Assessment Run 12 2004

Carcinoembryonic antigen (CEA)

The slide to be stained for Carcinoembryonic antigen (CEA) comprised:
A. Liver, B. Appendix, 1. Esophagus, 2. Appendix, 3. Malignant mesothelioma, epithelial type, 4. Colon adenocarcinoma, high grade, 5. Colon adenocarcinoma, low grade.

Criteria for assessing a CEA staining as optimal included: A moderate to strong, distinct cytoplasmic and membranous staining of enterocytes of the appendiceal mucosa and the superficial squamous epithelial cells of the oesophagus, as well s a strong, distinct cytoplasmic and membranous staining of the two colon adenocarcinomas. Staining reaction should not be seen in any other cells including neutrophils, histiocytes (with the exception of those in the vicinity of CEA-positive tumour cells), liver cells (bile canaliculi) or the malignant mesothelioma.

If a slight staining reaction in neutrophils and histiocytes was seen in an otherwise optimal staining, it was accepted as 'good'. In case of strong reaction in neutrophils and histiocytes as well as staining of bile canaliculi (as demonstrated with all polyclonal antibodies [pAbs] and a few monoclonals [mAbs]), the antibody was considered inappropriate, because of its capability of cross reacting with other CEA-like proteins.

75 laboratories submitted stains. Of these 15 used a CEA antibody considered inappropriate. Assessing the remaining 60 stains, 37 were found optimal (61 %), 15 good (25 %), 7 borderline (12 %) and 1 (2 %) poor.

The following appropriate mAbs were used:
Clone II-7 from DakoCytomation (56)
Clone Col-1 from Zymed (n=3)
Clone BW 431/26 from Behring (n=1).

In this assessment optimal staining could be obtained both with clone II-7 (36 out of 56 (64 %)) and clone Col-1. (1 out of 3).

To obtain an optimal staining with clone II-7, all used HIER with either Tris-EDTA/EGTA pH 9 as the heating buffer, (29 out of 36 (81%) were optimal), Citrate pH 6 – 7,3 (4 out of 10 (40%) were optimal), TRS pH 6 (DakoCytomation)(3 out of 3 were optimal), or CC1 (Ventana Benchmark)(1 out of 2 was optimal). None of the 4 laboratories using proteolytic pre-treatment obtained an optimal staining.
The mAb clone II-7 was typically used in a dilution of 1:50 – 1:500.
The optimal protocol for the mAb clone Col-1 was based on HIER using Tris-EDTA/EGTA pH 9 as the heating buffer with a primary antibody dilution of 1:100.

The insufficient stains typically displayed a positive cytoplasmic reaction of the high grade colon adenocarcinoma, whereas the membranous reaction of the low grade colon adenocarcinoma generally was too weak or totally negative.

When excluding the inappropriate stains, the most frequent causes of insufficient staining were:
- Inappropriate epitope retrieval (proteolytic pre-treatment)
- Omission of epitope retrieval
- Too low concentration of the primary antibody.

The following mAbs were considered inappropriate for the above mentioned reasons: clone 12-140-10, clone B01-94-11M, clone C260, clone TF-3H8-1, and clone ZC23.
Using an inappropriate CEA-antibody, neutrophils, histiocytes and sometimes bile canaliculi were stained, and a heterogeneous staining reaction of the malignant mesothelioma was observed as well.

In 27 studies (Ordóñez, AJSP 2003;27:1031) comprising a total of 878 cases of malignant mesothelioma (MM), 58 cases (7%) were found positive. However, in 14 of the studies, comprising 458 cases of MM, no CEA positive case was found, while in the remaining 13 studies, 3-45% of the cases were positive! The present study emphasizes the importance of selecting the right mAbs.

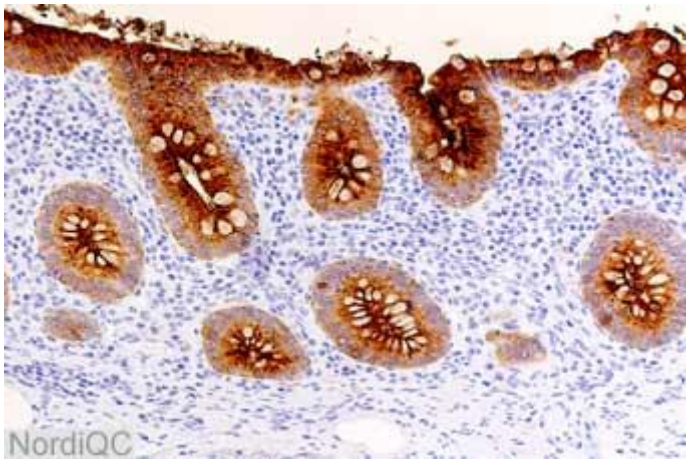

Fig. 1a
Optimal CEA staining of the appendix using clone II7. The enterocytes of the appendiceal mucosa reveal a strong apical cytoplasmic staining and a weaker basal reaction. No reaction is seen in neutrophile granulocytes.

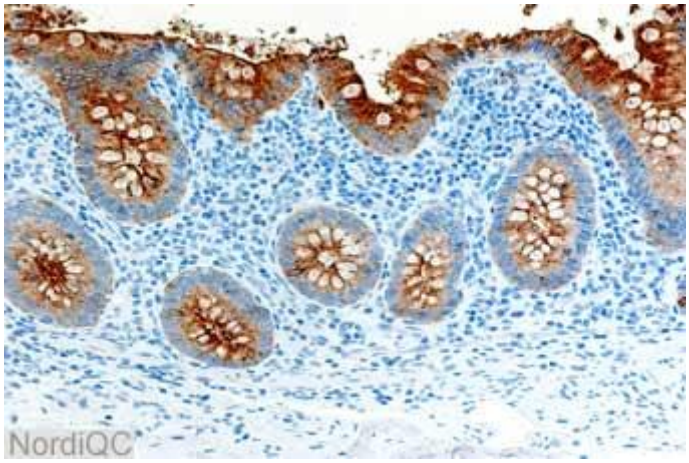

Fig. 1b
CEA staining of the appendix using clone II7 and an insufficient protocol (same field as in Fig 1a). The enterocytes reveal a moderate apical cytoplasmic staining but almost no staining of the basal part of the cells. Compare with Fig. 3b.

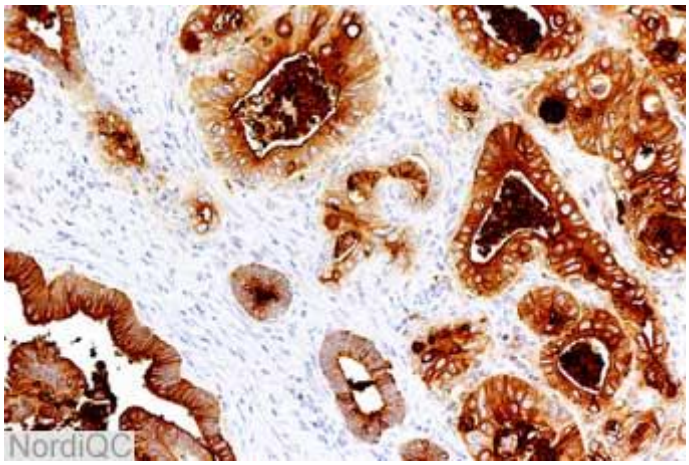

Fig. 2a
Optimal CEA staining of the low grade colon adenocarcinoma. A moderate to strong cytoplasmic staining is seen in almost all of the neoplastic cells. Same protocol as in Fig. 1a.

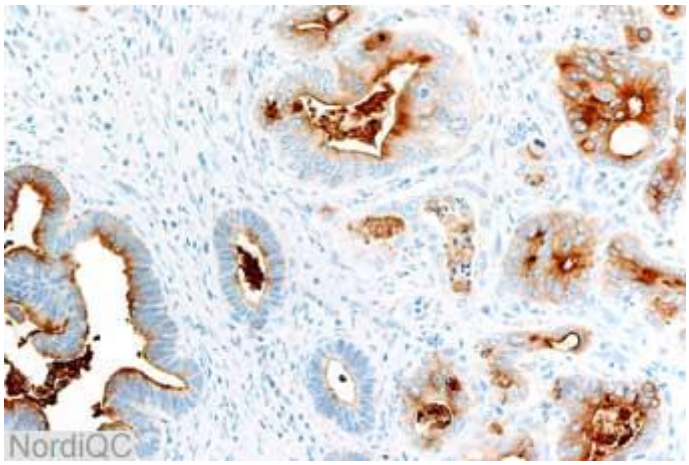

Fig. 2b
Insufficient CEA staining of the low grade colon adenocarcinoma. The staining is considerable weaker than in Fig. 2a (same field). Also compare Fig. 3b. Same protocol as in Fig. 1b.

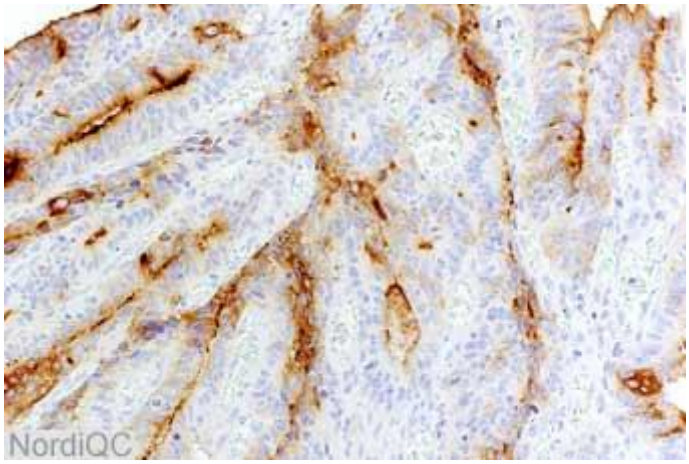

Fig. 3a
Optimal CEA staining of the high grade colon adenocarcinoma. A moderate predominantly membranous staining is seen in the majority of the neoplastic cells. Same protocol as in Fig. 1a.

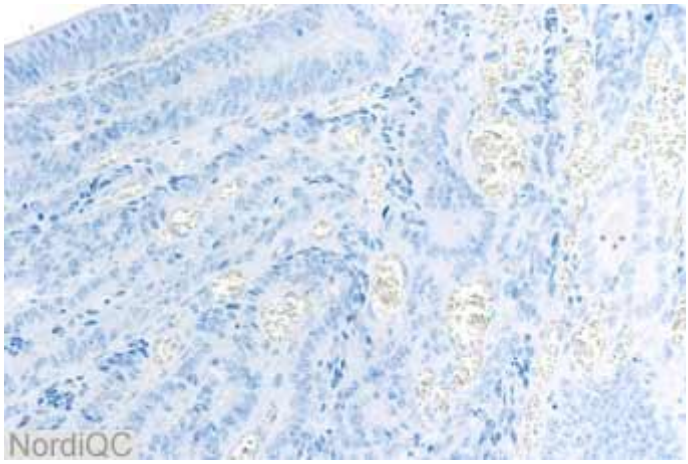

Fig. 3b
Insufficient CEA staining of the high grade colon adenocarcinoma. The neoplastic cells are all virtually negative. Compare with Fig. 3a (same field). Same protocol as in Fig. 1b and 2b.

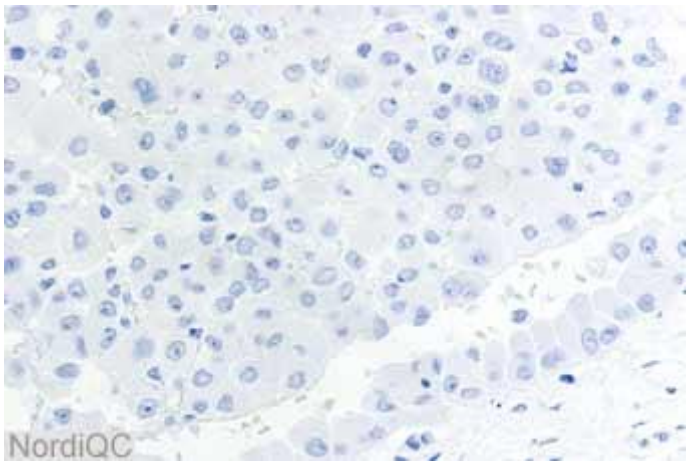

Fig. 4a
Optimal CEA staining of the malignant mesothelioma. No reaction is seen in the neoplastic cells. Same protocol as in Fig. 1a.

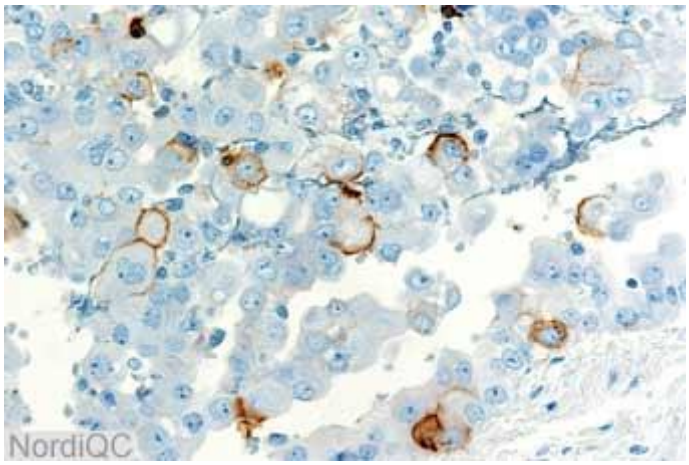

Fig. 4b
Staining of the malignant mesothelioma using an inappropriate monoclonal CEA Ab. A proportion of the neoplastic cells reveal staining.

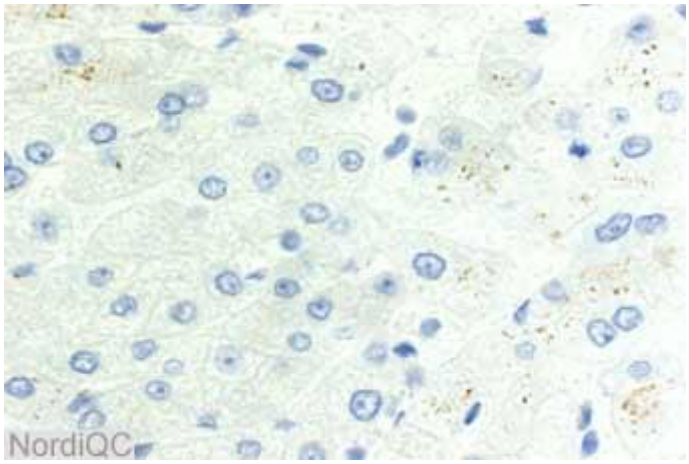

Fig. 5a
Optimal CEA staining of the liver. No staining reaction is seen in the bile canaliculi or Kuppfer cells. Same protocol as in Fig. 1a.

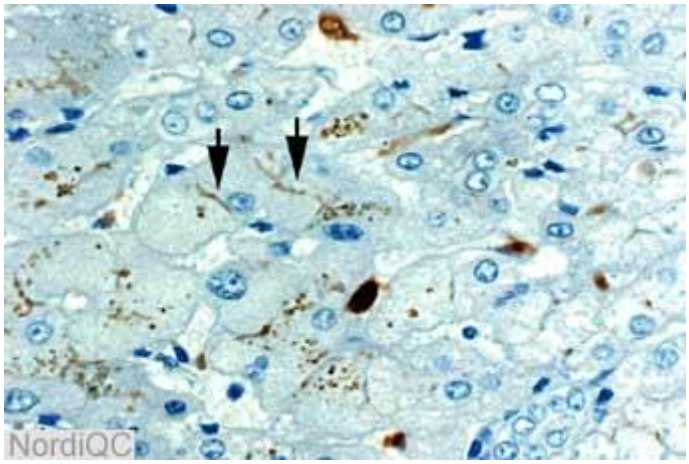

Fig. 5b
Staining of the liver using an inappropriate monoclonal CEA Ab. Kuppfer cells and bile canaliculi also are stained.

SN/MV/LE 28-11-2004