Assessment Run 8 2003

Cytokeratin 20 (CK20)

The slide to be stained for cytokeratin 20 (CK20) comprised:
1: liver, 2-3: colon adeno-carcinoma, 4: breast ductal carcinoma, 5: Merkel cell carcinoma, 6: stomach fundal mucosa.
Criteria for assessing a CK20 staining as optimal included: A strong and distinct cytoplasmic reaction of the gastric epithelium (surface and foveolae), the well differentiated colon adenocarcinoma (almost all tumour cells), the poorly differentiated colon adenocarcinoma (scattered cells), and the Merkel cell tumour (dot like staining reaction of most cells identified at a low magnification). The liver and ductal breast carcinoma should be negative.

71 laboratories submitted stains. At the assessment 24 achieved optimal staining (34 %), 40 good (56 %), 7 borderline (10 %) and 0 poor staining (0 %).

mAb clone Ks20.8 was used by all laboratories, obtained from either DakoCytomation (62 labs.), NeoMarkers (3 labs.), Novocastra (2 labs.), Ventana (2 labs.), BioGenex (1 lab.) or ProGen (1 lab.).
An optimal staining was only obtained when pre-treatment was used. Laboratories using HIER, proteolytic pre-treatment, or HIER followed by proteolytic pre-treatment achieved almost equal results, when an optimal dilution of the primary antibody was chosen. In the protocols based on HIER, all but one laboratory used Tris-EDTA/EGTA pH 9 as the heating buffer.
Proteinase K and Ventana Protease 1 were the most frequently used enzymes for proteolytic pre-treatment in protocols giving an optimal result.
Using proteolytic pre-treatment the optimal dilution was 1:25 – 1:100 , while using HIER +/- proteolytic pre-treatment the optimal dilution was 1:50 – 200. Almost all laboratories were able to demonstrate CK20 in the well differentiated colon adenocarcinoma, while in the borderline cases the low differentiated colon adenocarcinoma stained negative.

The most frequent causes of insufficient stainings were:
-No pre-treatment used
-Too low or too high concentration of the primary Ab.
-False positive staining reaction due to endogenous biotin (HIER in combination with a biotin based detection system).

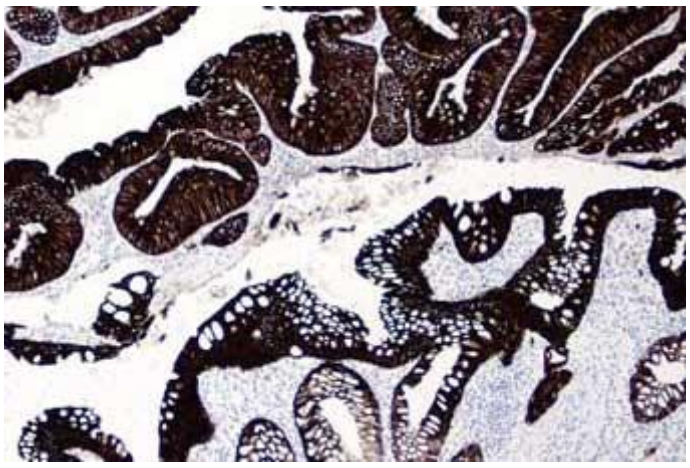

Fig. 1a
An optimal staining for CK20 mAb clone Ks20.8 of the well differentiated colon adenocarcinoma. All the tumour cells are stained without any background reaction.

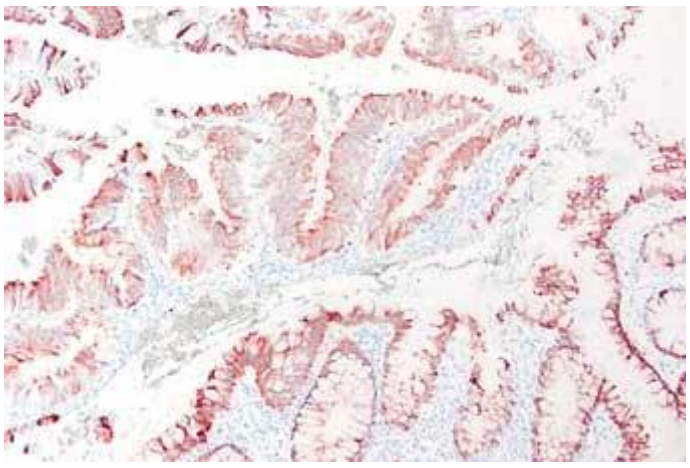

Fig. 1b
An insufficient staining for CK20 mAb clone Ks20.8 of the well differentiated colon adenocarcinoma. The tumour cells are only moderately stained (also compare fig. 2b)

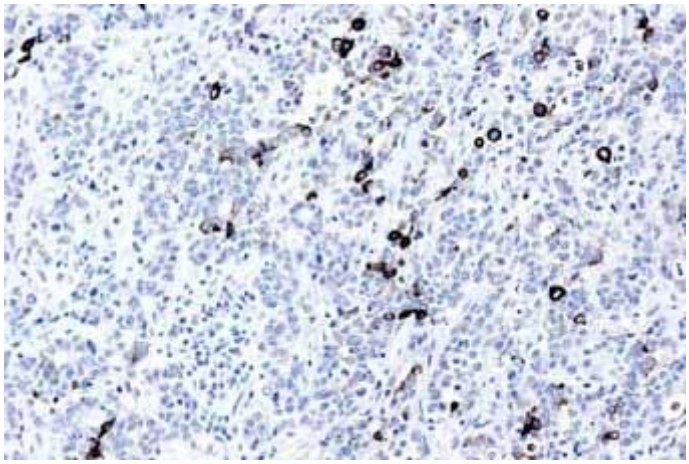

Fig. 2a
An optimal staining for CK20 mAb clone Ks20.8 of the low differentiated colon adenocarcinoma. The staining is focal but strong.

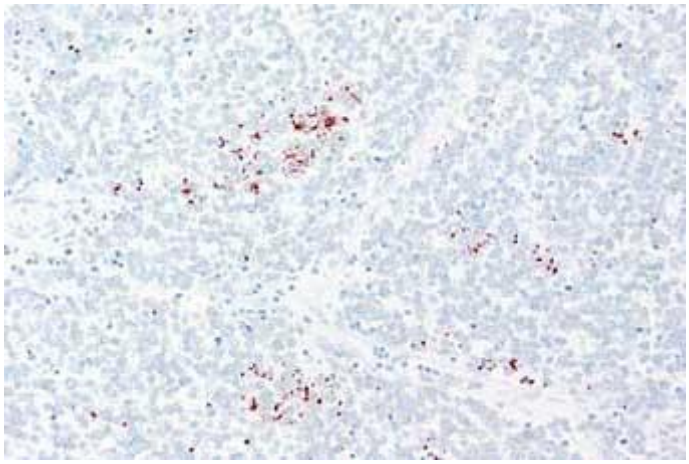

Fig. 2b
An insufficient staining for CK20 mAb clone Ks20.8 of the low differentiated colon adenocarcinoma. The tumour cells are negative. Only an unspecific reaction of the macrophages is seen.

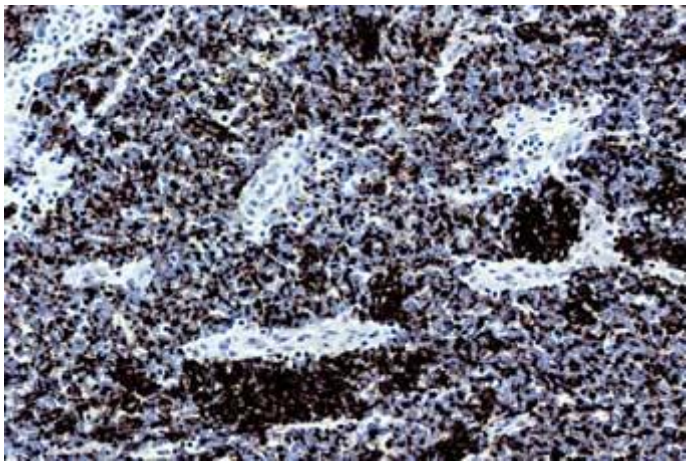

Fig. 3
An optimal staining for CK20 mAb clone Ks20.8 of the Merkel cell tumor. The tumour cells show the charateristic dot like positivity.

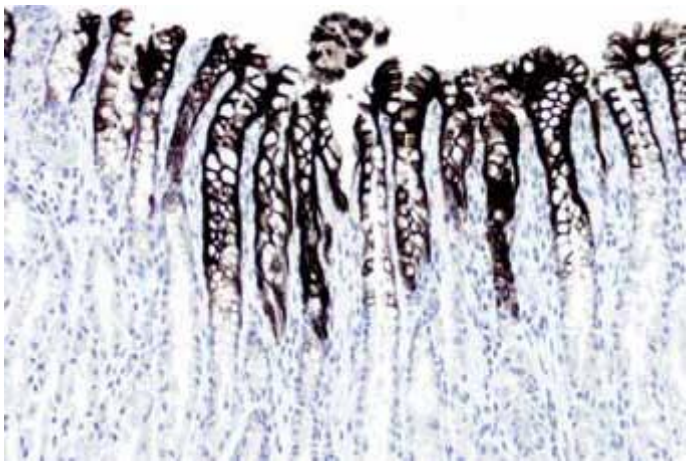

Fig. 4
An optimal staining for CK20 mAb clone Ks20.8 showing a strong and distinct cytoplasmic reaction of the gastric surface epithelium.

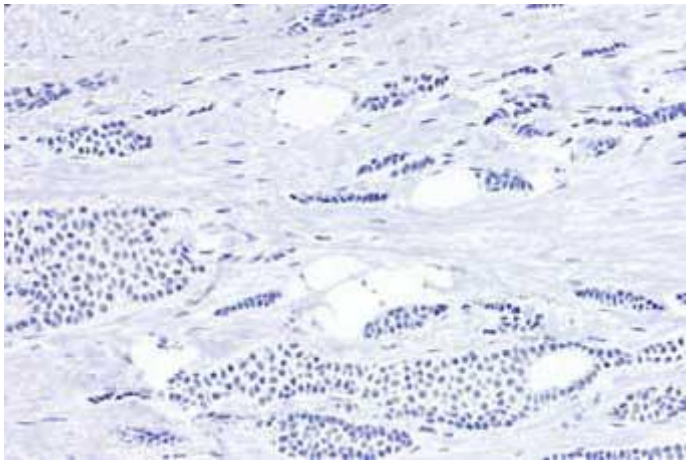

Fig. 5a
An optimal staining for CK20 mAb clone Ks20.8 in of the breast carcinoma. The tumour cells are all negative.

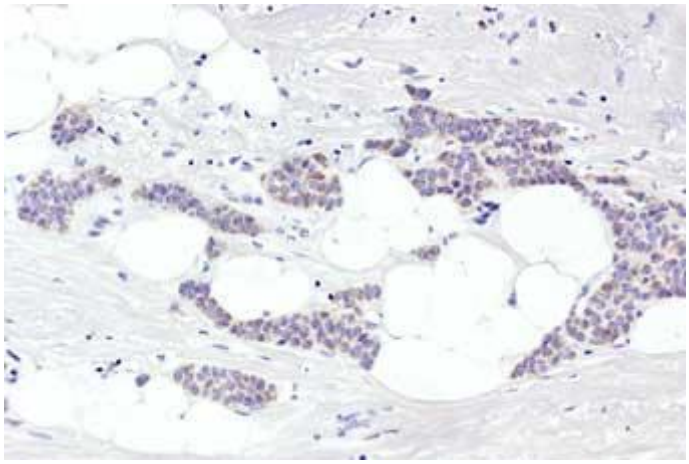

Fig. 5b
An insufficient staining for CK20 in the tumour cells of the breast carcinoma. The tumour cells are weakly positive due to endogenous biotin. A biotin based detection system has been used in combination with an efficient HIER procedure but without suppression of endogenous biotin.

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