Epithelial cell-cell adhesion molecule (Ep-CAM)

Material

The slide to be stained for Ep-CAM comprised:

1. Appendix, 2. Kidney, 3. Adrenal gland, 4. Lung carcinoid, 5 & 6. Renal clear cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

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

- A strong, distinct, predominantly membranous staining of virtually all the columnar epithelial cells of the appendix.
- A moderate to strong predominantly membranous staining of the epithelial cells of the renal collecting tubules, and an at least weak basolateral staining of the epithelial cells of the proximal tubules of the kidney.
- A moderate to strong, distinct, predominantly membranous staining of virtually all the neoplastic cells of the lung carcinoid.
- An at least weak predominantly membranous staining of scattered neoplastic cells in the two renal cell carcinomas.

145 laboratories participated in this assessment. 4 laboratories used an inappropriate Ab. Out of the remaining 141 labs 45 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for EpCAM, run 32**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-EP4**	90 3 2 1	Dako NeoMarkers Diagnostic Biosystems Master Diagnostica	10	28	34	24	40 %	82 %
mAb clone **MOC-31**	8 3 1	Dako Leica/Novocastra Euro-Diagnostika	4	3	2	3	58 %	100 %
mAb clone **VU-1D9**	2 2	Euro-Diagnostika NeoMarkers	2	1	1	0	-	-
rmAb clone **E144**	1	Abcam	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **Ber-EP4 IS/IR637**	14	Dako	1	12	1	0	93 %	92 %
mAb clone **Ber-EP4 760-4383**	8	Ventana/Cell Marque	0	1	3	4	13 %	-
mAb clone **Ber-EP4 248M-97**	1	Cell Marque	0	0	1	0	-	-
mAb clone **Ber-EP4 N1554**	1	Dako	0	0	0	1	-	-
mAb clone **MOC-31 790-4561**	2	Ventana	0	0	2	0	-	-
mAb clone **MOC-31 PM403**	1	Biocare	1	0	0	0	-	-
mAb clone **MOC-31 MON-RTU1097**	1	Monosan	0	0	0	1	-	-
Total	141		18	45	44	34	-	-
Proportion			13 %	32 %	31 %	24 %	45 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs

mAb clone **Ber-EP4**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution (TRS) low pH 6.1 (Dako) (8/21)* or Diva Decloaker pH 6.2 (Biocare) (2/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 18 out of 22 (82 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **MOC-31**: The protocols giving an optimal result were all based on HIER using either TRS high pH 9 (3-in-1) (Dako) (1/1), TRS high pH 9 (Dako) (1/1), TRS low pH 6.1 (Dako) (1/2) or EDTA/EGTA pH 8 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:20-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 out of 8 (100 %) laboratories produced a sufficient staining.

mAb clone **VU-1D9**: The protocols giving an optimal result were all based on HIER using Cell Conditioning 1 (BenchMark, Ventana (1/3)) or Citrate pH 6 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 4 (75 %) laboratories produced a sufficient staining.

Ready-To-Use Abs

mAb clone **Ber-EP4** (prod. no. IS/IR637, Dako): The protocol giving an optimal result was based on HIER in PT-Link using TRS low pH 6.1 and an incubation time of 20 min. in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 12 out of 13 (92 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **MOC-31** (prod. no. PM403, Biocare): The protocol giving an optimal result was based on proteolytic pre-treatment using Pepsin 20 for min. at room temperature, an incubation time of 45 min. in the primary Ab and MACH4 (4U534) as the detection system

The most frequent causes of insufficient stains were:

- Inappropriate HIER buffer (an alkaline buffer for the mAb clone Ber-EP4)
- Less successful performance of the mAb clone Ber-EP4 on the BenchMark, Ventana platform.
- Proteolytic pre-treatment
- Too low concentration of the primary Ab
- Use of low sensitive detection systems

In this assessment and in concordance to the previous NordiQC assessments, virtually all laboratories were able to demonstrate Ep-CAM in the columnar epithelial cells of the appendix, whereas the prevalent feature of the insufficient staining results was a too weak or false negative staining of the neoplastic cells of the lung carcinoid and in particular of the two renal cell carcinomas. For the most widely used mAb clone Ber-EP4 the proportion of sufficient results was highly influenced by the pre-treatment applied and the IHC platform used. First of all the pass rate was significantly lower if proteolytic pre-treatment was used compared to HIER. If proteolytic pre-treatment was used 10 out of 36 protocols (28 %) were assessed as sufficient and none of these were optimal. If HIER was applied, 42 out of 75 protocols (56 %) were assessed as sufficient, out of which 11 (15 %) were optimal. A significant difference in the overall performance for the mAb clone Ber-EP4 was also related to the HIER buffer and thus the IHC platform applied. If HIER was based on TRS low pH 6.1 (Dako) and subsequently IHC was performed by an open IHC platform such as the Autostainer (Dako or LabVision) 28 out of 33 laboratories (85 %) produced a sufficient staining, out of which 11 (33 %) were optimal. Using the fully automated platform BenchMark XT or BenchMark Ultra, Ventana, and HIER based on Cell Conditioning 1, pH 8.5, 19 out of 19 protocols were assessed as insufficient.
As an alternative to TRS low pH 6.1, Dako, it was observed that HIER based on DIVA decloaker solution pH 6.2 , Biocare gave an optimal staining result in 2 out of 2 protocols for the mAb clone Ber-EP4.
The most successful and robust assay for Ep-CAM based on the mAb clone Ber-EP4 in this assessment was obtained by the Ready-To-Use (RTU) system from Dako giving a pass rate of 93 % (13 out of 14 laboratories)out of which 11 % (1 laboratory) were assessed as optimal. In comparison the RTU system from Ventana based on the same mAb gave a pass rate of 13 % (1 out of 8 laboratories) and none were assessed as optimal.

The mAb clone MOC31 and VU-1D9 gave a slightly different staining pattern in both the normal kidney and in the two renal clear cell carcinomas. In the kidney the epithelial cells of the collecting tubules showed a moderate to

strong membranous staining similar to the pattern seen for the mAb clone Ber-EP4, but also an increased staining in the basolateral membranes of the epithelial cells of the proximal tubules. The mAb clone MOC31 and VU-1D9 also typically labelled a higher proportion of the neoplastic cells in the two renal cell carcinomas. For the mAb MOC31 an optimal staining typically was obtained by HIER in e.g. TRS low pH 6.1 (Dako), but an optimal result could also be obtained by other HIER buffers with an alkaline pH as EDTA pH 8 and TRS High pH 9 (Dako) and by the use of proteolytic pre-treatment.

The mAb clone VU-1D9 was the only marker for Ep-CAM giving an optimal staining result on both an open IHC platform, Autostainer (LabVision), using HIER in Citrate pH 6 and a fully automated platform, BenchMark Ultra (Ventana), using HIER in Cell Conditioning 1.

Kidney was the most reliable positive control for Ep-CAM. A moderate to strong membranous staining must be seen in the epithelial cells of the collecting ducts, whereas optimally an at least weak basolateral membranous staining should be seen in the epithelial cells of the proximal tubules. Appendix can not be recommended as the columnar epithelial cells have a high expression of EP-CAM and thus will not identify a protocol with a too low sensitivity.

This was the 3rd NordiQC assessment of Ep-CAM (see table 3). A significant decrease in the proportion of sufficient results was seen in this run compared to the previous runs. This is most likely related to a combination of more challenging material circulated and many new laboratories participating for the first time. For the laboratories participating for the first time the pass rate was 37 % (25 out of 67), whereas the pass rate was 51 % (38 out of 74 laboratories) for the laboratories participating in previous runs.

Table 2. **Proportion of sufficient results for EpCAM in the four NordiQC runs performed**

	Run 17 2006	Run 23 2008	Run 32 2011
Participants, n=	74	78	141
Sufficient results	54 %	63 %	45 %

Conclusion
The mAb clones Ber-EP4, MOC31 and VU-1D9 can all be used for the demonstration of Ep-CAM. HIER in TRS low pH 6.1 (Dako) or Diva Decloaker pH 6.2 (Biocare) seems mandatory for an optimal performance of the mAb clone Ber-EP4. For the mAb clones MOC31 and VU-1D5, HIER in either an alkaline buffer or a non-alkaline buffer such as Citrate pH 6 could be used to obtain an optimal performance. Kidney is a recommendable positive control: A moderate to strong membranous staining must be seen in the epithelial cells of the renal collecting tubules while an at least weak membranous staining should be seen in the epithelial cells of the proximal tubules.
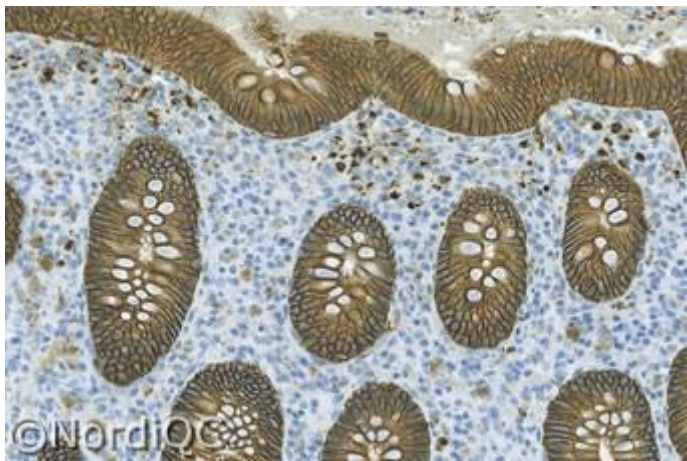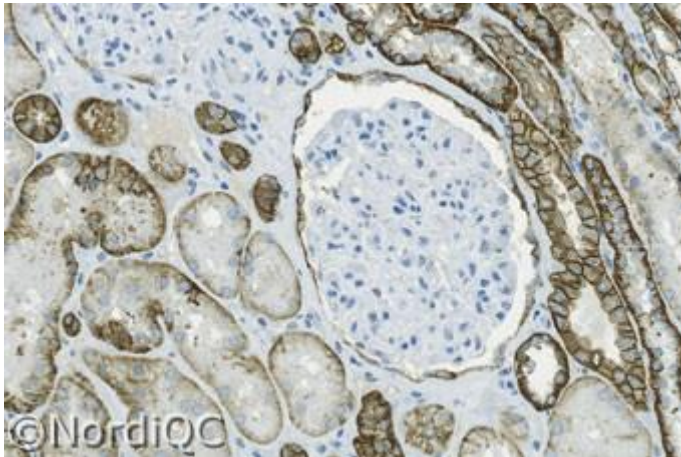

Fig. 1a
Optimal staining for Ep-CAM in the appendix using the mAb clone Ber-EP4 optimally calibrated and with HIER in DIVA epitope retrieval solution pH 6.2 (Biocare). The enterocytes show a strong distinct predominantly membranous staining. Macrophages having engulfed epithelial cells show an intracytoplasmic staining.
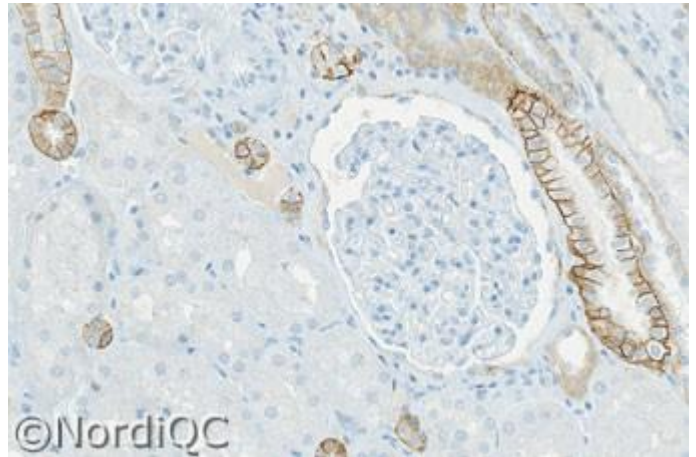
Fig. 1b
Staining for Ep-CAM in the appendix using an insufficient protocol based on the mAb clone Ber-ER4 with HIER in an alkaline buffer, CC1 pH 8.5 Ventana, same field as in Fig. 1a. The enterocytes show a strong distinct predominantly membranous staining. However also compare with Figs. 2b – 4b, same protocol. As an insufficient staining for Ep-CAM was seen in both renal cell carcinomas, appendix can not be recommended as a positive control due to a too high expression of Ep-CAM.

Fig. 2a
Optimal staining for Ep-CAM of the normal kidney using same protocol as in Fig. 1a.
The epithelial cells of the renal collecting tubules and the Bowman capsule show a moderate to strong membranous staining, while the epithelial cells of the proximale tubules only show a weak predominantly basolateral reaction.

Fig. 2b
Insufficient staining for Ep-CAM of the normal kidney using same protocol as in Fig. 1b, same field as in Fig. 2a. Only the epithelial cells of the collecting tubules are demonstrated. Also compare with Figs. 3b and 4b.
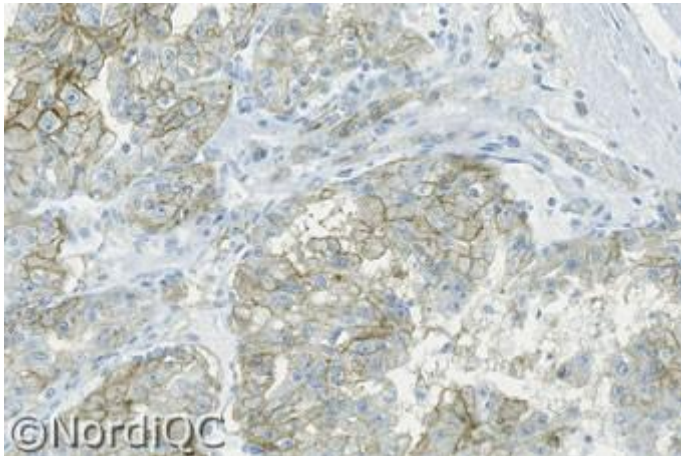
Fig. 3a
Optimal staining for Ep-CAM in the renal clear cell carcinoma no. 5 using same protocol as in Figs. 1a - 2a. The majority of the neoplastic cells show a moderate and distinct membranous reaction.
No background reaction is seen.
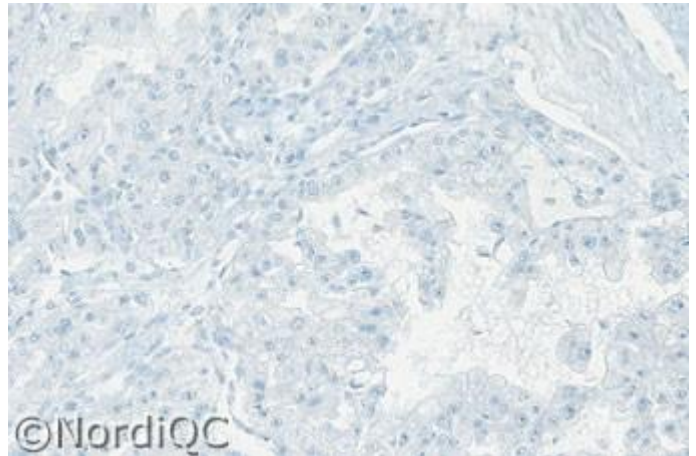
Fig. 3b
Insufficient staining staining for Ep-CAM in the renal clear cell carcinoma no. 5 using same protocol as in Figs. 1b – 2b, same field as in Fig. 3b.
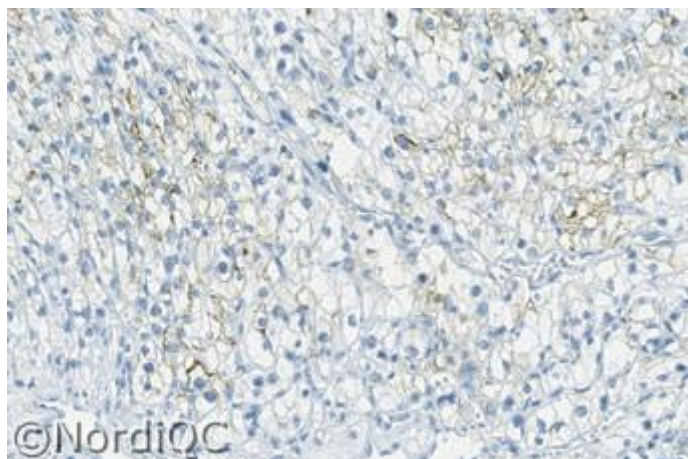The neoplastic cells are all false negative.

Fig. 4a
Optimal Ep-CAM staining of the renal cell carcinoma no. 6 using same protocol as in Figs. 1a – 3a. Scattered neoplastic cells show a weak to moderate distinct membranous reaction.
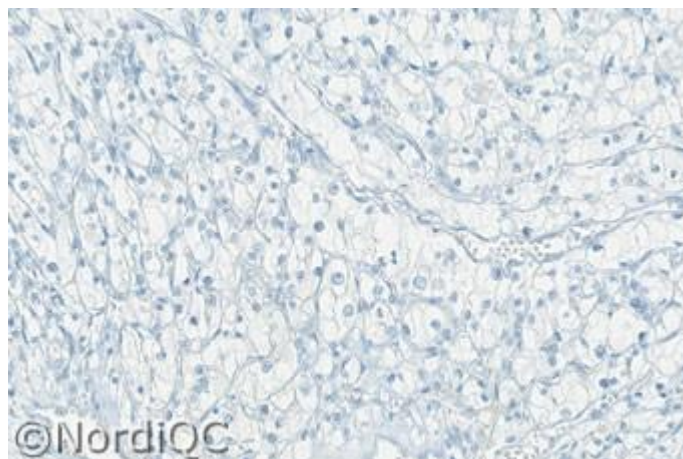

Fig. 4b
Insufficient Ep-CAM staining of the renal cell carcinoma no. 6 using same protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
The neoplastic cells are all false negative.

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