

## Epithelial cell-cell adhesion molecule (EpCAM)

### Material

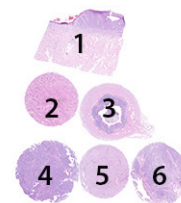
The slide to be stained for EpCAM comprised:

1. Basal cell carcinoma (BCC), 2. Kidney, 3. Appendix, 4. Colon adenocarcinoma,
5. Renal clear cell carcinoma (RCCC), 6. Small Cell Lung Carcinoma (SCLC)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing EpCAM staining as optimal included:

- A strong and distinct, predominantly membranous staining reaction of virtually all columnar epithelial cells in the appendix.
- A moderate to strong, predominantly membranous staining reaction of virtually all epithelial cells in the renal distal convoluted tubules.
- An at least weak, predominantly basolateral staining reaction of epithelial cells in the proximal tubules and membranous staining of epithelial cells lining the Bowman capsule in the kidney.
- A moderate to strong and distinct, predominantly membranous staining reaction of virtually all neoplastic cells in the BCC and colon adenocarcinoma.
- An at least moderate, predominantly membranous staining reaction of the vast majority of neoplastic cells in the RCCC and SCLC.



### Participation

Number of laboratories registered for EpCAM, run 56	265
Number of laboratories returning slides	258 (97%)

### Results

258 laboratories participated in this assessment. Two participants used an inappropriate Ab. Of the remaining 256 laboratories, 57% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Less successful performance of mAb clone Ber-EP4 on BenchMark and BOND IHC platforms
- Proteolytic pre-treatment
- Too low concentration of the primary Ab
- Low sensitive detection systems

### Performance history

This was the fifth NordiQC assessment of EpCAM and, as shown in Table 2, the pass rate increased significantly compared to the latest run 45, 2015.

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

	Run 17 2006	Run 23 2008	Run 32 2011	Run 45 2015	Run 56 2019
Participants, n=	74	78	141	192	256
Sufficient results	54%	63%	45%	43%	57%

### Conclusion

The mAb clones **BS14**, **Ber-EP4**, **MOC31** and **VU-1D9** could all be used to obtain an optimal staining result. For the most widely used Ab for demonstration of EpCAM (clone Ber-EP4), HIER in special formulated buffers as TRS low pH 6.1 (Dako) provided the highest proportion of sufficient and optimal results. The mAb clones BS14, MOC31 and VU-1D9 could provide an optimal staining result in standard HIER buffers. Based on the performance, and for laboratories struggling with optimization of Ber-EP4 or MOC-31 on the Bond (Leica) or BenchMark (Ventana) platform, both BS14 and VU-1D9 could be better alternatives. For all clones applied within a laboratory developed (LD) assay, use of sensitive 3-step polymer/multimer detection systems provided the highest proportion of sufficient and optimal results. The Dako RTU system GA637 (Omnis) based on mAb clone Ber-EP4 was superior to all other RTU systems.

Kidney and tonsil are recommendable as positive and negative tissue controls for EpCAM. In kidney, virtually all epithelial cells lining the collecting tubules must show a moderate to strong, predominantly membranous staining reaction, whereas an at least weak, predominantly basolateral, staining reaction must be seen in the majority of epithelial cells in the proximal tubules and also in scattered epithelial cells lining the Bowman capsule. In tonsil, lymphocytes and smooth muscle cells of the vessels should be negative and only dispersed squamous epithelial cells should be demonstrated.

Table 1. **Antibodies and assessment marks for EpCAM, run 56**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. <sup>1</sup>	Suff. OPS <sup>2</sup>
mAb clone <b>BS14</b>	10	Nordic Biosite	9	1	0	0	100%	100%
mAb clone <b>Ber-Ep4</b>	69	Dako						
	6	Cell Marque	14	13	21	28	36%	93%
	1	Diagnostic Biosystems						
mAb clone <b>MOC-31</b>	23	Dako						
	5	Cell Marque	10	10	7	2	69%	71%
	1	Diagnostic Biosystems						
mAb clone <b>VU-1D9</b>	5	Thermo Scientific						
	3	Merck Millipore	9	0	1	0	90%	100%
	1	Immunologic						
	1	Novus Biologicals						
rmAb clone <b>EPR20532-225</b>	1	Abcam	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone <b>Ber-Ep4 760-4383</b>	16	Ventana/Cell Marque	1	6	6	3	44%	100%
mAb clone <b>Ber-Ep4 248M-98</b>	49	Cell Marque	5	13	16	15	37%	-
mAb clone <b>Ber-Ep4 IR/IS637</b>	18	Dako	5	9	3	1	78%	87%
mAb clone <b>Ber-Ep4 IR/IS637<sup>3</sup></b>	6	Dako	1	2	2	1	-	-
mAb clone <b>Ber-Ep4 GA637</b>	27	Dako	26	1	0	0	100%	100%
mAb clone <b>Ber-Ep4 GA637<sup>3</sup></b>	2	Dako	0	1	1	0	-	-
mAb <b>Ber-Ep4 PM107</b>	1	Biocare	1	0	0	0	-	-
mAb <b>Ber-Ep4 MAD-001709QD</b>	2	Master Diagnostica	0	2	0	0	-	-
mAb clone <b>Ber-Ep4 PDM131</b>	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone <b>MOC-31 790-4561</b>	3	Ventana	1	2	0	0	-	-
mAb clone <b>MOC-31 248M-18</b>	2	Cell Marque	2	0	0	0	-	-
mAb clone <b>VU-1D9 8230-C010</b>	2	Sakura FineTek	2	0	0	0	-	-
mAb clone <b>MX066 MAB-0850</b>	1	Maxin	1	0	0	0		
Total	256		87	60	58	51	-	
Proportion			34%	23%	23%	20%	57%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of sufficient stains with optimal protocol settings only, see below.

3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

### Detailed analysis of EpCAM, Run 56

The following protocol parameters were central to obtain optimal staining:

## Concentrated antibodies

mAb clone **BS14**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/2)\*, Cell Conditioning 1 (CC1, Ventana) (1/1), Tris-EDTA pH 9 (1/1) or CC1 followed by enzymatic pre-treatment with Protease 3 (Ventana) (5/6) as retrieval procedures. The mAb was typically diluted in the range of 1:100-1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 of 7 (100%) laboratories produced a sufficient staining result. The one laboratory assessed as good applied proteolytic pre-treatment (Protease 3) before HIER in CC1.

\* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **Ber-Ep4**: Protocols with optimal results were typically based on HIER using TRS pH 6.1 (3-in-1) (Dako) (10/14) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 13 of 14 (93%) laboratories produced a sufficient staining result. Optimal results could also be obtained by using HIER in TRS pH 9 (3-in-1) (Dako), CC1 (Ventana) or citrate buffer pH6 followed by proteolytic pre-treatment.

mAb clone **MOC-31**: Protocols with optimal results were typically based on HIER using TRS pH 6.1 (3-in-1) (Dako) (4/6), CC1 (Ventana) (3/14), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (2/6) or Tris-EDTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100. Using these protocol settings, 17 of 24 (71%) laboratories produced a sufficient staining result.

mAb clone **VU-1D9**: Protocols with optimal results were based on HIER using CC1 (Ventana) (7/8) or TRS pH 6.1 (3-in-1) (Dako) (2/2) as retrieval buffer. The mAb was diluted in the range of 1:80-500. Using these protocol settings, 8 of 8 (100%) laboratories produced a sufficient staining result (all assessed as optimal).

Table 3. **Proportion of optimal results for EpCAM for the most commonly used antibodies as concentrate on the four main IHC systems\***

Concentrated antibodies	Dako Autostainer Link/Classic		Dako Omnis		Ventana BenchMark GX /XT/ Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone <b>Ber-EP4</b>	-	4/7** (57%)	-	3/4	2/16*** (13%)	0/1	-	0/3
mAb clone <b>MOC-31</b>	-	1/1	-	3/5 (60%)	2/11 (18%)	-	-	2/6 (33%)
mAb clone <b>BS14</b>	-	-	2/2	-	4/5*** (80%)	-	-	-
mAb clone <b>VU-1D9</b>	-	-	-	1/1	6/6 (100%)	-	-	-

\* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

\*\* (number of optimal results/number of laboratories using this buffer).

\*\*\* Protocols without or combined with proteolytic pre-treatment (see description above).

## Ready-To-Use antibodies and corresponding systems

mAb clone **Ber-Ep4**, product no. **760-4383**, Ventana, BenchMark XT/Ultra:

One protocol with an optimal result was based on HIER in CC1 (efficient heating time 32 min. at 94°C) followed by enzymatic pre-treatment with Protease 3 (4 min. at 36°C), 60 min. incubation of the primary Ab and OptiView (760-700) as detection systems. Using these protocol settings 1 of 1 (100%) laboratory produced a sufficient staining result (optimal).

mAb clone **Ber-Ep4**, product no. **IS637/IR637**, Dako, Autostainer+/Autostainer Link:

Protocols with optimal results were based on HIER in PT-Link using TRS pH 6.1 (efficient heating time 10-20 min. at 96-99°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 13 of 15 (87%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Ber-Ep4**, product no. **GA637**, Dako, Omnis:

Protocols with optimal results were typically based on HIER using TRS pH 6.1 (efficient heating time 30 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (GV800+GV821) as detection system. Using these protocol settings, 23 of 23 (100%) laboratories produced an optimal result.

mAb clone **Ber-Ep4**, product no. **PM107**, Biocare Medical, IntelliPATH:

One protocol with an optimal result was based on HIER in Diva Decloaker (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 HRP Polymer (MRH534 + UP534) as detection system.

mAb clone **MOC-31**, product no. **790-4561**, Ventana, BenchMark XT/Ultra:

One protocol with an optimal result was based on HIER in CC1 (efficient heating time 36 min. at 98°C), 32 min. incubation of the primary Ab and UltraView with amplification (760-500+760-080) as detection system.

mAb clone **VU-1D9**, product no. **8230-C010**, Sakura FineTek, Tissue-Tek Genie:

Protocols with optimal results were based on HIER in PT-Link using Tissue-Tek Genie Citrate Antigen Retrieval Solution (efficient heating time 38 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit, DAB (8826-K250) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems ( $\geq 10$  assessed protocols). The performance of the individual assays were evaluated as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations but also as laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for EpCAM for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
BenchMark XT/Ultra mAb Ber-EP4 <b>760-4383</b>	(0/1)	(0/1)	47% (7/15)	7% (1/15)
Autostainer +/Link mAb Ber-EP4 <b>IS/IR637</b>	80% (8/10)	20% (2/10)	75% (6/8)	38% (3/8)
Omnis mAb Ber-EP4 <b>GA637</b>	100% (23/23)	100% (23/23)	(4/4)	(3/4)

\* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

\*\* Significant modifications: retrieval method, retrieval duration and Ab incubation time altered  $>25\%$ , detection kit – only protocols performed on the specified vendor IHC stainer integrated.

## Comments

In concordance with the previous NordiQC assessments for EpCAM, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 94% of the insufficient results (103 of 109). Too weak staining result in combination with either background and/or false positive reaction were seen in 4% of the insufficient results (4 of 109). The remaining insufficient results were characterized by poor signal-to-noise ratio and/or false positive staining reaction compromising interpretation. Virtually all participating laboratories were able to stain EpCAM in high-level antigen expressing cells as columnar epithelial cells of appendix, neoplastic cells in the BCC and colon adenocarcinoma, whereas demonstration of EpCAM in neoplastic cells of the RCCC and SCLC was more challenging and only seen when appropriate protocol settings were applied.

Used within a LD assay, the mAb clone Ber-EP4 was the most widely used antibody for the demonstration of EpCAM. As described in the previous report (Run 45, 2015), proportion of sufficient results was highly influenced by the pre-treatment conditions and the IHC platform used. If enzymatic pre-treatment was used in this assessment, the pass rate was only 5% (1/22). In comparison, if HIER was applied, 50% (21/42) of the protocols were assessed as sufficient, of which 29% (12/42) were optimal. For participants applying combined antigen retrieval (HIER and proteolytic pre-treatment), the pass rate was 42% (5/12). Performing enzymatic treatment is not without problems, as several parameters may affect efficiency of the digestion procedure e.g. fixation time of specimens in formalin, enzyme concentration and digestion time. Of the 22 protocols based on enzymatic pre-treatment alone, it was observed that 41% (9/22) were challenged by impaired morphology and especially the fragile neoplastic cells of the SCLC were affected.

As shown in Table 3, and indicated in previous assessments, the performance and level of analytical sensitivity for mAb clone Ber-EP4 is significantly improved if HIER is based on the special formulated buffers, TRS pH 6.1 (Dako) and Diva pH 6.2 (Biacore) compared to standard HIER buffers. Inevitable, this will impact the performance of IHC platforms lacking the possibility to perform HIER in these special

formulated buffers. Consequently, when applying optimal dilution range 1:25-1:100 (see above), only 33% of (4/12) protocols based on HIER in CC1 (Ventana) or BERS1 (Leica) on the fully automated platforms BenchMark XT/ Ultra (Ventana) or Bond MAX/III (Leica) provided a sufficient result of which one (8%) was optimal. In comparison, if staining was performed on either Dako Autostainer or Omnis, 91% (10/11) of the protocols based on HIER in the TRS low pH 6.1 (Dako) were assessed as sufficient of which 55% (6/11) were optimal. For IHC platforms without access to the specially formulated HIER buffers, identification of robust Abs providing the expected reaction pattern using standard HIER buffers is highly needed. In this assessment and as shown in Table 3, the mAb clones BS14 and VU-1D9 might be alternatives to Ber-EP4 (MOC-31 can also be used but produced a significantly lower pass rate and proportion of optimal results compared to mAb BS14 and VU-1D9), as these Abs could provide an optimal staining result applying standard HIER buffers for the respective IHC platforms from BenchMark and BOND. Internal studies performed in NordiQC reference laboratories, have confirmed that the mAb clone BS14 seems robust and can be used for the demonstration of EpCAM on platforms deprived from access to these special formulated HIER buffers. In this run and using the mAb BS14 within a LD assay, 60% (6/10) protocols were based on combined retrieval typically applying HIER in CC1 followed by proteolysis in P3 and OptiView as detection system. Using this combination, 83% (5/6) of the protocols were assessed as optimal. In total, combining Run 45 and Run 56, 100% (12/12) of the laboratories using this clone were able to produce a sufficient staining result of which 92% (11/12) were given an optimal mark. These results underline, that the antigenic epitope recognized by the mAb BS14 is less critical in term of which antigen retrieval procedure is used for demonstration of EpCAM and more importantly, that standard HIER buffers as e.g. BERS2 (Leica) or CC1 (Ventana) may be sufficient to produce optimal results. Similarly, the mAb VU-1D9 also seems robust. Stained on BenchMark Ultra (Ventana) applying HIER in CC1 and OptiView as the detection system, all (7/7) protocols were assessed as optimal.

Irrespective of the clone applied within a LD assay, use of 3-step polymer/multimer based detection systems gave an increased number of sufficient results compared to 2-step systems. Using a 3-step multimer/polymer detection system as e.g. OptiView (Ventana) or EnVision FLEX+ (Dako), 68% (48/71) of the protocols were assessed as sufficient (45% optimal) compared to a pass rate of 55% (16/29) of which 31% (9/29) were optimal if 2-step systems as e.g. UltraView (Ventana) EnVision FLEX (Dako) were used.

The Ready-To-Use (RTU) system GA637 (Dako, Omnis), based on the mAb clone Ber-EP 4 provided a superior performance compared to all other RTU systems (see Table 1). Applying the official protocol recommendation given by the vendor (see Table 3), HIER in TRS low pH 6.1 and Envision Flex+ as the detection system, all protocols were assessed as optimal (23/23). The IR/IS637 RTU system (Autostainer) based on the same clone, provided 78% (14/18) of sufficient results but a significantly lower proportion of optimal results (28%). This deviation in performance is most likely caused by the official recommendations for the protocol settings for the RTU system IS/IR637, which is based on the use of a 2-step polymer based detection system providing a reduced analytic sensitivity compared to the 3-step EnVision Flex+ detection system being recommended for the corresponding RTU system on the Omnis variant

The RTU system 760-4383 (BenchMark XT/Ultra, Ventana) also based on the mAb Ber-EP4 was challenged by the lack of the special formulated buffers required for optimal performance for this mAb, consequently providing a low proportion of sufficient and optimal results, 44% and 6%, respectively. Only one laboratory followed the recommended protocols settings as listed in the official package insert (Ab incubation for 16 min., HIER in CC1 32 min. and UltraView as detection system) and was assessed as insufficient (see Table 4). The majority of participants used laboratory modified protocol settings of which one was assessed as optimal. The protocol was based on HIER in CC1 for 32 min. followed by enzymatic pre-treatment in Protease 3 for 4 min., Ab incubation for 60 min. and OptiView as detection system. Using similar settings, two additional protocols were assessed as good.

This was the fifth assessment of EpCAM in NordiQC (see Table 2). EpCAM has been used for many years, and the marker is still challenging although the pass rate increased to 57% in this run compared to a pass rate of 43% in the previous run 45, 2015. The most important parameters influencing the final outcome in negative direction was: 1) Challenging tissue included in the block, especially demonstration of EpCAM in the neoplastic cells of the RCCC and of the SCLC were difficult, 2) The access to special formulated buffers is critical if applying the mAb clone Ber-EP4 or MOC-31. In total, 64% (148/232) of participants using these clones performed the test on either a Bond (Leica) or BenchMark (Ventana) platform, making it difficult to optimize the protocols without access to appropriate HIER buffers 3) A large proportion of laboratories applied protocol settings based on enzymatic pre-treatment, providing a pass rate of only 5% (1/22) of which none were optimal.

Laboratories should apply an Ab that work on the in-house IHC platform, calibrate the protocols correctly and stain according to the expected antigen level of the recommended control material (see below).



## Controls

Kidney and tonsil are recommendable as positive and negative tissue controls for EpCAM. In kidney, virtually all epithelial cells lining the collecting tubules must show a moderate to strong predominantly membranous staining reaction, whereas an at least weak predominantly basolateral staining reaction must be seen in the majority of epithelial cells in the proximal tubules and also in scattered epithelial cells lining the Bowman capsule. In tonsil, no staining reaction should be seen in lymphocytes or smooth muscle cells of the vessels and only dispersed squamous epithelial cells should be demonstrated.

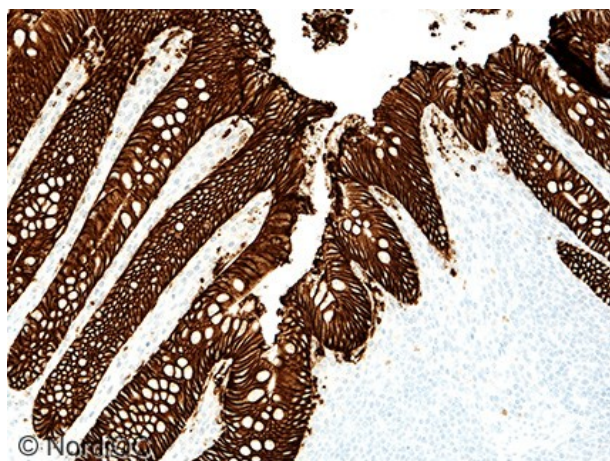


Fig. 1a (x200)  
Optimal EpCAM staining of the appendix using the mAb VU-1D9 optimally calibrated, HIER in an alkaline buffer (CC1, Ventana) and OptiView (Ventana) as the detection system - same protocol used in Figs. 2a-5a. All columnar epithelial cells show strong membranous staining reaction.

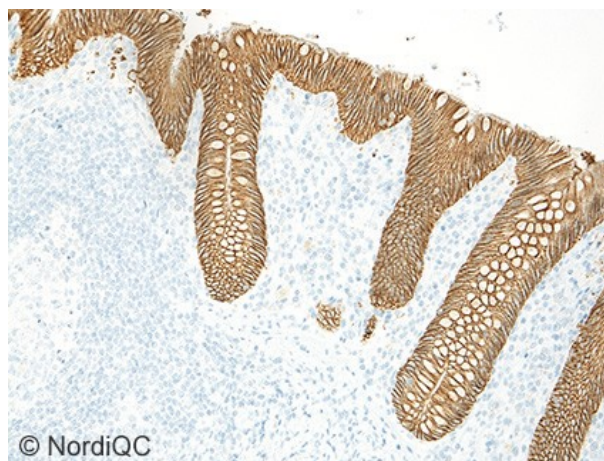


Fig. 1b (x200)  
Insufficient EpCAM staining of the appendix using the mAb VU-1D9 (too diluted), HIER in CC1 (Ventana) and the low sensitive UltraView (Ventana) as the detection system - same protocol used in Figs. 2b-5b. Although the epithelial cells are distinctively stained, the intensity is reduced, which is critical in relation to low expressing tissue/cell structures - compare Fig. 2a-5b.

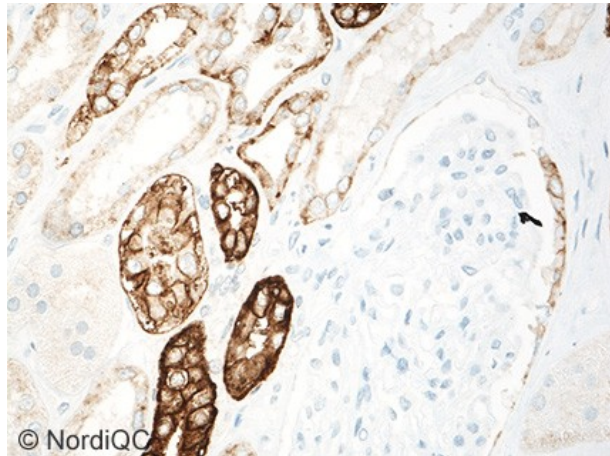


Fig. 2a (x400)  
Optimal EpCAM staining reaction of the kidney using same protocol as in Fig. 1a. The epithelial cells of the distal convoluted tubules show a moderate to strong membranous staining reaction, while the epithelial cells of the Bowman capsule and proximal tubules only show a weak predominantly basolateral reaction.

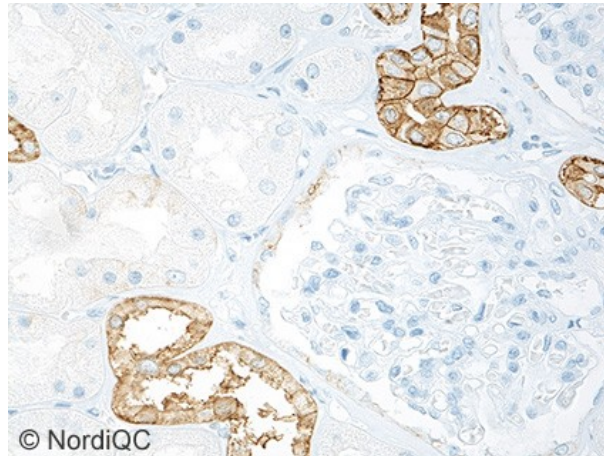


Fig. 2b (x400)  
Insufficient EpCAM staining reaction of the kidney using same protocol as in Fig. 1b. Only the epithelial cells of the distal convoluted tubules are demonstrated, whereas epithelial cells of the Bowman capsule and proximal tubules are completely negative or only faintly positive - compare with Fig. 2a.



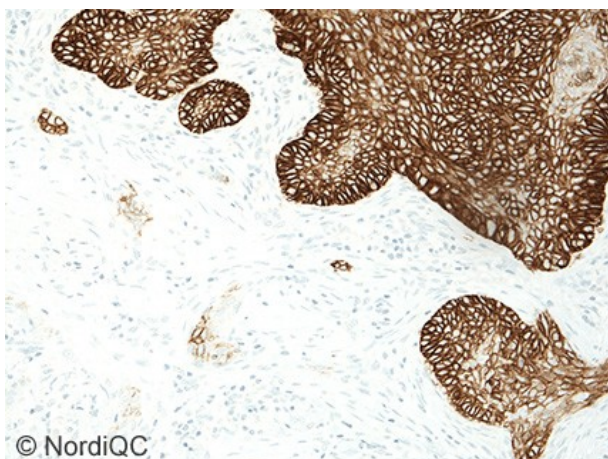


Fig. 3a (x200)  
Optimal EpCAM staining of the BCC using the same protocol as in Fig. 1a-2a. Virtually all neoplastic cells show a strong predominantly membranous staining reaction. Dispersed tumor cells seated in the stromal compartment display weak to moderate staining intensity.

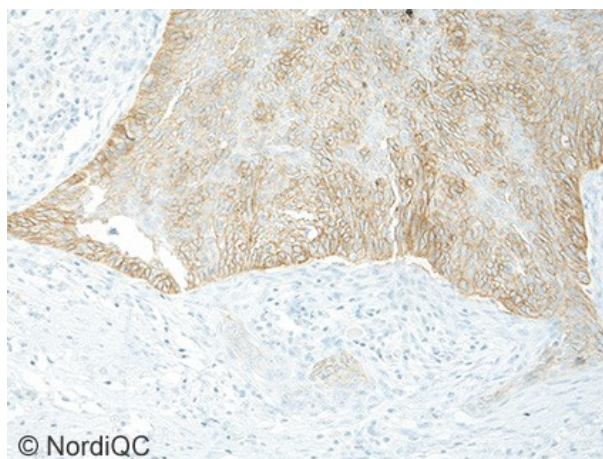


Fig. 3b (x200)  
Insufficient EpCAM staining of the BCC using the same protocol as in Fig. 1b-2b. The proportion of stained neoplastic cells is reduced, intensity is too weak and a fraction of tumor cells in the stromal compartment is false negative - compare with Fig. 3a.

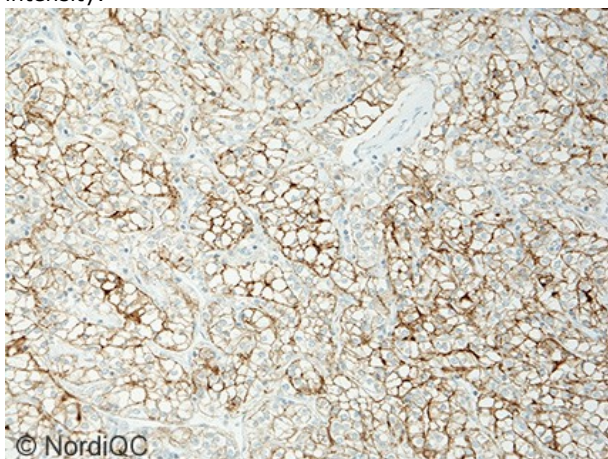


Fig. 4a (x200)  
Optimal EpCAM staining of the RCCC using the same protocol as in Fig. 1a-3a. Virtually all neoplastic cells show an at least moderate, distinct membranous staining reaction.

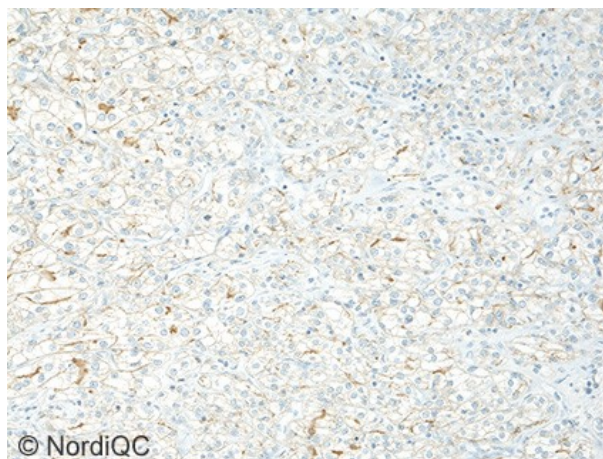


Fig. 4b (x200)  
Insufficient EpCAM staining of the RCCC using the same protocol as in Fig. 1b-3b. The staining pattern is focal and dot-like, whereas the vast majority of the neoplastic cells are false negative, not displaying the expected distinct membranous staining reaction as seen in Fig. 4a.



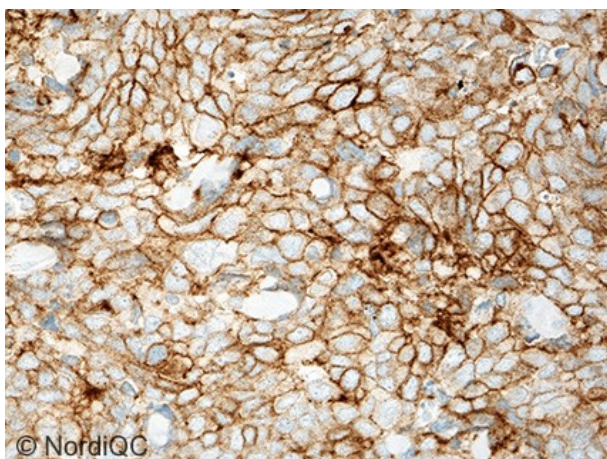


Fig. 5a (x400)  
Optimal EpCAM staining of the SCLC using the same protocol as in Fig. 1a-4a. All neoplastic cells display moderate to strong, distinct membranous staining reaction.

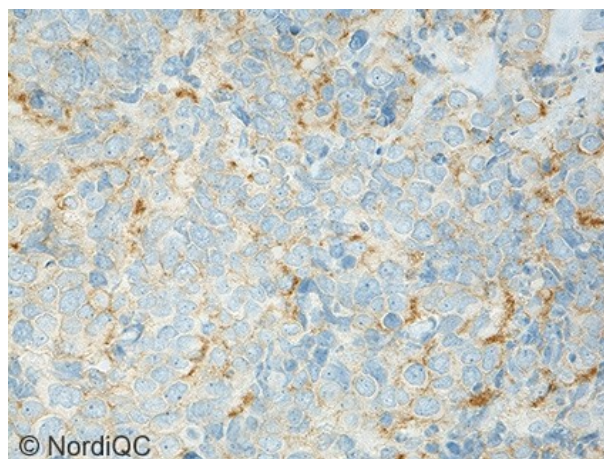


Fig. 5b (x400)  
Insufficient EpCAM staining of the SCLC using the same protocol as in Fig. 1b-4b. Same staining pattern is seen as in Fig. 4b. Virtually all neoplastic cells are false negative, lacking the specific membranous staining pattern as displayed in Fig. 5a.

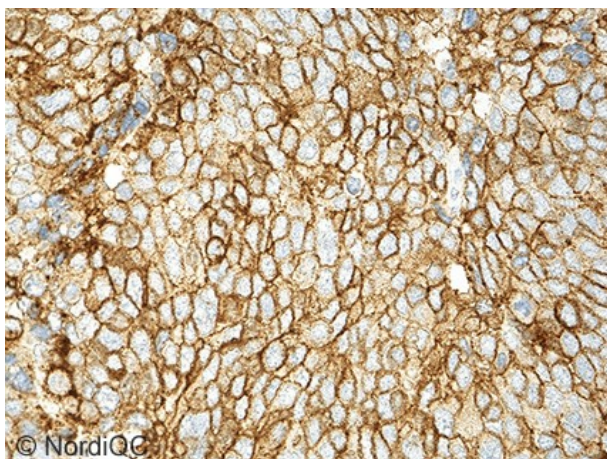


Fig. 6a (x400)  
Optimal EpCAM staining of the SCLC using the mAb BS14 on the Omnis (Dako), optimally calibrated, HIER in an alkaline buffer (TRS pH9, Dako) and EnVision Flex+ (Dako) as detection system. The protocol showed the expected staining pattern (all cores). As for mAb VU-1D9, this Ab could be an alternative to the challenging mAbs Ber-EP4 and MOC-31 on platforms (e.g. BOND/Leica and BenchMark/Ventana) deprived from use of the special formulated low pH HIER buffers e.g. TRS Low pH 6.1 (Dako) - see comments.

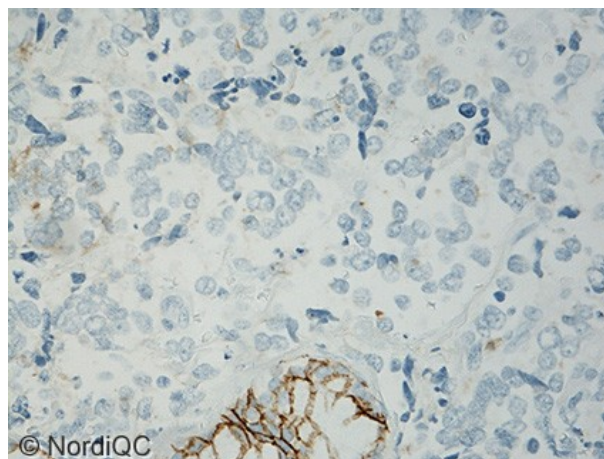


Fig. 6b (x400)  
Insufficient EpCAM staining of the SCLC using a protocol based on proteolytic pre-treatment. The neoplastic cells are all false negative as the fragile membranes have been digested by the enzymatic treatment. Only remnants of normal epithelial structures are demonstrated. The use of proteolytic pre-treatment should be avoided as the vast majority of laboratories (95%) applying these procedures were unable to produce a sufficient result.

MB/LE/RR/MV/SN 08.07.2019