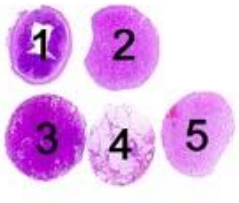
Assessment Run 17 2006

Epithelial cell-cell adhesion molecule (Ep-CAM)

The slide to be stained for Epithelial protein Cell Adhesion Molecule (Ep-CAM) comprised:
1. Appendix, 2. Kidney, 3. Lung adenocarcinoma, 4. Malignant mesothelioma, epithelial subtype, 5. Clear cell renal carcinoma. All specimens were fixed in 10 % NBF.



Criteria for assessing an Ep-CAM staining as optimal included:

- A strong, distinct predominantly membranous staining of the columnar epithelial cells in the appendix, the renal collecting tubules, and the Bowman capsule
- A strong, distinct predominantly membranous staining of the lung adenocarcinoma and the clear cell renal carcinoma.

74 laboratories submitted stains. At the assessment 17 achieved optimal marks (24 %), 21 good (30 %), 17 borderline (24 %) and 15 (21 %) poor marks. Four used an inappropriate Ab (low molecular weight cytokeratin) and were not assessed.

The following Abs were used:
mAb clone **Ber-EP4** (Dako, n=65)
mAb clone **MOC31** (Dako, n=4; Euro-diagnostika, n=1)

Optimal staining for Ep-CAM in this assessment was obtained with both the mAb clone **Ber-EP4** (16 out of 65) and the mAb clone **MOC31** (1 out of 5). In optimal stains, no differences in staining patterns were seen between these two Abs.

With mAb clone **Ber-EP4** the protocols giving an optimal staining result were based on heat induced epitope retrieval (HIER) either as single pre-treatment or combined with proteolytic pre-treatment.

Using HIER as single pre-treatment, only Target Retrieval Solution pH 6,1 (TRS, Dako) could be used as HIER buffer to obtain an optimal result. Using HIER in TRS the mAb clone **Ber-EP4** typically was diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed. In these settings 14 out of 32 (44 %) were assessed as optimal and all 32 were sufficient (optimal or good). Using a combined pretreatment, HIER in TRS was followed by Proteinase K. Ber-EP4 was diluted 1:100. Using these settings 2 out of 2 obtained an optimal mark.

With mAb clone **MOC31** the optimal result was based on HIER in TRS, the Ab was diluted 1:50.

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary Ab
- Inappropriate pretreatment (i.e., HIER in other buffers than TRS; proteolytic pretreatment)

The prevalent feature of an insufficient staining was a too weak or false negative staining of the lung adenocarcinoma and the clear cell renal carcinoma. In general almost all laboratories were capable of demonstrating Ep-CAM in the columnar epithelial cells of the appendix, indicating that appendix/colon cannot be recommended as a reliable positive control for Ep-CAM. In this assessment normal kidney was an applicable and valid quality indicator for Ep-CAM, as the ability to demonstrate Ep-CAM in the Bowman's capsule and the collecting tubules was characteristic for laboratories obtaining an optimal mark, and the structures typically were negative in the insufficient stains. In this assessment the malignant mesothelioma was positive for Ep-CAM irrespective of the immunohistochemical method applied and the assessment mark obtained, stressing that markers for Ep-CAM should be used in a broad Ab panel for the differential diagnosis of mesothelioma and adenocarcinoma. Actually several laboratories were not capable to detect Ep-CAM in the lung adenocarcinoma but demonstrated Ep-CAM in the malignant mesothelioma!

It should be noticed that the most frequent cause of insufficient staining in this assessment was the choice of pre-treatment. HIER in other buffers than TRS or proteolytic pre-treatment gave an insufficient result in 30 out 32 laboratories. This observation is in concordance with the previous assessment of Ep-CAM/Ber-EP4 in run 6 in

which only 3 out of 26 obtained an optimal mark using proteolytic pre-treatment, compared to HIER in Target Retrieval Solution pH 6.1 which resulted in an optimal mark for 13 out of 18 laboratories.

Ep-CAM was also assessed in Run 6 2002, in which 48 laboratories participated. Out of these 11 laboratories (23 %) had an insufficient staining. The proportion of insufficient staining in this run increased to 32 out of 70 laboratories (45 %). The main cause for the decrease in pass rate is most likely the large number of new participants, which almost all used an inappropriate or less efficient pre-treatment. Using an appropriate pre-treatment as HIER in TRS all of 34 laboratories obtained a sufficient result.

Conclusion
The mAb clones **Ber-EP4** and **MOC31** are applicable markers for Ep-CAM. HIER in TRS pH 6.1 is highly recommended for optimal performance with both Abs. Kidney should be the preferred positive control.

Erratum: In a previous version of this page, it was indicated that the proximal tubules expressed Ep-CAM. However, the staining reaction is in the collecting tubules, as also illustrated in Fig. 4a and described in the Epitopes section (Ep-CAM).

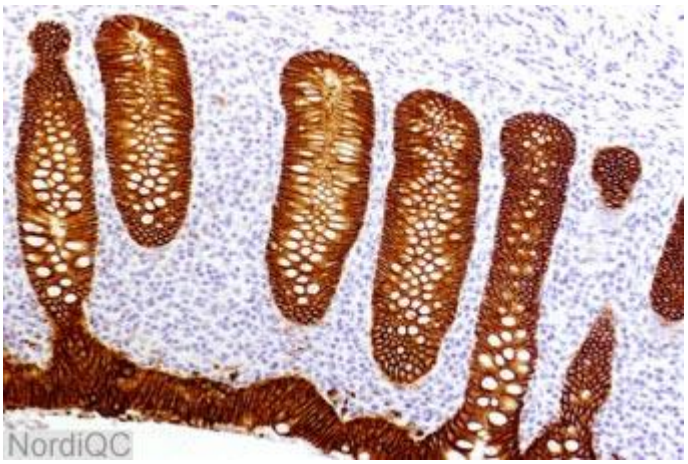


Fig. 1a
Optimal staining for Ep-CAM (mAb clone Ber-EP4) in the appendix. The enterocytes show a strong distinct predominantly membraneous staining. The protocol is based on HIER in TRS pH 6,1.

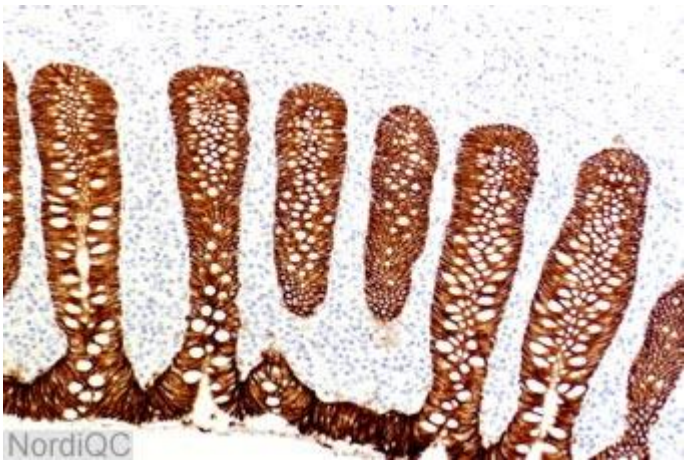


Fig. 1b
Staining for Ep-CAM (mAb clone Ber-EP4) in the appendix using an insufficient protocol. The enterocytes are well demonstrated. However, compare with Fig. 2b – same protocol (based on proteolytic pre-treatment).

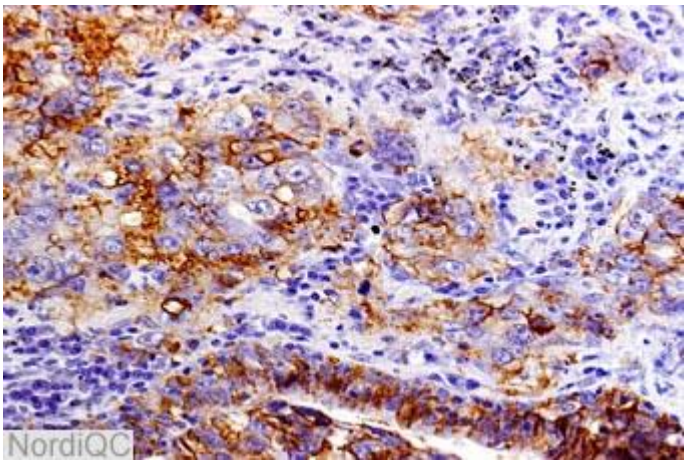


Fig. 2a
Optimal staining for Ep-CAM of the lung adenocarcinoma. The majority of tumour cells show a moderate to strong, distinct staining (same protocol used in Fig. 1a). Remnants of the bronchiolar cells (lower right corner) are also demonstrated.

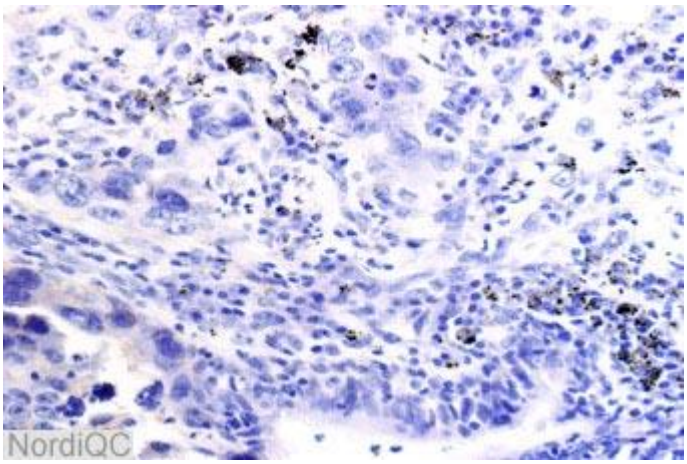


Fig. 2b
Insufficient staining for Ep-CAM of the lung adenocarcinoma. The tumour as well as the bronchiolar cells are negative (same protocol as in Fig. 1b). The false negative reaction may be due to a lower Ep-CAM antigen expression compared to the appendix and/or a longer fixation time in NBF.

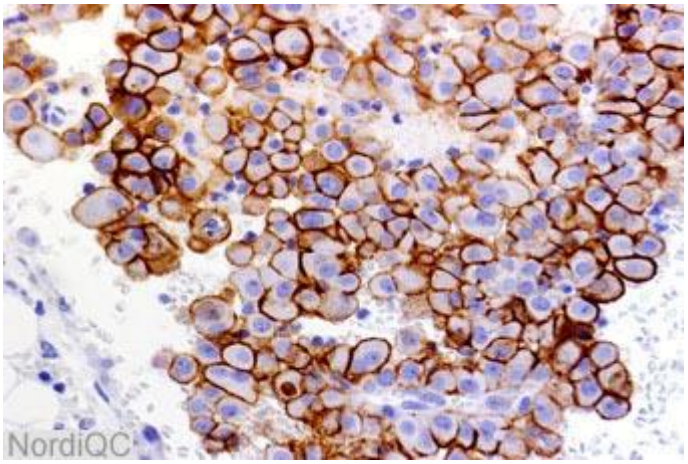


Fig. 3a
Optimal staining for Ep-CAM in the malignant mesothelioma using same protocol as in Fig 1a and 2a. The majority of the neoplastic cells show a strong, distinct membraneous staining.

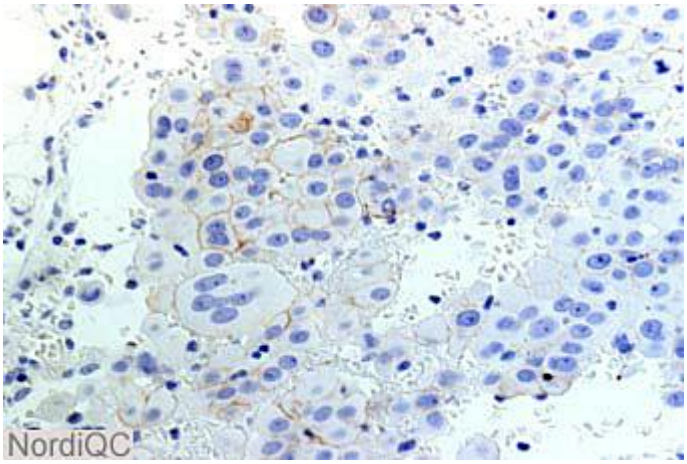


Fig. 3b
Insufficient staining for Ep-CAM in the malignant mesothelioma using same protocol as in Fig 1b and 2b. A faint membraneous staining of some tumour cells is seen.

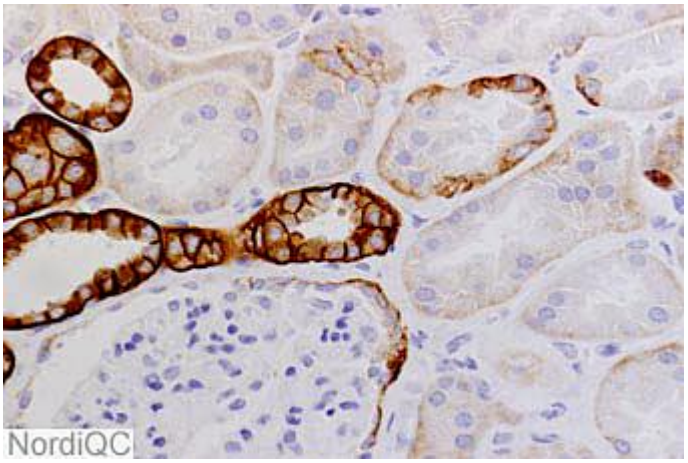


Fig. 4a
Optimal staining for Ep-CAM in the normal kidney using same protocol as in Fig 1a-3a. A quality indicator for Ep-CAM is the ability to demonstrate Ep-CAM in the renal collecting tubules (basolateral reaction) and the Bowman capsule.

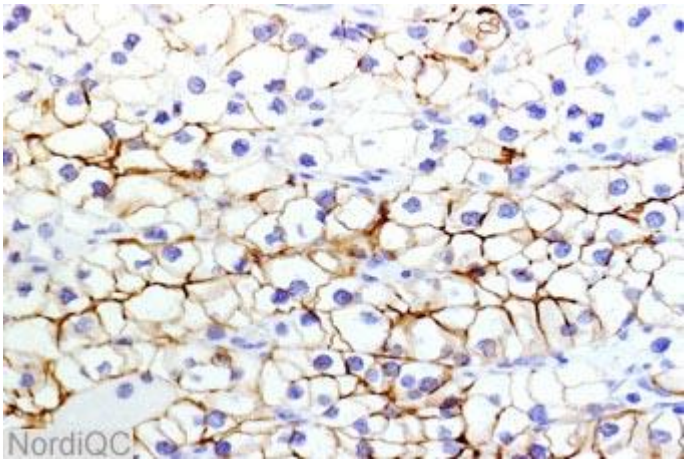


Fig. 4b
Optimal staining for Ep-CAM in the renal clear cell carcinoma using same protocol as in Fig 1a-4a. The majority of the neoplastic cells show a distinct membraneous reaction.

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