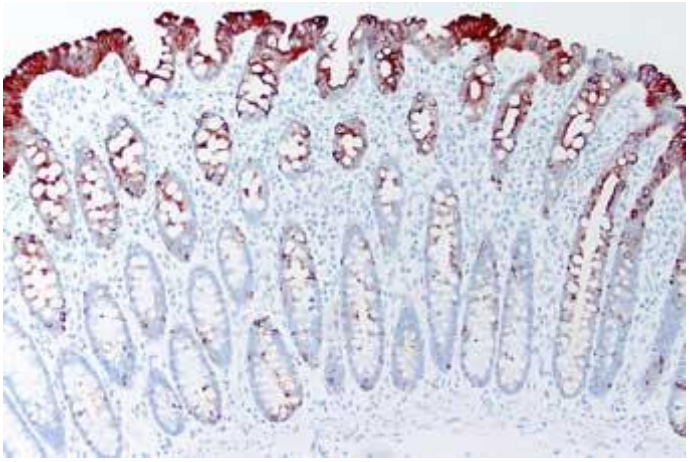

Fig. 3b
Insufficient staining of normal colon mucosa (same field as in Fig. 3a) using mAb CAM5.2. The crypt cells are mainly negative.

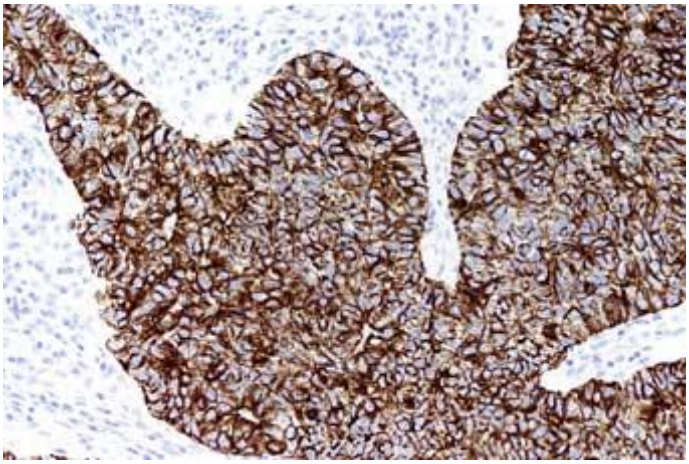

Fig. 4a
Optimal staining of neuroendocrine carcinoma using mAb DC10. All neoplastic cells are strongly stained (same protocol as in Fig. 3a).

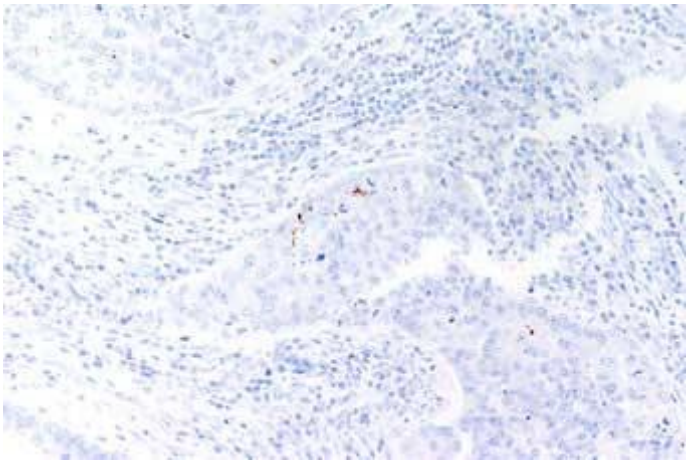

Fig. 4b
Insuffcient staining of neuroendocrine carcinoma (same field as in Fig. 4a) using mAb CAM5.2. The neoplastic cells are almost unstained (same protocol as in Fig. 3b).

SN/MV/LE 17-12-2003