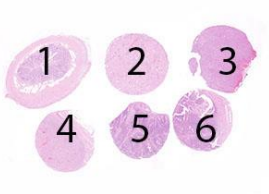
Assessment Run 69 2023

Epithelial cell-cell adhesion molecule (EpCAM)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for EpCAM performed by the NordiQC participants, identifying adenocarcinomas of unknown origin and differentiation between basal cell carcinoma and squamous cell carcinoma.
Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of EpCAM antigen densities (see below).

Material
The slide to be stained for EpCAM comprised:



1. Appendix, 2. Kidney, 3. Skin squamous cell carcinoma, 4. Renal clear cell carcinoma (RCCC), 5. Basal cell carcinoma (BCC), 6. Colon adenocarcinoma

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 weak to moderate, predominantly membranous staining reaction of the vast majority of neoplastic cells in the RCCC.

Participation

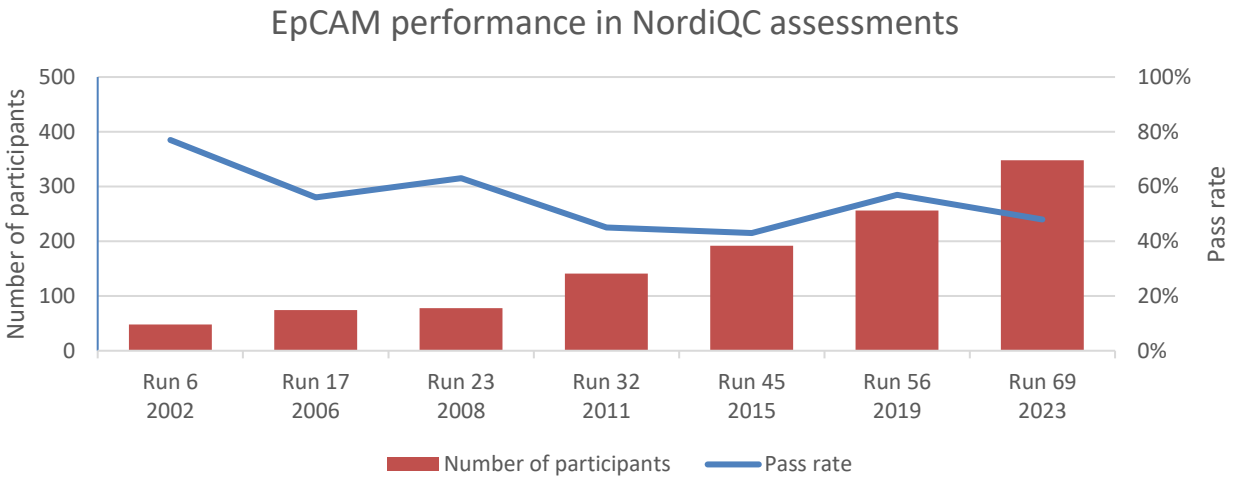
Number of laboratories registered for EpCAM, run 69	372
Number of laboratories returning slides	348 (94%)

Results
348 laboratories participated in this assessment. Three participants used an inappropriate Ab. Of the remaining 345 laboratories, 48% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of mAb clone Ber-EP4 on BenchMark and BOND IHC platforms
- Too low concentration of the primary Ab
- Low sensitive detection systems
- Less successful performance of newly introduced primary antibodies

Performance history
This was the seventh NordiQC assessment of EpCAM and, as shown in Graph 1, the pass rate decreased compared to the latest run 56, 2023.

Graph 1. **Proportion of sufficient results for EpCAM in the seven NordiQC runs performed**



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/Agilent) provided the highest proportion of sufficient and optimal results. The mAb clones BS14, MOC-31 and VU-1D9 could provide an optimal staining result in standard HIER buffers. Based on the performance, and for laboratories struggling with optimization of the clones Ber-EP4 or MOC-31 on the BenchMark (Ventana/Roche) platforms, both clones 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/Agilent RTU system GA637 (Omnis) based on mAb clone Ber-EP4 was superior to all other RTU systems.

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.

Table 1. **Antibodies and assessment marks for EpCAM, run 69**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BS14**	25	Nordic Biosite	19	6	0	0	100%	76%
mAb clone **Ber-Ep4**	40 8 1	Dako/Agilent Cell Marque Thermo Scientific	5	11	30	3	33%	10%
mAb clone **MOC-31**	16 1 2 1	Dako/Agilent Biocare Medical Cell Marque Leica Biosystems	1	14	4	1	75%	5%
mAb clone **VU-1D9**	9 1 4 1	Thermo Scientific Merck Millipore Diagnostic Biosystems Monosan	4	8	2	1	80%	27%
mAb clone **C-10**	2	Santa Cruz	0	0	2	0	-	-
mAb clone **ZM131**	1	Zeta Corporation	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **Ber-Ep4 760-4383**[3]	13	Ventana/Roche	0	0	13	0	0%	0%
mAb clone **Ber-Ep4 760-4383**[4]	86	Ventana/Roche	1	20	60	5	24%	1%
mAb clone **Ber-Ep4 248M-98**	15	Cell Marque	0	1	13	1	7%	0%
mAb clone **Ber-Ep4 IR/IS637**[3]	1	Dako/Agilent	0	0	1	0	-	-
mAb clone **Ber-Ep4 IR/IS637**[4]	15	Dako/Agilent	3	2	10	0	33%	20%
mAb clone **Ber-Ep4 GA637**[3]	44	Dako/Agilent	12	30	2	0	95%	27%
mAb clone **Ber-Ep4 GA637**[4]	16	Dako/Agilent	3	8	5	0	69%	19%
mAb clone **Ber-Ep4 PM107**	3	Biocare Medical	0	0	1	2	-	-
mAb clone **Ber-Ep4 MAD-001709QD**	3	Master Diagnostica	0	0	2	1	-	-
mAb clone **Ber-Ep4 PDM131**	2	Diagnostic Biosystems	0	0	0	2	-	-
mAb clone **Ber-Ep4 P-E002**	1	Quartett	0	0	0	1	-	-
mAb clone **Ber-Ep4 BMS048**	1	Zytomed Systems	0	1	0	0	-	-
mAb clone **Ber-Ep4 GM080402**	2	Gene Tech	0	1	1	0	-	-
mAb clone **MOC-31 790-4561**[3]	3	Ventana/Roche	0	1	2	0	-	-
mAb clone **MOC-31 790-4561**[4]	10	Ventana/Roche	2	6	2	0	80%	20%
mAb clone **MOC-31 248M-18**	4	Cell Marque	0	1	3	0	-	-
mAb clone **MOC-31 CEM-0051**	1	Celnovte	0	0	1	0	-	-
mAb clone **VU-1D9 PDM077**	2	Diagnostic Biosystems	0	1	0	1	-	-
mAb clone **VU-1D9 8230-C010**	3	Sakura FineTek	0	1	2	0	-	-
mAb clone **BS14 8377-C010**	1	Sakura FineTek	1	0	0	0	-	-
mAb clone **SPM491 ab228023**	1	abcam	0	0	0	1	-	-

mAb clone **MX066 MAB-0850**	1	Fuzhou Maxin	0	1	0	0	-	-
mAb clone **BP6056 BX50051**	1	Biolynx	0	0	1	0	-	-
mAb clone **IHC567 IHC567-7/25**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **EP155 AN820**	1	BioGenex	0	0	0	1	-	-
rmAb clone **BY118 BFM-0436**	1	Bioin Biotechnology	0	0	1	0	-	-
rmAb clone **DA056 RMA1A068**	1	Shenzhen Dartmon Biotechnology	0	0	0	1	-	-
Total	345		51	113	159	22		
Proportion			15%	33%	46%	6%	48%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).

Detailed analysis of EpCAM, Run 69
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/Agilent) (1/2)*, Cell Conditioning 1 (CC1, Ventana/Roche) (6/7), CC1 followed by enzymatic pre-treatment with Protease 2 or 3 (Ventana/Roche) (10/13) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (1/2) 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 25 of 25 (100%) laboratories produced a sufficient staining result.
* (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/Agilent) (5/12) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:75 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 6 (83%) laboratories produced a sufficient staining result.

mAb clone **MOC-31**: One protocol with optimal result was based on HIER using TRS pH 6.1 (3-in-1) (Dako/Agilent) (1/5). The mAb was diluted 1:50 and a 3-layer detection system was applied. Only one laboratory used these protocol settings.

mAb clone **VU-1D9**: Protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (4/7) as retrieval buffer. The mAb was typically diluted in the range of 1:400-500. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.

Table 2. **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 / Ultra Plus**			**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 2/3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **BS14**	-	-	1/2**	-	6/7 (86%)	10/13 (77%)	-	1/2	-
mAb clone **Ber-EP4**	0/1	1/1	-	5/6 (83%)	0/2	0/4	0/1	0/1	0/2
mAb clone **MOC-31**	0/1	-	-	1/3	0/4	-	-	-	0/1
mAb clone **VU-1D9**	-	-		0/1	4/5 (80%)		-	0/1	-

* 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).

Ready-To-Use antibodies and corresponding systems
mAb clone **Ber-Ep4**, product no. **760-4383**, Ventana/Roche, BenchMark XT/Ultra/Ultra Plus:
One protocol with an optimal result was based on HIER in CC1 (efficient heating time 16 min. at 100°C) followed by enzymatic pre-treatment with Protease 3 (4 min. at 36°C), 32 min. incubation of the primary Ab and OptiView (760-700) as detection systems. Only one laboratory used these protocol settings.

mAb clone **Ber-Ep4**, product no. **IS637/IR637**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 6.1 or TRS pH 9 (efficient heating time 20 min. at 97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 3 of 5 (60%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Ber-Ep4**, product no. **GA637**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 6.1 (efficient heating time 30 min. at 97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800+GV821) as detection system. Using these protocol settings, 45 of 47 (96%) laboratories produced a sufficient result.

mAb clone **MOC-31**, product no. **790-4561**, Ventana/Roche, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER in CC1 (efficient heating time 32 min. at 99°C) or a combined pre-treament using HIER in CC1 (efficient heating time 32 min. at 100°C) followed by enzymatic pre-treatment with Protease 3 (4 min. at 36°C), 28-32 min. incubation of the primary Ab and OptiView with OptiView Amplification (760-700+760-099) as detection system. Only two laboratories used these protocol settings.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed 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 3. **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 **760-4383**	0% (0/13)	0% (0/13)	25% (21/85)	1% (1/85)
Autostainer +/Link mAb Ber-EP4 **IS/IR637**	(0/1)	(0/1)	40% (4/10)	30% (3/10)
Omnis mAb Ber-EP4 **GA637**	95% (42/44)	27% (12/44)	73% (11/15)	27% (3/11)
BenchMark XT/Ultra mAb MOC-31 **790-4561**	(1/3)	(0/3)	80% (8/10)	20% (2/10)

* 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 90% of the insufficient results (162 of 181). False positive reaction was seen in 1% of the insufficient results (2 of 181). The remaining insufficient results were characterized by poor signal-to-noise ratio or excessive background 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 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 reports (Run 45, 2015 and Run 56, 2019), 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 0% (0/12). In comparison, if HIER was applied as single pretreament, 46% (13/28) of the protocols were assessed as sufficient, of which 18% (5/28) were optimal. For participants applying combined antigen retrieval (HIER and proteolytic pre-treatment), the pass rate was 33% (3/9). 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 subtype, concentration and digestion time.

As shown in Table 2, 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/Agilent) and Diva pH 6.2 (Biocare Medical) 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. Only 33% of (5/15) protocols based on HIER in CC1 (Ventana/Roche), BERS1 or BERS2 (Leica Biosystems) on the fully automated platforms BenchMark XT/ Ultra (Ventana/Roche) or Bond MAX/III (Leica Biosystems) provided a sufficient result of which none was optimal. In comparison, if staining was performed on either Dako Autostainer or Omnis, 66% (6/9) of the protocols based on HIER in the TRS low pH 6.1 (Dako/Agilent) provided a result assessed as sufficient of which 56% (5/9) 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 and previous assessments and as shown in Table 2, the mAb clones BS14 and VU-1D9 might be alternatives to Ber-EP4 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. 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.

Using the mAb BS14 within a LD assay, 52% (13/25) of the protocols were based on combined retrieval typically applying HIER in CC1 followed by proteolysis in P3, OptiView as detection system and performed on BenchMark (Ventana/Roche). Using this combination, 77% (10/13) of the protocols were assessed as optimal. In total, combining Run 45, Run 56 and this Run 69, 100% (37/37) of the laboratories using this clone were able to produce a sufficient staining result of which 81% (30/37) were given an optimal mark, regardless of what platform was used. 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 Biosystems) or CC1 (Ventana/Roche) can produce optimal results. Similarly, the mAb VU-1D9 also seems robust. Stained on BenchMark Ultra (Ventana/Roche) applying HIER in CC1 all (7/7) protocols provided sufficient results.

The use of mAb clone MOC-31 within a LD assay has slightly decreased in this run, from 29 in Run 56 till 20 laboratories in this Run 69. The proportion of sufficient staining results has increased from 69% to 75%, however, the proportion of optimal results has significantly dropped from 71% to 5%, despite similar protocol settings being applied in both runs.

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 based detection system as e.g. OptiView (Ventana/Roche) or EnVision FLEX+ (Dako/Agilent), 64% (58/91) of the results were assessed as sufficient (30% optimal) compared to a pass rate of 48% (10/21) of which 10% (2/21) were optimal if 2-step systems as e.g. UltraView (Ventana/Roche) or EnVision FLEX (Dako/Agilent) were used.

The Ready-To-Use (RTU) system GA637 (Dako/Agilent, Omnis), based on the mAb clone Ber-EP4 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, a pass rate of 95% (42/44) was obtained. The IR/IS637 RTU system (Autostainer) based on the same clone, provided a significantly lower pass rate of 36% (4/11), 27% optimal. 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 most widely used RTU system 760-4383 (BenchMark XT/GX/Ultra/Ultra Plus, Ventana/Roche) 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, 21% and 1%, respectively. 13 laboratories 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 all results were all assessed as insufficient (see Table 3). The majority of participants used laboratory modified protocol settings giving a pass rate of 25% (21/85) of which one result was assessed as optimal.

The Ventana/Roche RTU system 790-4561 based on mAb clone MOC-31 (BenchMark Ultra/Ultra Plus), gave an overall pass rate of 69% (9/13), 15% optimal. As seen in Table 3, the majority of laboratories modified the protocol settings, with a pass rate of 80% (8/10), 20% optimal.

This was the seventh assessment of EpCAM in NordiQC (see Graph 1). EpCAM has been used for many years, and the marker is still challenging. The pass rate decreased to 48% in this run, compared to 57% in run 56, 2019, but comparable 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 was difficult, 2) The limited access to special formulated buffers if applying the mAb clone Ber-EP4 on non Dako/Agilent IHC platforms. , and 3) A large proportion of laboratories applied protocol settings based on enzymatic pre-treatment, providing a pass rate of only 16% (6/38) of which none were optimal.
Laboratories should apply an Ab that work on the in-house IHC platform, calibrate the protocols correctly accordingly to the expected antigen level of the recommended control material and diagnostic purpose.

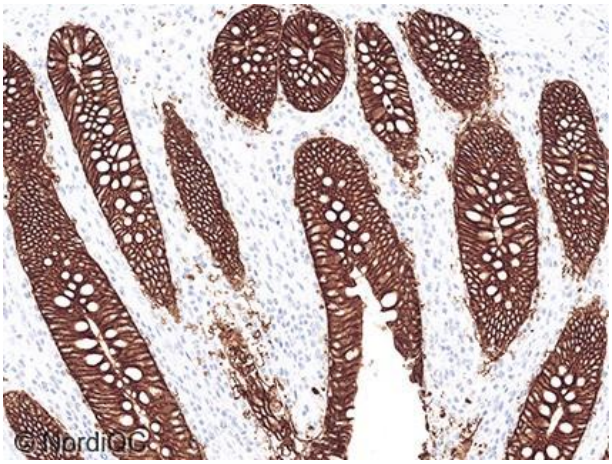


Fig. 1a
Optimal EpCAM staining reaction of the appendix using the mAb clone BS14 optimally calibrated, HIER in an alkaline buffer (CC1) and OptiView as detection system on a BenchMark platform (Ventana/Roche) - same protocol used in Figs. 2a-5a. All columnar epithelial cells show strong membranous staining reaction.

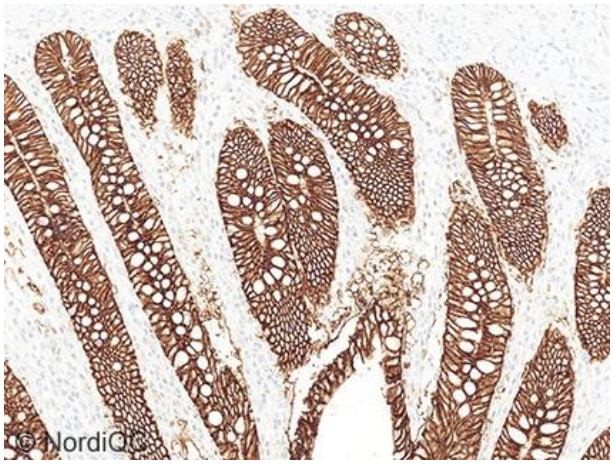


Fig. 1b
EpCAM staining reation of the appendix using the mAb clone Ber-EP4 as an RTU (760-4383, Ventana/Roche), using HIER in CC1 and UltraView as the detection system as recommended - same protocol used in Figs. 2b-4b. Although the epithelial cells are distinctively stained, the intensity is reduced, which is critical in relation to low expressing tissue/cell structures – also compare Figs. 2a-4b.

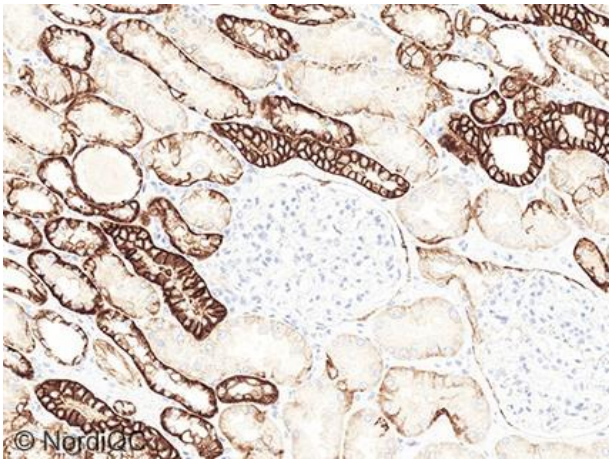


Fig. 2a
Optimal EpCAM staining reaction of the kidney using same protocol as in Fig. 1a. The epithelial cells of the distal convoluted tubules show a moderate to strong membranous staining reaction, while the epithelial cells of the Bowman capsule and proximal tubules only show a weak but distinct predominantly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

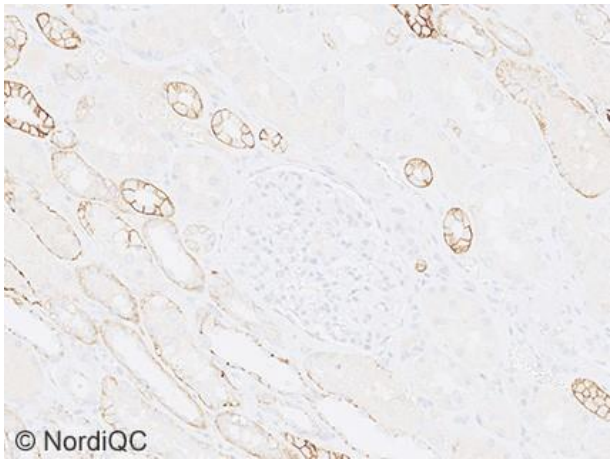


Fig. 2b
Insufficient EpCAM staining reaction of the kidney using same protocol as in Fig. 1b. Only the epithelial cells of the distal convoluted tubules are demonstrated, whereas epithelial cells of the Bowman capsule and proximal tubules are only faintly positive - compare with Fig. 2a, same area.

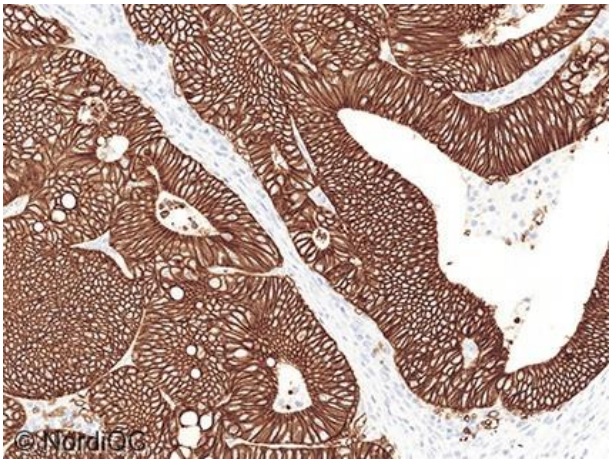


Fig. 3a
Optimal EpCAM staining of the colon adenocarcinoma using the same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a strong predominantly membranous staining reaction.

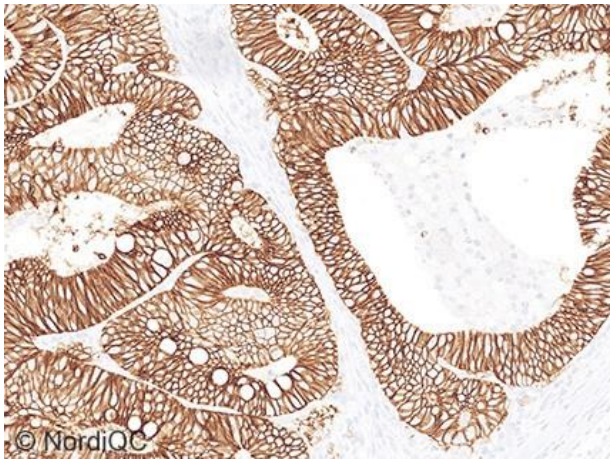


Fig. 3b
EpCAM staining of the colon adenocarcinoma using the same protocol as in Figs. 1b and 2b. The intensity is reduced but a distinct result is obtained – however compare with Fig. 4b, same protocol but in a tumour with low antigen expression level.

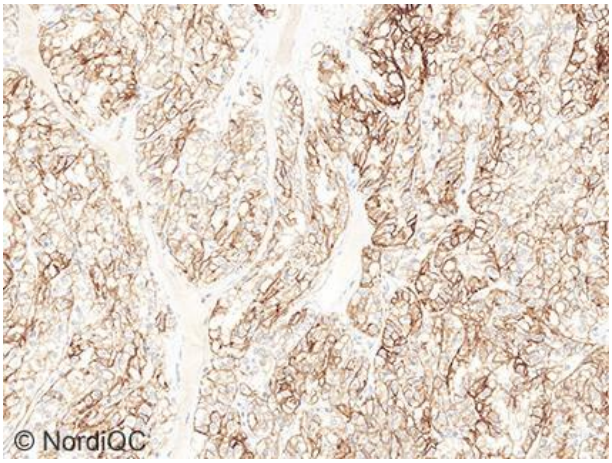


Fig. 4a
Optimal EpCAM staining reaction of the RCCC using the same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show an at least moderate, distinct membranous staining reaction.

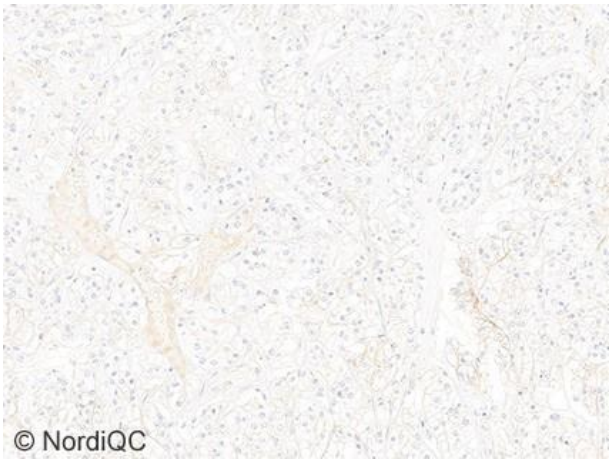


Fig. 4b
Insufficient EpCAM staining reaction of the RCCC using the same protocol as in Figs. 1b-3b. The vast majority of the neoplastic cells are false negative, not displaying the expected distinct membranous staining reaction as seen in Fig. 4a, same area.

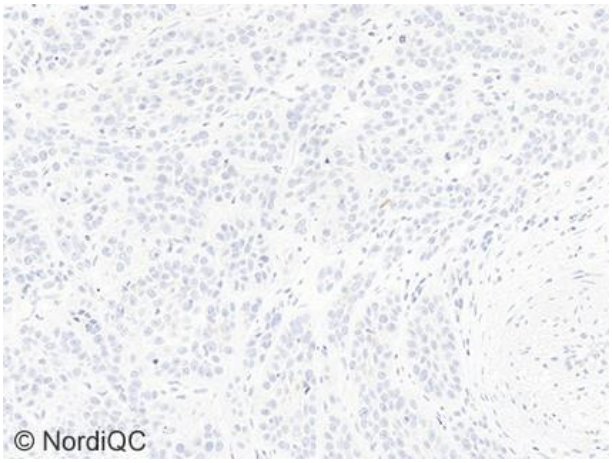


Fig. 5a
Optimal EpCAM staining reaction of the skin squamous cell carcinoma using the same protocol as in Figs. 1a-4a. No staining reaction is seen.

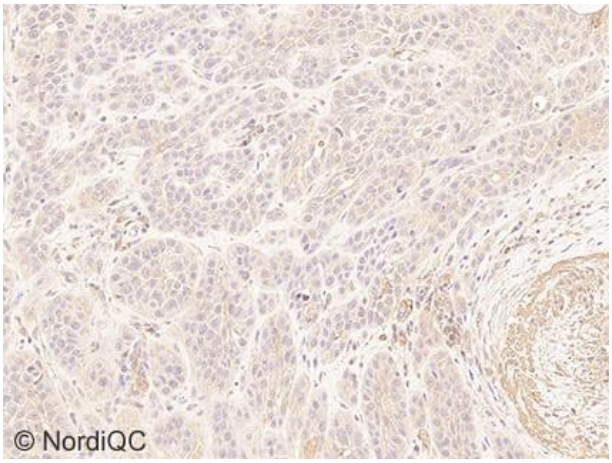


Fig. 5b
Insufficient EpCAM staining reaction of the skin squamous cell carcinoma using the mAb clone VU-1D9 as an RTU (PDM077, Diagnostic Biosystems), HIER in BERS1 and Bond Refine as detection system on the Bond platform (Leica Biosystems). In general, a poor signal-to-noise ratio is seen, complicating the interpretation. Same insufficient staining pattern is seen as in Figs. 6a-b.

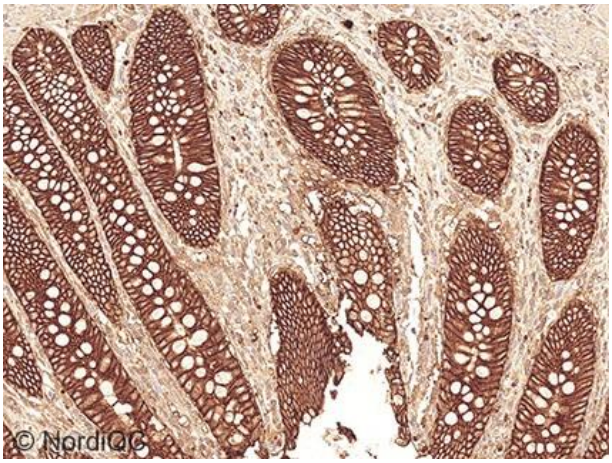


Fig. 6a
Insufficient EpCAM staining reaction of appendix, using the same protocol as in Fig. 5b. Columnar epithelial cells show strong membranous staining reaction, however, all inter-epithelial cells are false positive. See Fig. 1a for optimal result.

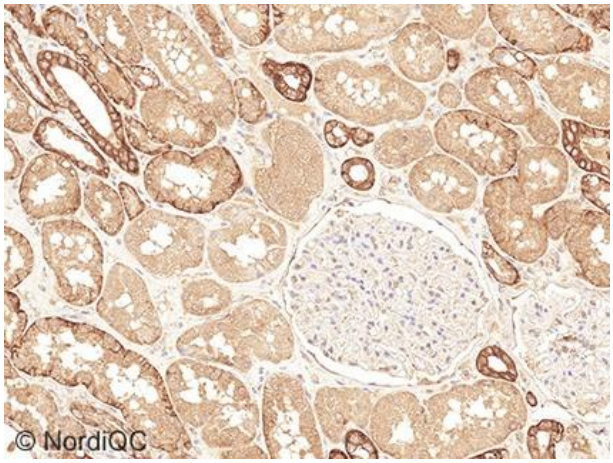


Fig. 6b
Insufficient EpCAM staining reaction of kidney using the same protocol as in Figs. 5b and 6a. An aberrantly cytoplasmic staining reaction is seen in all tubules, whereas the epithelial cells of the Bowman capsule are false negative. For optimal result, see Fig. 2a.

HLK/LE/SN 24.11.2023