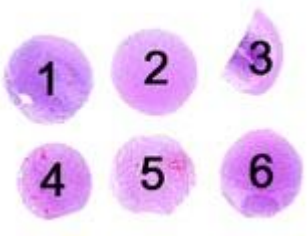
Assessment Run 23 2008

Epithelial cell-cell adhesion molecule (Ep-CAM)

The slide to be stained for epithelial cell-cell adhesion molecule (Ep-CAM) comprised:
1. Colon adenocarcinoma, 2. Kidney, 3. Appendix, 4. Clear cell renal cell carcinoma, 5. Chromophobe renal cell carcinoma, 6. Ileal carcinoid.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a membranous Ep-CAM staining as optimal included:

- A strong, distinct, predominantly membranous staining of virtually all the columnar epithelial cells in the appendix.
- A moderate to strong predominantly membranous staining of the epithelial cells of the renal collecting tubules, and of scattered epithelial cells lining the Bowman capsule.
- A strong, distinct, predominantly membranous staining of virtually all the neoplastic cells of the colon adenocarcinoma and the ileal carcinoid.
- A moderate to strong predominantly membranous staining of the majority of the neoplastic cells of the two renal cell carcinomas.

83 laboratories submitted stains. 5 laboratories used an inappropriate Ab (such as low molecular weight cytokeratin). At the assessment of 78 laboratories, 29 achieved optimal marks (37 %), 20 good (26 %), 13 borderline (17 %) and 16 poor marks (20 %).

The following Abs were used:
mAb clone **Ber-EP4** (Dako, n=67; NeoMarkers/Thermo Scientific, n=4)
mAb clone **MOC-31** (Dako, n=4; Novocastra, n=1)
mAb clone **KS1/4** (BD Pharmingen, n=1)
mAb clone **VU-1D9** (Euro-Diagnostica, n=1)

Optimal staining for Ep-CAM in this assessment was obtained with the mAb clones **Ber-EP4** (27 out of 71), **MOC-31** (1 out of 5) and **VU-1D9** (1 out of 1).

With mAb clone Ber-EP4 the optimal protocols were based on either heat induced epitope retrieval (HIER) or enzymatic pre-treatment. HIER was the most successful epitope retrieval method (Table 1).

Table 1. **The proportion of sufficient and optimal results with HIER and proteolytic pre-treatment, respectively.**

	HIER		**Proteolysis**	
	Sufficient	Optimal	Sufficient	Optimal
mAb **Ber-EP4**	76% (37/49)	51% (25/49)	36% (8/22)	9% (2/22)

The most robust HIER buffer was Target Retrieval Solution low pH 6.1 (Dako, TRS, S1699/1700) as 32 out of 33 stains (97%) gave a sufficient result (out of which 22 [67%] were optimal). Citrate pH 6.0 gave a sufficient result in 3 out of 8 results (of which 2 [25%] were optimal).
HIER in an alkaline buffer such as Tris-EDTA/EGTA pH 9.0 or Cell Conditioning 1 (Ventana) gave no sufficient results out of 8.
Using HIER, the mAb was typically used in the range of 1:25 – 1:500 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) Ab.
The two optimal results with proteolytic pre-treatment were based on either 0.1 % Trypsin or Bond™ Enzyme (Bond, Leica Microsystems). Using proteolytic pre-treatment, the mAb was used in the range of 1:25 - 1:100.

With mAb clone MOC-31 the optimal protocol was based on HIER in Target Retrieval Solution pH 6.1 (Dako, TRS) and a dilution of 1:25 of the primary Ab.

With mAb clone VU-1D9 the optimal protocol was based on enzyme pre-treatment in Protease Type XIV 0.03 and a dilution of 1:100 of the primary Ab.

The most frequent causes of insufficient staining were:
- Inappropriate HIER buffer (alkaline)
- Insufficient proteolytic pre-treatment
- Excessive proteolytic pre-treatment
- Too low concentration of the primary Ab.

The majority of the laboratories were able to demonstrate Ep-CAM in the columnar epithelial cells of the appendix and the neoplastic cells of the colon adenocarcinoma and the carcinoid, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the two renal cell carcinomas, particular the clear cell type. When proteolysis was used, it generally gave either a too weak reaction in the renal cell carcinomas due to insufficient pre-treatment or a false negative reaction due to excessive proteolysis where the cytoplasm and cell membranes were partly or totally eroded.
In accordance with the previous assessments for Ep-CAM normal kidney was an applicable control for Ep-CAM, as the ability to demonstrate Ep-CAM in the collecting tubules and epithelial cells lining the Bowman's capsule was characteristic for laboratories obtaining an optimal mark.

Table 2. **Pass rate for Ep-CAM in three consecutive assessments.**

	Run 6 2002	Run 17 2006	Run 23 2008
Participants, n=	48	74	78
Sufficient results	77%	54%	63%

Table 2 shows the proportion of sufficient results in the two previous runs and the current run. The reason for the pass rate being lower in the two latest runs compared to the first run is probably a more challenging material. The specifically tailored recommendations given to the laboratories obtaining an insufficient mark in run 17 did have a positive impact: 24 laboratories which obtained an insufficient result in run 17, submitted a new Ep-CAM stain in run 23. 10 of these followed the recommendation, of which 8 improved to good or optimal (80 %). 10 laboratories did not follow the recommendation, and only 2 of these (20 %) obtained a sufficient staining in run 23. 4 laboratories changed their entire IHC system. Of these 3 obtained a sufficient mark.
The tailored recommendations for Ep-CAM to the laboratories are relative problematic for NordiQC. The most robust method appears to be based on HIER in Target Retrieval Solution low pH 6.1, (Dako), which is adaptable to all open IHC systems. For "closed" staining systems with on-board HIER using the reagents developed and optimized for the respective IHC-system (e.g., Bond™, Leica Microsystems and BenchMark XT, Ventana), NordiQC has not been able to identify any robust protocol in the three Ep-CAM assessments. For these platforms, only proteolysis has been used to obtain an optimal result, but as shown in table 1, the success rate is very low. NordiQC will collaborate with the respective companies to develop useful protocols for Ep-CAM.

Conclusion
The mAb clones **Ber-EP4**, **MOC31** and **VU-1D9** are all useful Abs for Ep-CAM. HIER in Target Retrieval Solution low pH 6.1 (Dako) is recommended for optimal performance for Ber-EP4 and MOC31. Kidney is an applicable control: At least a moderate membranous staining should be seen in the epithelial cells of the renal collecting tubules and scattered epithelial cells lining the Bowman capsule.

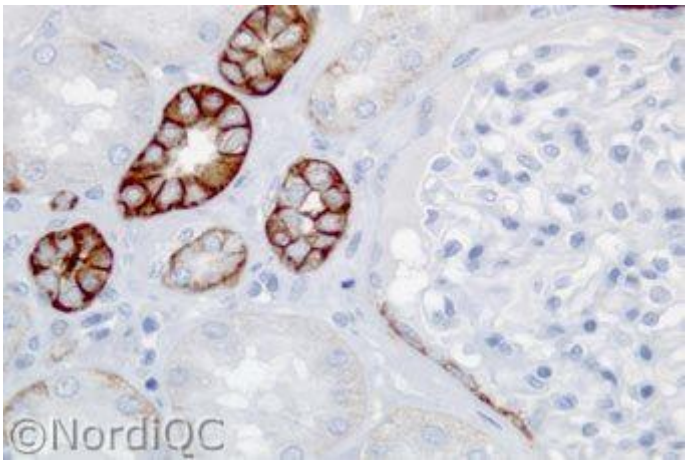


Fig. 1a
Optimal staining for Ep-CAM of the normal kidney using the mAb clone Ber-EP4 with HIER in TRS low pH 6.1 (Dako). The epithelial cells of the renal collecting tubules and the Bowman capsule show a distinct basolateral staining while the epithelial cells of the proximal tubules only show a weak reaction.

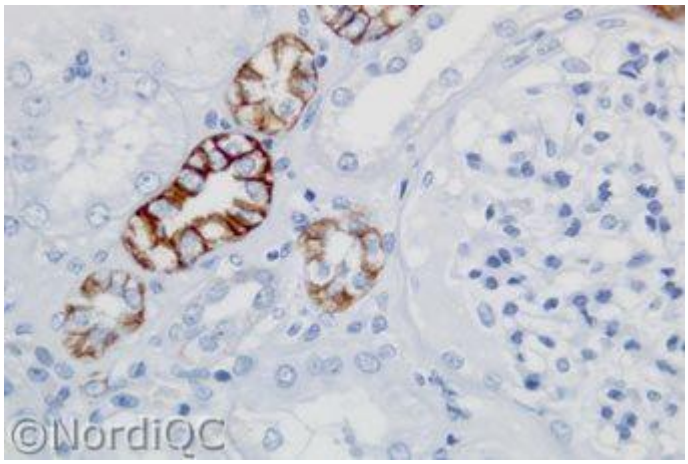


Fig. 1b
Staining for Ep-CAM of the normal kidney using the mAb clone Ber-EP4 with an insufficient protocol based on proteolytic pre-treatment – same field as in Fig. 1a. Only the epithelial cells of the collecting tubules are demonstrated while the proximal tubules and Bowman capsule are negative. Also compare with Fig. 2b and 3b.

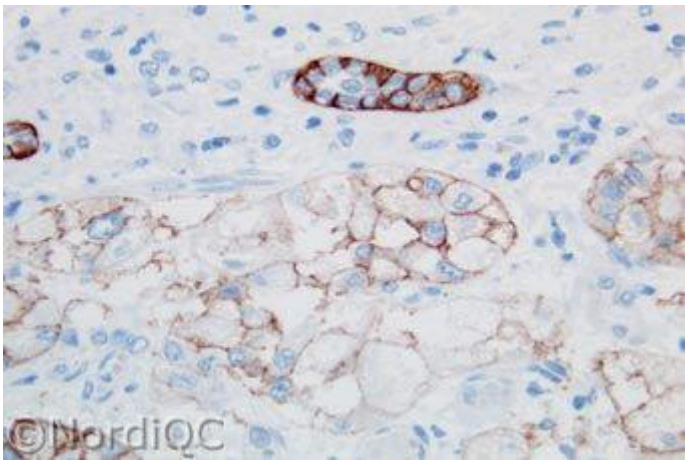


Fig. 2a
Optimal Ep-CAM staining of the chromophobe renal cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate distinct membranous reaction with no background reaction.

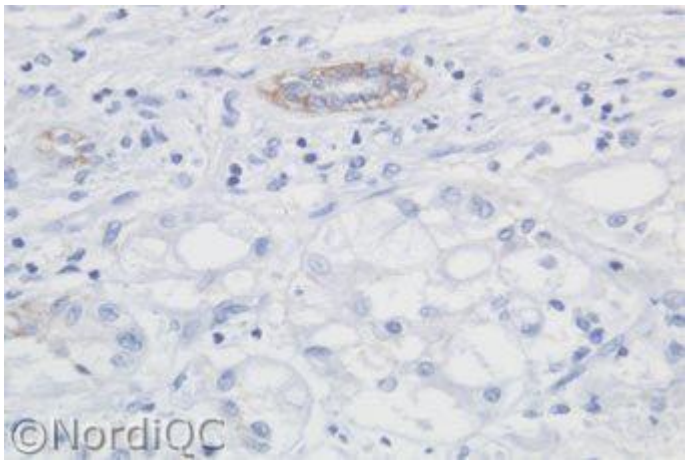


Fig. 2b
Insufficient Ep-CAM staining of the chromophobe renal cell carcinoma using same protocol as in Fig. 1b. Only the normal epithelial cells of the entrapped collecting tubules show a weak reaction, while the neoplastic cells are negative – same field as in Fig. 2a.

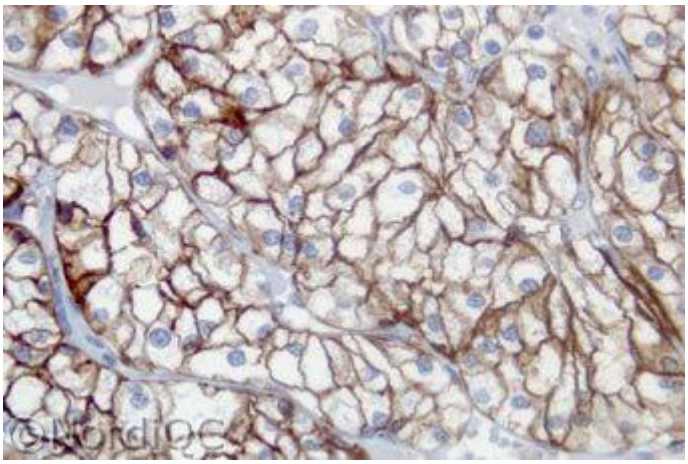


Fig. 3a
Optimal staining for Ep-CAM in the renal clear cell carcinoma using same protocol as in Fig 1a-2a. Virtually all the neoplastic cells show a distinct membraneous reaction.

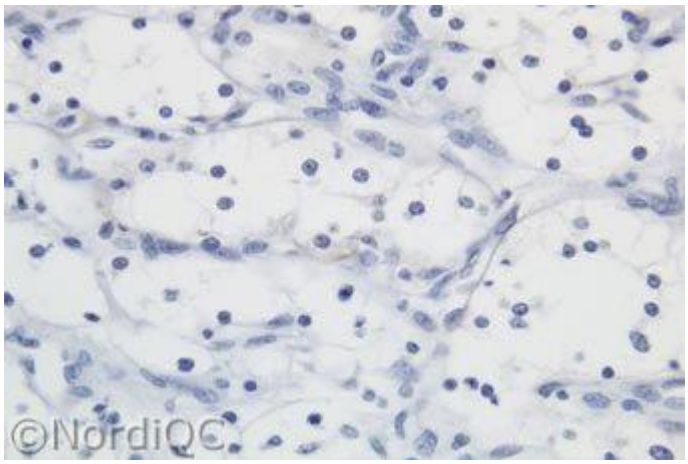


Fig. 3b
Insufficient staining for Ep-CAM in the renal clear cell carcinoma using same protocol as in Fig. 1b – 2b. The neoplastic cells are all false negative (same protocol used in Figs. 1b and 2b) as the fragile membranes have been digested by the proteolytic pre-treatment.

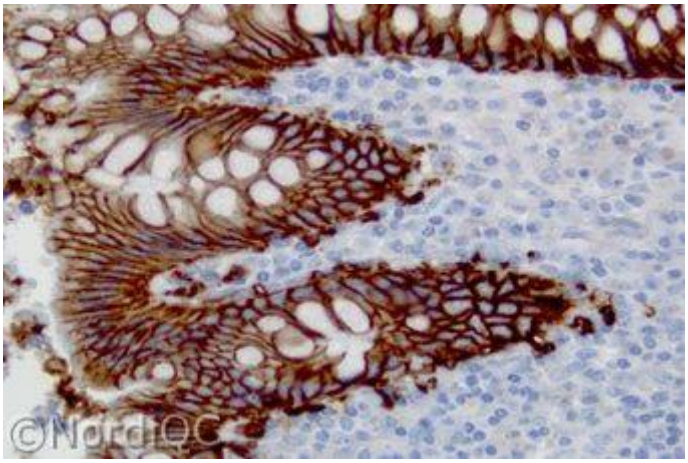


Fig. 4a
Optimal staining for Ep-CAM in the appendix using same protocol as in Figs 1a – 3a. The enterocytes show a strong distinct predominantly membraneous staining.

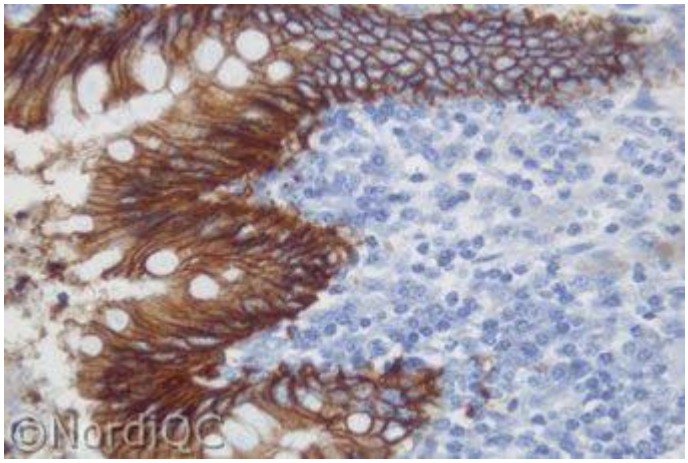


Fig. 4b
Staining for Ep-CAM in the appendix using same insufficient protocol as in Figs 1b – 3b. The enterocytes show a strong distinct predominantly membraneous staining - in spite of the renal cell carcinomas being false negative. Thus, appendix can **not** be recommended as control.

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