Assessment Run 8 2003

Cytokeratin 7 (CK7)

The slide to be stained for cytokeratin 7 (CK7) comprised:
1: liver, 2-3: colon adeno-carcinoma, 4: breast ductal carcinoma, 5: Merkel cell carcinoma, 6: stomach fundal mucosa.
Criteria for assessing a CK7 staining as optimal included: A strong and distinct cytoplasmic reaction in the cells expected to stain: tumour cells of the ductal breast carcinoma, bile duct epithelium in the liver, fundic gastric epithelium (heterogeneous staining, particularly chief cells in base of glands, focal staining in surface and foveolar epithelium), the low differentiated colon adenocarcinoma (widespread staining). The well differentiated colon adenocarcinoma should be negative or give a weak focal staining only. Normal liver cells and the Merkel cell tumour should be negative. Normal endothelial cells may be focally positive.

71 laboratories submitted stainings. At the assessment 36 achieved optimal staining (50 %), 26 good (37 %), 7 borderline (10 %) and 2 poor staining (3 %).
The most used Ab for CK7 was mAb OV-TL 12/30 obtained from either DakoCytomation (63), Novocastra (1), BioGenex (1) or Immunotech (1). 3 used mAb clone K72.7 (NeoMarkers), 2 mAb clone LP5K (Novocastra, Ventana), and 1 used mAb clone K72 (Ventana).

Optimal results were achived with mAbs OV-TL 12/30, K72.7 and K72.
Optimal results with mAb OV-TL 12/30 was obtained with proteolytic pre-treatment, HIER and a combination of these two retrieval techniques.
Using proteolytic pre-treatment, optimal stainings were achieved with Protease 1 (Ventana), Pronase E (Sigma) and Proteinase K (DakoCytomation).
Using HIER, optimal stainings were achieved with MWO, pressure cooker, water bath and autoclave, provided the use of Tris-EDTA/EGTA pH 9 or EDTA pH 8 as the heating buffer.
HIER with Citrate pH 6 as the heating buffer only resulted in an optimal result, if the HIER was followed by a (gentle) proteolytic pre-treatment.
The optimal dilution of mAb OV-TL 12/30 was in the range of 1:50 – 500 for both proteolytic pre-treatment and HIER.
mAb clone K72.7 gave an optimal staining using HIER with Tris-EDTA/EGTA pH 9 (2 of 2), while proteolytic pre-treatment appeared inferior (1 of 1).
mAb clone K72 gave an optimal staining using HIER with Citrate pH 6 (1 of 1).

The most frequent causes of insufficient stainings were:
-No pre-treatment used
-HIER with Citrate pH 6 (mAb OV-TL 12/30)
-False positive reaction due to endogenous biotin (HIER combined with a biotin based detection system without biotin blocking)
-Inappropriate choice of antibody for CK7 demonstration.

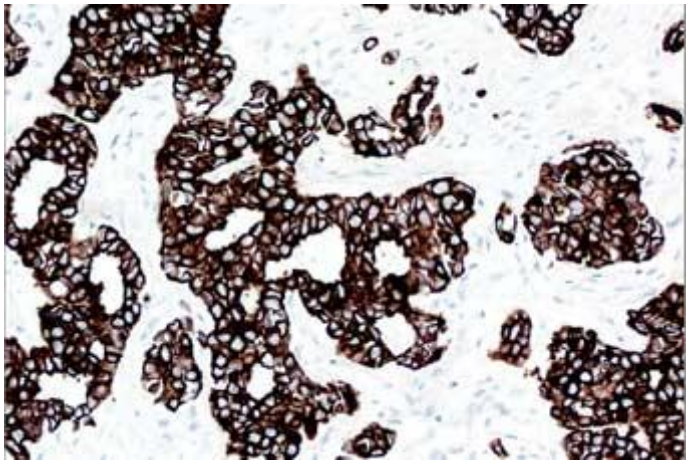

Fig. 1a
Optimal CK7 staining (mAb clone OV-TL 12/30) of the ductal breast carcinoma. All the tumour cells are strongly stained.

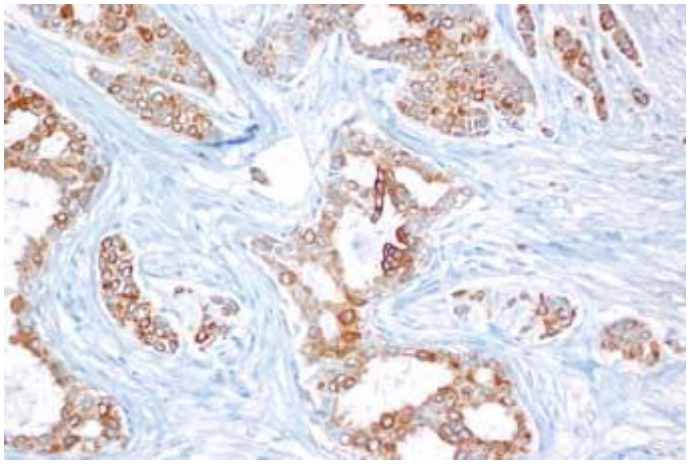

Fig. 1b
CK7 staining (mAb clone OV-TL 12/30) of the ductal breast carcinoma, using an insufficient protocol. The tumour cells are weakly to moderately stained. Furthermore, compare with Fig. 2b.

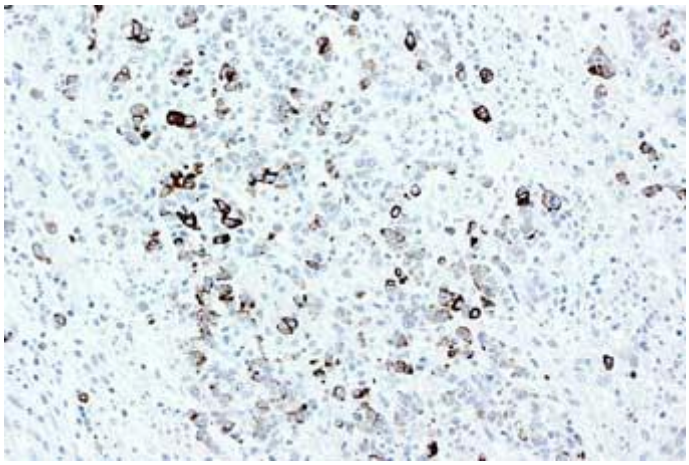

Fig. 2a
Optimal CK7 staining (mAb clone OV-TL 12/30) of the low differentiated colon adenocarcinoma. The tumour cells are stained in a heterogeneous pattern.

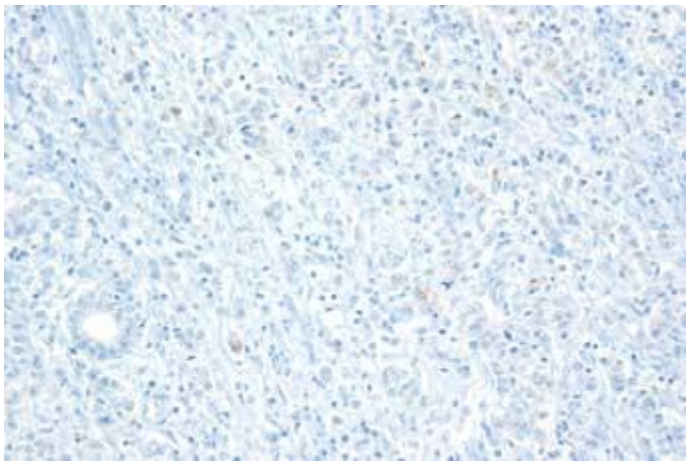

Fig. 2b
Insufficient CK7 staining (mAb clone OV-TL 12/30) of the low differentiated colon adenocarcinoma. Almost all tumour cells are unstained.

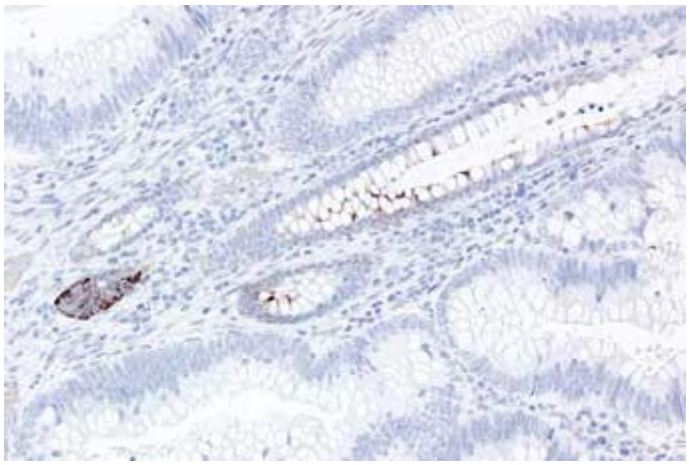

Fig. 3
Optimal CK7 staining (mAb clone OV-TL 12/30) of the well differentiated colon adenocarcinoma. The tumour cells are unstained, while a focal staining of entrapped crypts is seen.

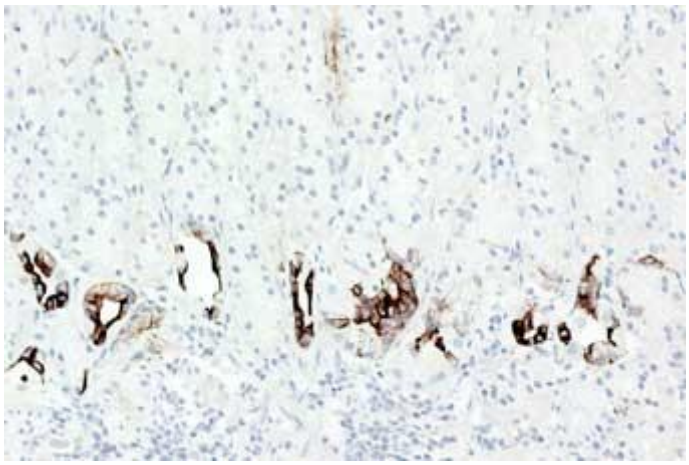

Fig. 4a
Optimal CK7 staining (mAb clone OV-TL 12/30) of the fundic gland bottoms.

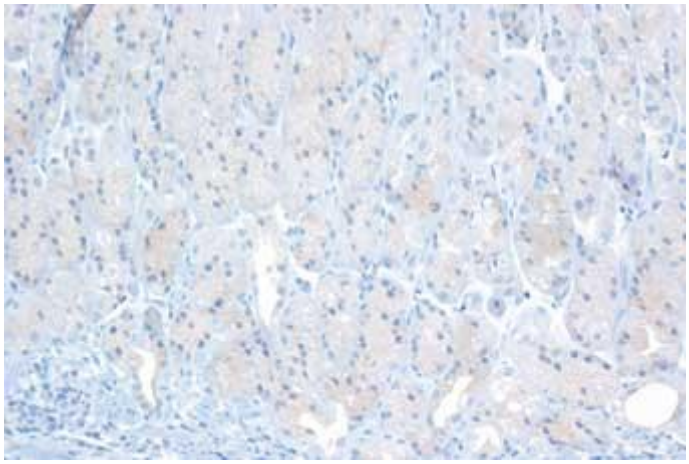

Fig. 4b
Insufficient CK7 staining (mAb clone OV-TL 12/30). Same protocol as in fig. 1b and 2b. The fundic gland bottoms are negative, whereas a weak false positive reaction is seen in the glands due to endogenous biotin. Compare with Fig. 4a.

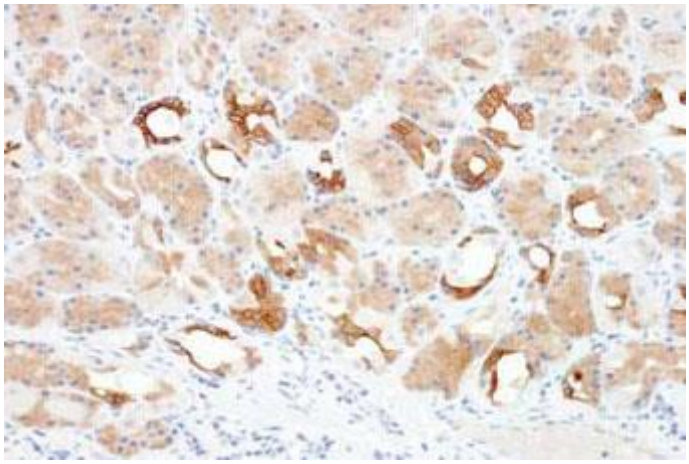

Fig. 4c
Insufficient CK7 staining (mAb clone OV-TL 12/30). The fundic gland bottoms are stained but there is also a strong false positive reaction due to endogenous biotin reaction (biotin based detection system using an efficient HIER procedure without biotin blocking).

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