Assessment Run 10 2004

Epitelial Membrane Antigen (EMA)

The slide to be stained for epithelial membrane antigen (EMA) comprised:
1. Peritoneal malignant mesothelioma, 2. Lung adenocarcinoma, 3. Meningioma, 4. Glioblastoma, 5. Tonsil.

Criteria for assessing an EMA staining as optimal included:
A strong and distinct predominantly membranous staining of the malignant mesothelioma, a strong cytoplasmic staining of the lung adenocarcinoma and squamous epithelium of the tonsil, a heterogenous predominantly membranous staining of the meningioma, and a widespread dot-like cytoplasmic staining of the glioblastoma.

78 laboratories submitted stainings. At the assessment 20 achieved optimal staining (26 %), 26 good (33 %), 14 borderline (17 %) and 19 poor staining (24%).

The following mAbs were used:
clone E29 (DakoCytomation, n=70; Cell Margue, n=1)
clone Mc5 (Ventana, n=4; BioGenex, n=1)
clone ZCE113 (Zymed, n=1)
clone GP1.4 (Novocastra, n=1)

In this assessment optimal stainings could only be obtained with mAb clone E29 and the use of HIER (primarily MWO with Tris-EDTA/EGTA pH 9 as the heating buffer, 17/20). E29 was used in the range of 1:40 – 2.000 depending on the total sensitivity of the used protocol.

In almost all protocols, the neoplastic cells of the lung adenocarcinoma and the malignant mesothelioma were stained appropriately whereas the demonstration of EMA in the meningioma and especially the dot-like positivity of the neoplastic cells of the glioblastoma was achieved only in the stainings based on optimal protocols. With the insufficient protocols the normal perineurial cells and plasmacells were weakly stained or unstained indicating that these cells may serve as a reliable positive control for EMA.

The most frequent causes of insufficient stainings (often in combination) were:
- Inappropriate choice of primary Ab
- No epitope retrieval or proteolytic pretreatment
- Insufficient HIER (too short heating time, particularly in combination with citrate pH 6)
- Too low concentration of the primary antibody.

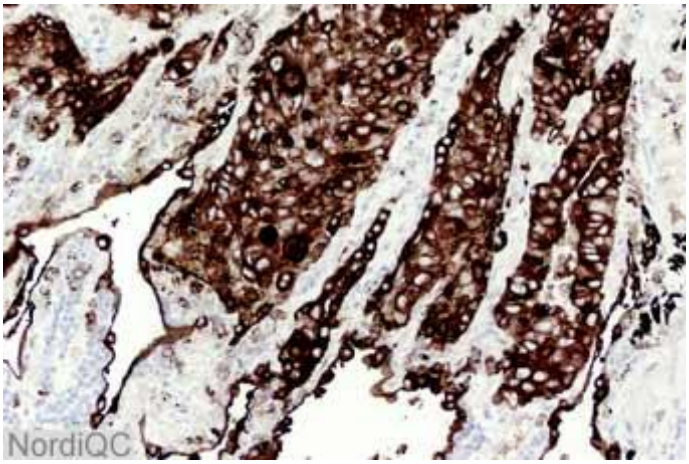

Fig. 1a
Optimal EMA staining (mAb clone E29) of the lung adenocarcinoma. All the tumour cells show a strong cytoplasmic staining.

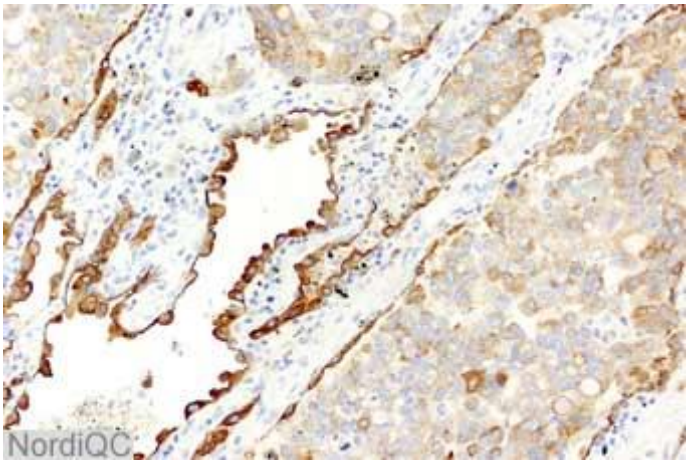

Fig. 1b
Insufficient EMA staining of the neoplastic cells in the lung adenocarcinoma. The cells are weakly stained. However, compare with Fig. 3b and 4b (same protocol).

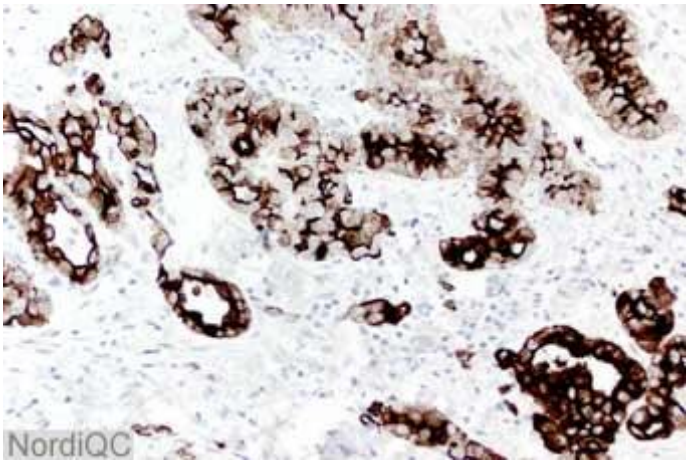

Fig. 2a
Optimal EMA staining (mAb clone E29) of the malignant mesothelioma. All the tumour cells show a strong predominantly membranous staining, but focally also a cytoplasmic staining is seen.

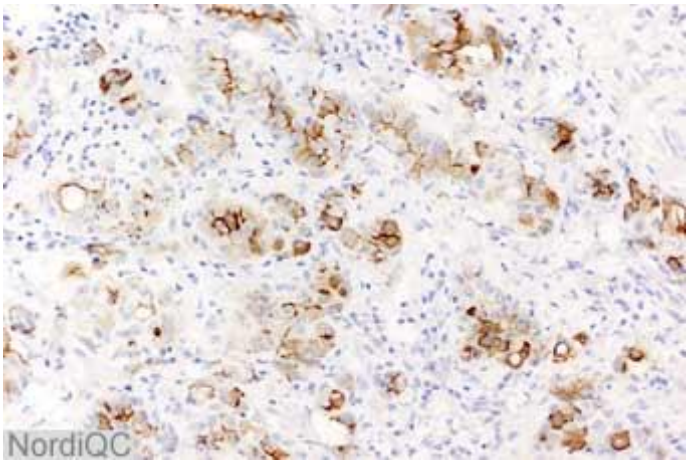

Fig. 2b
Insufficient EMA staining of the malignant mesothelioma. The tumour cells show a moderate membranous staining. However, compare with Fig. 3b and 4b (same protocol).

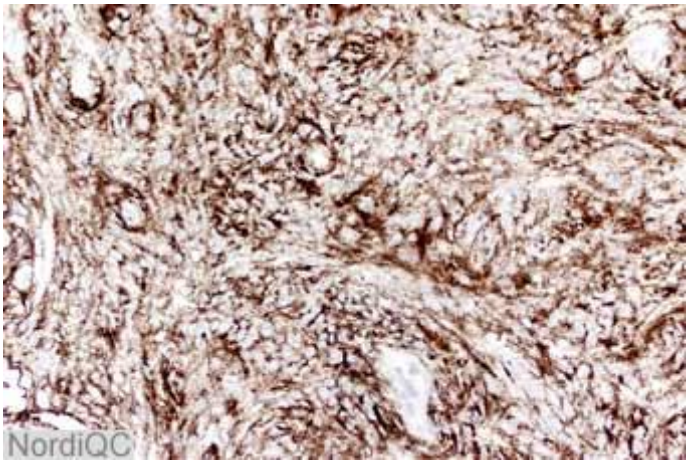

Fig. 3a
Optimal EMA staining (mAb clone E29) of the meningioma. Almost all of the cells show a strong staining.

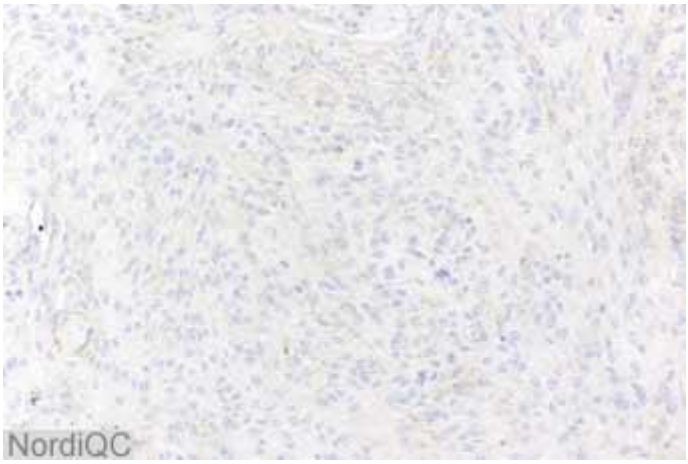

Fig. 3b
Insufficient EMA staining of the meningioma. The tumour is virtually unstained (same protocol as in Fig. 1b and 2b).

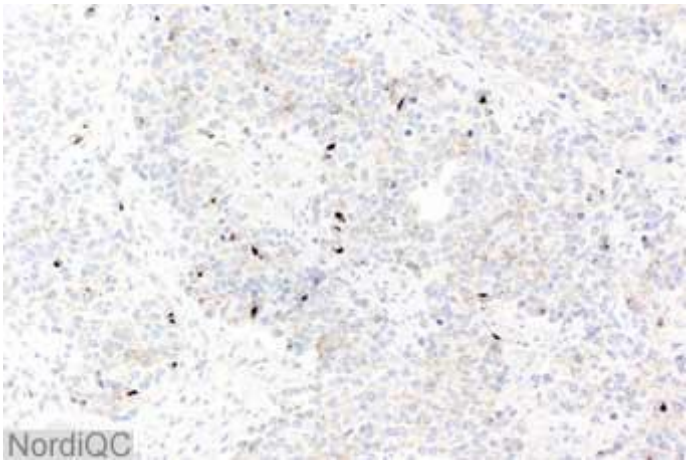

Fig. 4a
Optimal EMA staining (mAb clone E29) of the neoplastic cells in the glioblastoma. Most tumour cells show a weak cytoplasmic reaction. Focally the cells reveal a strong dot-like reaction.

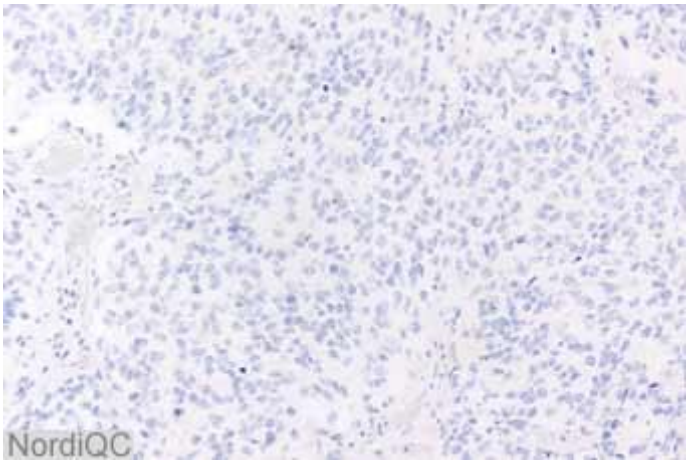

Fig. 4b
Insufficient EMA staining of the glioblastoma. The tumour is virtually unstained (same protocol as in Fig. 1b and 2b)

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