Assessment Run 8 2003

Pan Cytokeratin (CK-PAN)

The slide to be stained for pan-cytokeratin (pan-CK) comprised:
1: liver, 2: lung squamous cell carcinoma, 3: lung small cell lung carcinoma (SCLC), 4: appendix, 5: adrenal gland, 6: testicular seminoma, 7: parotid gland.

Criteria for assessing a pan-CK staining as optimal included: A strong and distinct staining of the large majority of epithelial cells in the appendix, parotid gland, and biliary tract of the liver, while most hepatocytes should reveal at least a moderate membranous staining and the adrenal gland at least a focal staining. In most tumour cells of the squamous cell carcinoma and the small cell lung carcinoma, a strong and distinct staining should be seen, dot-like in the latter The seminoma should focally be strongly labelled. A slight overstaining of cells with many epitopes was accepted (this is almost inevitable in order to avoid false negative stainings).

72 laboratories submitted stainings. At the assesment 15 achieved optimal staining (21 %), 23 good (32 %), 24 borderline (33 %) and 10 (14%) poor staining.
The following pan-CK markers were used:

MNF116	DakoCytomation (14)
KL1	Immunotech (8), Serotec (1), Biomeda (1)
AE1/AE3	DakoCytomation (30), Zymed (2), Boehringer Mannheim (4), Biogenex (1), Biomeda (1)
AE1/AE3 + PCK26	Ventana (3)
AE1/AE3 + 5D3	BioCare (1)
NCL-Pan-CK	Novocastra (4)
Pan-CK Ab2	NeoMarkers (1)
Polyclonal Z0622	DakoCytomation (1)

Optimal stainings were obtained with the clones AE1/AE3 (DakoCytomation), AE1/AE3 + 5D3 (BioCare) and KL1 (Serotec and Immunotech). When using these, all protocols were based on HIER with Tris-EDTA/EGTA pH 9 (14 labs.) or EDTA pH 8 (1 lab.) as the heating buffer.
mAb clone AE1/AE3 with proteolytic pre-treatment (Proteinase K, Pronase E, Trypsin or equivalent) gave inferior results with a consistent negative reaction in cells with scarce amounts of low molecular weight cytokeratins type 8 (i.e., hepatocytes and a subset of acinar cells of the parotid gland). On the other hand the majority of protocols based on proteolytic pre-treatment were able to detect CK in the SCLC and the squamous cell carcinoma.
The cocktail AE1/AE3 + PCK26 (3 labs) was only used with proteolytic pre-treatment. Thus, it was not possible to evaluate the potential of this marker with HIER.
Using mAb clone MNF116 (14 labs.) in combination with proteolytic pre-treatment, a good (but not optimal) result could be obtained in this assessment.

The most frequent causes of insufficient stainings were:
-Inappropriate retrieval (Proteolysis for AE1/AE3)
-Too weak HIER (short heating time and/use of citrate pH 6)
-Too low concentration of primary antibody
-Inappropriate choice of pan-CK antibody
-Use of HIER for mAb MNF116

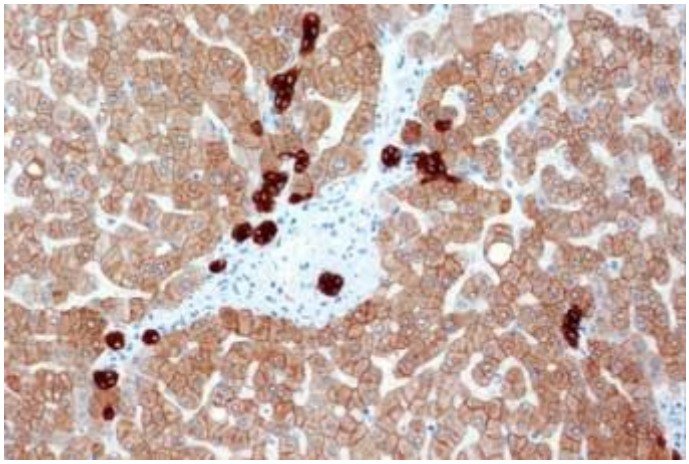

Fig. 1a
An optimal pan-CK staining of the liver using the mAb cocktail AE1/AE3 with HIER as pretreatment. The hepatocytes show a distinct membranous positivity and the bilary ducts are intensively stained. The background is clean.

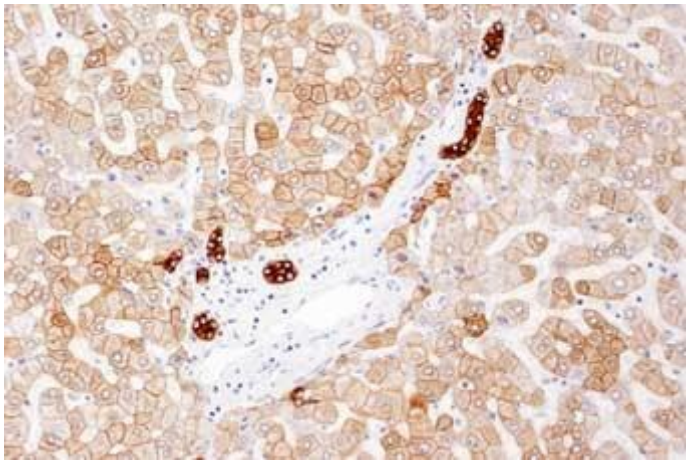

Fig. 1b
A good pan-CK staining of the liver using the mAb mAB MNF116 with proteolytic pretreatment. The majority of the hepatocytes show a distinct membranous positivity and the bilary ducts are strongly stained.

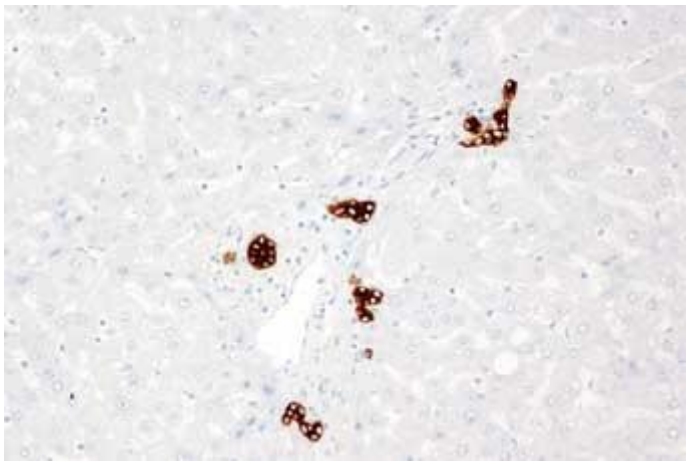

Fig. 1c
An insufficient pan-CK staining of the liver using the mAb cocktail AE1/AE3 with proteolytic pretreatment. The hepatocytes are false negative and only the biliary ducts are stained.

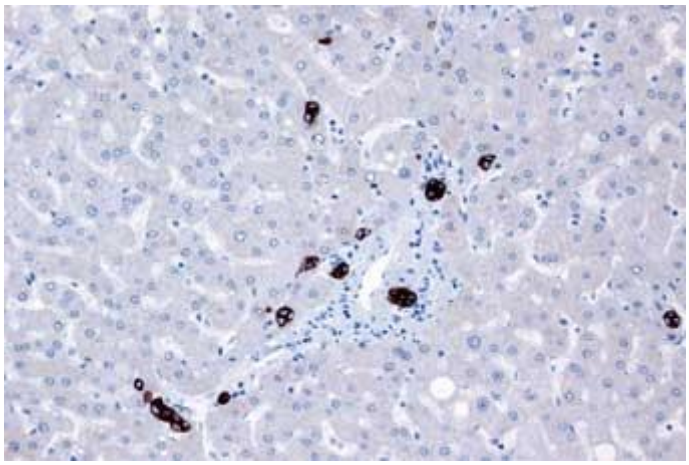

Fig. 1d
An insufficient pan-CK staining of the liver using the mAb cocktail AE1/AE3 with HIER as pretreatment, but with a too low concentration of the primary Ab. The hepatocytes are false negative and only the bilary ducts are stained.

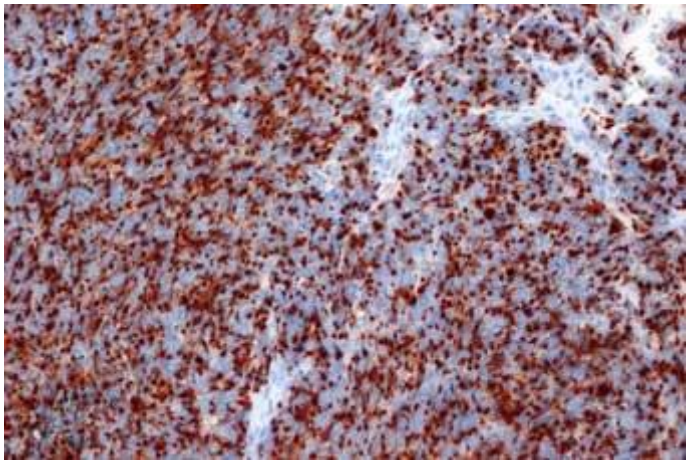

Fig. 2a
An optimal pan-CK staining of the SCLC using the mAb cocktail AE1/AE3 with HIER as pretreatment. All the tumour cells are strongly labelled with a characteristic dot like staining pattern.

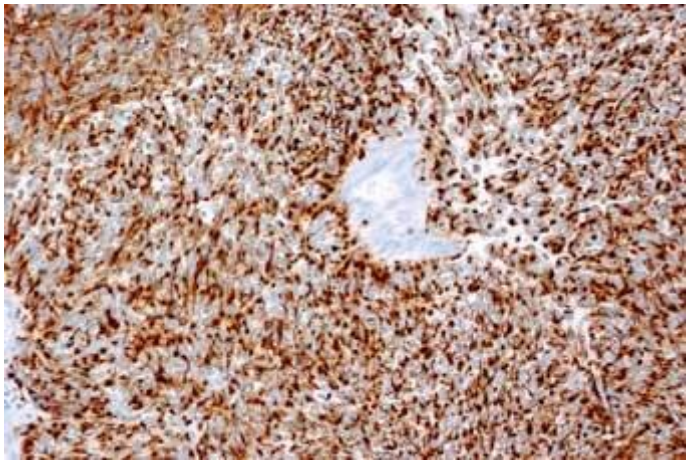

Fig. 2b
A pan-CK staining of the SCLC using the mAb cocktail AE1/AE3 with proteolytic pretreatment. All the tumour cells are strongly labelled with a characteristic dot like staining pattern (However, compare Fig. 1c).

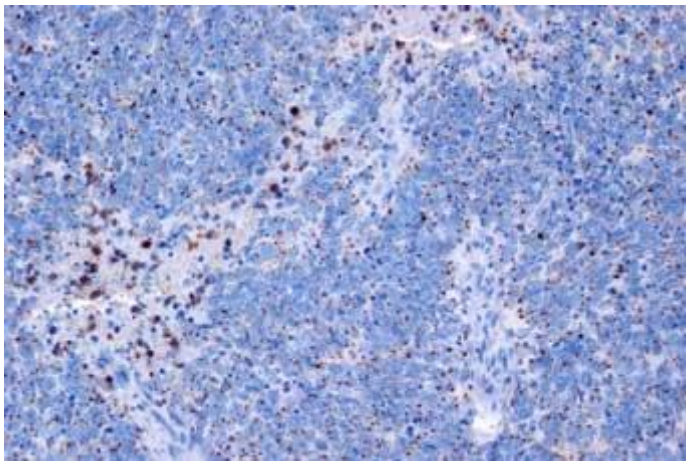

Fig. 2c
A good pan-CK staining of the SCLC using mAb MNF116 with proteolytic pretreatment. The majority of the tumour cells are labelled with a characteristic dot like staining pattern.

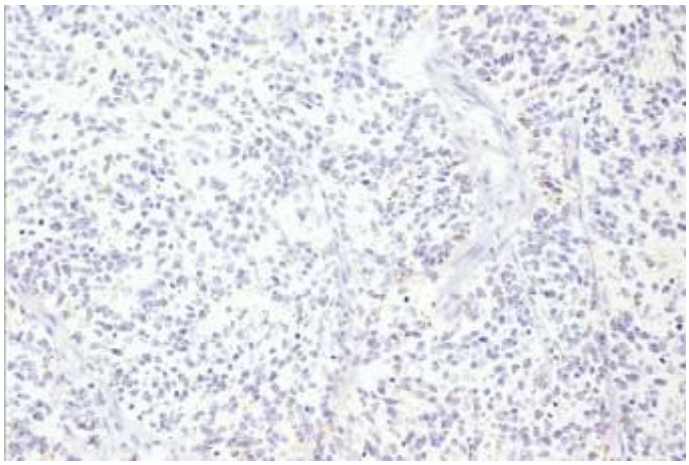

Fig. 2d
An insufficient pan-CK staining of the SCLC using mAb cocktail AE1/AE3 with HIER as pretreatment, but with a too low concentration of the primary Ab. The tumour cells all remain unstained.

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