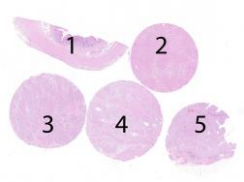
## Assessment Run 47 2016

# Carcinoembryonic antigen (CEA)

**Material**
The slide to be stained for CEA comprised:




1. Appendix, 2. Liver, 3. Colon adenocarcinoma, 4-5. Urothelial carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CEA staining as optimal included:

- An at least weak to moderate cytoplasmic staining reaction of the vast majority of columnar epithelial cells in the appendix with enhancement of the glycocalyx.
- A moderate to strong predominantly cytoplasmic staining reaction of virtually all neoplastic cells in the colon adenocarcinoma and the majority of neoplastic cells in the urothelial carcinoma tissue core no. 4.
- An at least weak to moderate predominantly cytoplasmic staining reaction focally of the neoplastic cells in the urothelial carcinoma tissue core no. 5.
- No staining in any other cells. Especially no staining reaction of non-specific cross-reacting antigen (NCA = CEACAM6) in leukocytes and biliary glycoprotein (BGP = CEACAM1) in bile canaliculi.

**Participation**

| | |
|---|---|
| Number of laboratories registered for CEA, run 47 | 294 |
| Number of laboratories returning slides | 276 (94%) |
| Number of laboratories returning slides using appropriate antibodies* | 255 (87%) |

*21 laboratories used a polyclonal antibody cross reacting with NCA and BGP. These were marked as "Inappropriate antibody" and not assessed further.

**Results**
255 laboratories participated in this assessment. 108 (42%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reaction were:

- Less successful primary Ab – all protocols (n=16) based on the mAb clones TF3H8-1 (n=13) and 12-140-10 (n=3) gave insufficient results.
- Less successful performance of the mAb clone II-7 (on all platforms, but especially on the BenchMark platform, Ventana).
- Inappropriate retrieval - omission of retrieval or use of proteolysis.
- Too low concentration of the primary Ab.
- Use of less sensitive detection systems.

**Performance history**
This was the fourth NordiQC assessment of CEA. A consistent decrease in the pass rate has been observed during these four runs as listed in table 2.

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

| | Run 12 2004 | Run 27 2009 | Run 37 2013 | Run 47 2016 |
|---|---|---|---|---|
| Participants, n= | 60 | 123 | 190 | 255 |
| Sufficient results | 86% | 75% | 59% | 42% |

**Conclusion**
The mAb clones **CEA31** and **COL-1** can both be recommended for demonstration of CEA, irrespective of IHC stainer platform. The mAb clone **II-7** was less successful. Although optimal staining results could be obtained (2 of 85)**,** this clone showed significantly lower analytical sensitivity compared to mAb clone CEA31 and COL-1. Irrespective of the clone applied, HIER in alkaline buffer was mandatory for optimal staining result. Appendix and liver are the recommended control tissues for CEA. In appendix, the vast

majority of columnar epithelial cells must show an at least weak to moderate intra-cytoplasmic staining reaction. No staining should be seen in liver.

Table 1. **Antibodies and assessment marks for CEA, run 47**

| Concentrated Antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **12-140-10** | 3 | Leica/Novocastra | 0 | 0 | 0 | 3 | - | - |
| mAb clone **CEA31** | 9<br>1 | Cell Marque<br>BioSB | 6 | 0 | 3 | 1 | 67% | 75% |
| mAb **COL-1** | 6<br>5<br>5<br>2<br>1<br>1 | Thermo/Neomarkers<br>Invitrogen/Zymed<br>Biocare<br>Immunologic<br>Zytomed<br>GeneTex | 11 | 7 | 2 | 0 | 90% | 94% |
| mAb **II-7** | 85 | Dako/Agilent | 2 | 19 | 60 | 4 | 25% | 58% |
| mAb **CEA88** | 2 | BioGenex | 0 | 0 | 1 | 1 | - | - |
| mAb **PARLAM 4** | 1 | Monosan | 0 | 0 | 1 | 0 | - | - |
| mAb **BS33** | 1 | Nordic Biosite | 0 | 0 | 1 | 0 | | |
| Ready-To-Use Antibodies | | | | | | | | |
| mAb clone **CEA31 760-4594** | 53 | Ventana/Cell Marque | 22 | 26 | 5 | 0 | 91% | 100% |
| mAb clone **CEA31 236M** | 4 | Cell Marque | 1 | 2 | 1 | 0 | - | - |
| mAb clone **COL-1 MAD-002095QD** | 2 | Master Diagnostica | 0 | 0 | 1 | 1 | - | - |
| mAb clone **COL-1 PM058** | 1 | Biocare | 0 | 0 | 1 | 0 | - | - |
| mAb clone **COL-1 Kit-0008** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| mAb clone **II-7 IR/IS622/GA622** | 47 | Dako/Agilent | 0 | 6 | 40 | 1 | 13% | - |
| mAb clone **II-7 PA0004** | 12 | Leica | 0 | 5 | 6 | 1 | 42% | - |
| mAb clone **TF3H8-1 760-2507** | 13 | Ventana/Roche | 0 | 0 | 0 | 13 | 0% | - |
| Total | 255 | | 43 | 65 | 122 | 25 | - | |
| Proportion | | | 17% | 25% | 48% | 10% | 42% | |

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

**Detailed analysis of CEA, Run 47**
The following protocol parameters were central to obtain optimal staining:

**Concentrated Antibodies**
mAb clone **CEA31**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (Ventana) (3/6) * or Target Retrieval Solution pH 9 (3-in-1) (Dako) (3/3). The mAb was typically diluted in the range of 1:100-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 8 (75%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **COL-1**: Protocols with optimal results were all based on HIER using either Target Retrieval Solution pH 9 (3-in-1) (Dako) (1/2), Cell Conditioning 1 (Ventana) (8/13), Bond Epitope Retrieval Solution 2 (Leica) (1/1) or Tris-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the

range of 1:100-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 16 of 17 (94%) laboratories produced a sufficient staining.

mAb clone **II-7**: Protocols with optimal results were all based on HIER using either TRS pH 9 (Dako) (1/12) or BERS 2 (Leica) (1/10) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 7 of 12 (58%) laboratories produced a sufficient staining.

Table 3. **Optimal results for CEA for the three most commonly used concentrated antibodies on the 3 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic / OMNIS** | | **Ventana BenchMark XT / Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|
| | 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 **II-7** | 1/17** (6%) | 0/2 | 0/35 (0%) | - | 1/10 (10%) | 0/4 (0%) |
| mAb clone **COL-1** | 1/2 | - | 8/13 (62%) | - | 1/1 | - |
| mAb clone **CEA31** | 3/3 | - | 3/6 (50%) | - | - | - |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

**Ready-To-Use Abs (RTU)**
mAb clone **CEA31** product no. **760-4594**, Ventana/Cell Marque, BenchMark GX, XT and Ultra: Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time 16-64 min.), 12-32 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 45 of 45 (100%) laboratories produced a sufficient staining.

**Comments**
In this assessment and in concordance with the previous NordiQC assessments of CEA, the prevalent features of an insufficient staining were either a generally too weak or false negative staining reaction of the cells and structures expected to be demonstrated or a false positive staining reaction. Too weak or false negative staining reaction was seen in 88% of the insufficient results (129 of 147 laboratories). Virtually all laboratories were able to demonstrate CEA in the colon adenocarcinoma, whereas the urothelial carcinoma no. 4 and in particular the urothelial carcinoma no. 5 were much more challenging and required an optimally calibrated protocol. Compared to the previous CEA assessment (Run 37 in 2013) a significant decrease in the pass rate was seen. The reason is unclear, but could be related to an increased number of new participants and, maybe more importantly, more challenging material circulated in the present assessment.

48% (122 of 255) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for CEA. The mAb clones II-7, CEA31 and COL-1 were the three most widely used. They could all be used to obtain an optimal staining, but the clones CEA31 and COL-1 provided a significantly higher proportion of optimal results, see table 1. The more challenging material circulated in this assessment clearly reveals a higher analytical sensitivity of the mAb clones CEA31 and COL-1 compared to mAb clone II-7 (see Fig. 1a – 4b). Only 2 of 85 (2%) laboratories using mAb clone II-7 produced optimal staining, whereas 55% (11 of 20) and 60% (6 of 10) produced optimal staining when using mAb clone COL-1 and CEA31, respectively. The two LD assays based on mAb clone II-7 giving optimal staining where both based on HIER in an alkaline buffer and a sensitive 3-step polymer detection system performed on either the Leica Bond platform or the Dako Autostainer platform. Despite similar protocol settings (range of Ab titre, 3-step multimer and HIER in alkaline buffer) no optimal results were seen with mAb clone II-7 on the Ventana BenchMark platform. This was in concordance with the previous NordiQC assessments of CEA.

Ready-To-Use (RTU) antibodies was used in 52% (133 of 255) of the laboratories. Optimal result could be obtained with the RTU systems based on the mAb clone CEA31 and the mAb clone COL-1. In concordance with the previous CEA assessment (run 37, 2013), the most successful and robust assay for CEA was obtained with the RTU system based on the mAb clone CEA31 from Ventana/Cell Marque (760-4594). The pass rate was 91% (48 of 53 laboratories) and 42% were assessed as optimal (22 of 53 laboratories). In contrast, the widely used RTU system based on the mAb clone II-7 from Dako (IR/IS/GA622) performed very poorly in this assessment. Only 13% of the laboratories passed (6 of 47) and none were assessed as optimal. In comparison, the same system had a pass rate of 61% (20 of 43) in the previously assessment in 2013. A similar decline in pass rate was not seen for the RTU system based on the mAb clone CEA31

from Ventana/Cell Marque (760-4594). The pass rate for mAb clone CEA31 (760-4594) dropped marginally from 92% in 2013 to 91% in this assessment. Data from both RTU- and LD assays indicate that more challenging material was circulated in Run 47, which highlights the lower analytical sensitivity of the mAb clone II-7 compared to the mAb clones CEA31 and COL-1.

In concordance with previous assessments, HIER was required in order to obtain sufficient results. Irrespective of the clone or IHC platform used, inappropriate retrieval, as omission of HIER (n=8 laboratories) or use of proteolytic pre-treatment (n=4 laboratories), gave insufficient results due to weak staining reactions (see Fig. 5a – 6b). Furthermore optimal results could only be obtained with use of HIER in an alkaline buffer.

False positive reaction was seen in 12% of the insufficient results (18 out of 147 laboratories). This was related to the following primary Ab clones: mAb clone 12-140-10 giving a cross reaction with non-specific cross-reacting antigen, (NCA; CEACAM6) in leucocytes and the mAb clones CEA88 and TF3H8-1 giving a cross reaction with both NCA and biliary glycoprotein (BGP; CEACAM1) (see Fig. 7a and 7b). All slides showing this positive reaction in either leukocytes and/or bile canaliculi were assessed as insufficient.

**Controls**
Appendix, in combination with liver, is the recommended positive and negative tissue controls for CEA. In the appendix the vast majority of epithelial cells must show an at least weak to moderate cytoplasmic staining reaction. If only the glycocalyx is demonstrated, inadequate staining in neoplasias with low CEA expression is seen (as observed in the urothelial carcinoma, tissue core no. 5 in this assessment – see Figs. 1a, 1b, 4a and 4b).
Liver is recommended as negative tissue control. Bile canaliculi and leucocytes must be negative with no cross reaction to BGP or NCA to verify the specificity of the primary Ab. Hepatocytes must be negative to verify a high signal-to-noise ratio.

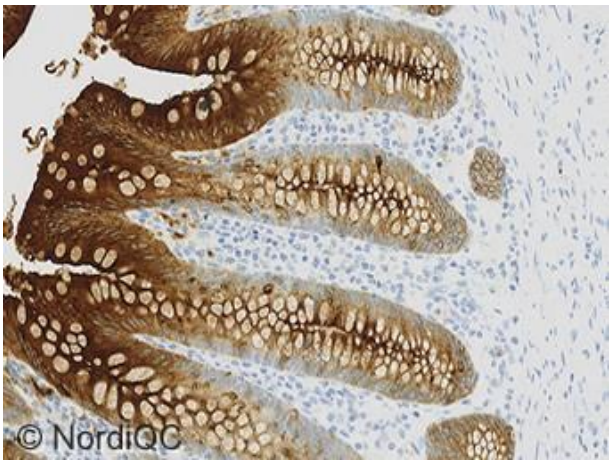


Fig. 1a (x200)
Optimal CEA staining of the appendix using the **mAb clone CEA31** diluted 1:100 and with an incubation time of 30 min. after HIER in an alkaline buffer (TRS pH 9, Dako). Staining was performed on the Dako Omnis using a 3-step polymer system (EnVision Flex+). A weak to moderate staining reaction is seen in the vast majority of the luminal epithelial cells of the appendix, whereas the glycocalyx show an intense staining reaction.
Also compare with Figs. 2a – 4a, same protocol. No background staining is seen.

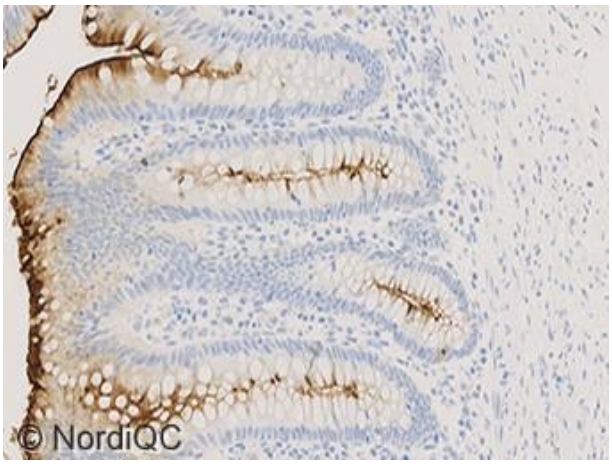


Fig. 1b (x200)
Insufficient CEA staining of the appendix using the **mAb clone II-7** in a RTU format (Dako GA622) with an incubation time of 25 min. after HIER in an alkaline buffer (TRS pH 9, Dako). Staining was performed on the Dako Omnis using a 3-step polymer system (EnVision Flex+). In spite of very similar protocol settings the "clone II-7"-protocol only demonstrates the glycocalyx distinctively, while the cytoplasmic compartment in the vast majority of epithelial cells is unstained - same field as in Fig. 1a. Also compare with Figs. 2b - 4b, same protocol.

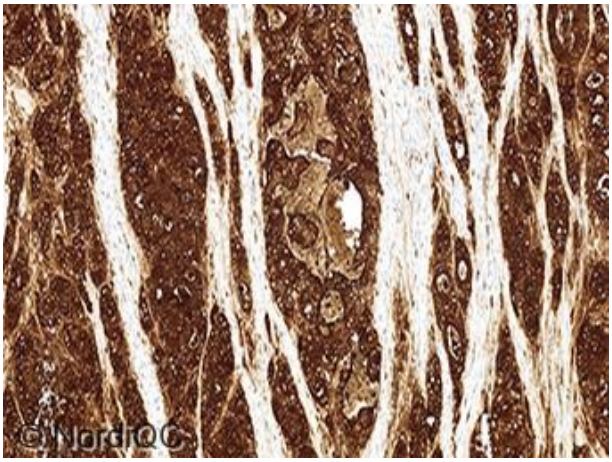



Fig. 2a (x200)
Optimal CEA staining of the colon adenocarcinoma with high level CEA expression using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction. Weak background staining in the vicinity of the neoplastic cells, due to diffusion of antigen, is seen and accepted.

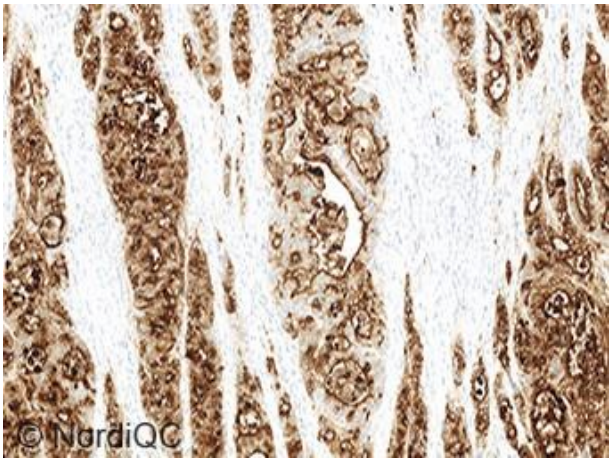



Fig. 2b (x200)
CEA staining of the colon adenocarcinoma with high level CEA expression using same insufficient protocol as in Fig. 1b – same field as in Fig. 2a. The intensity of the neoplastic cells demonstrated is reduced compared to the level expected and obtained in Fig. 2a.

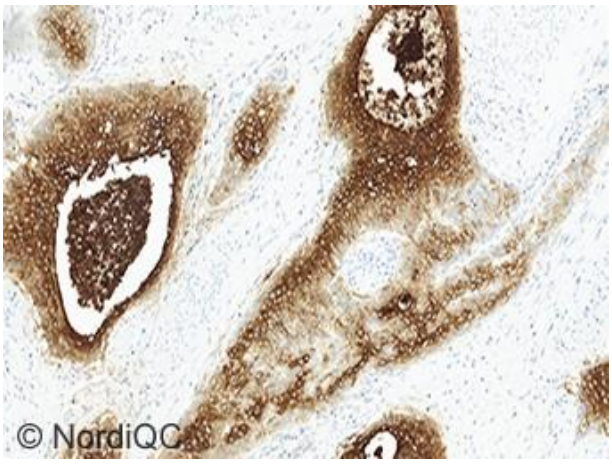



Fig. 3a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 4, using same protocol as in Figs. 1a and 2a. The majority of the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

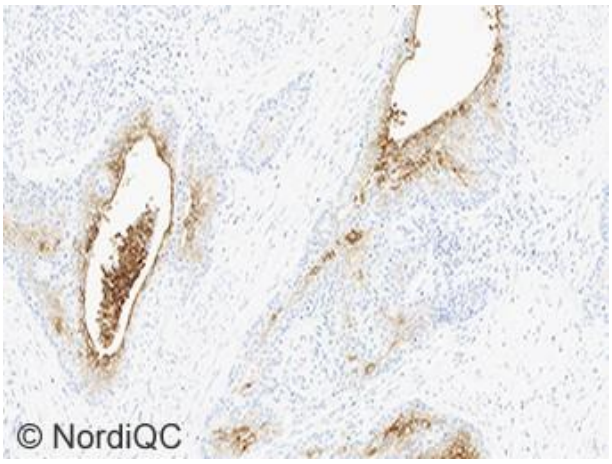



Fig. 3b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a.

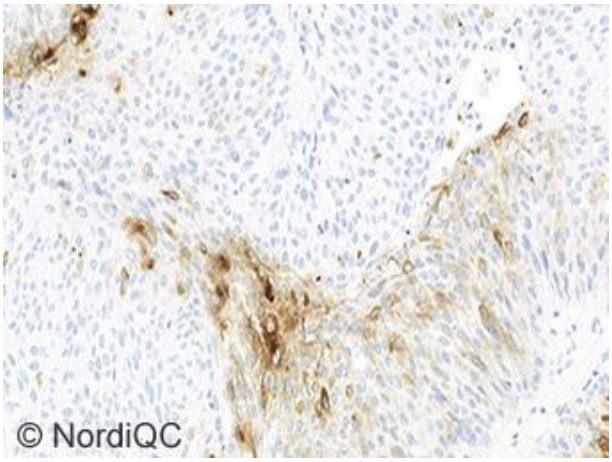



Fig. 4a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Figs. 1a - 3a. Focally the neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

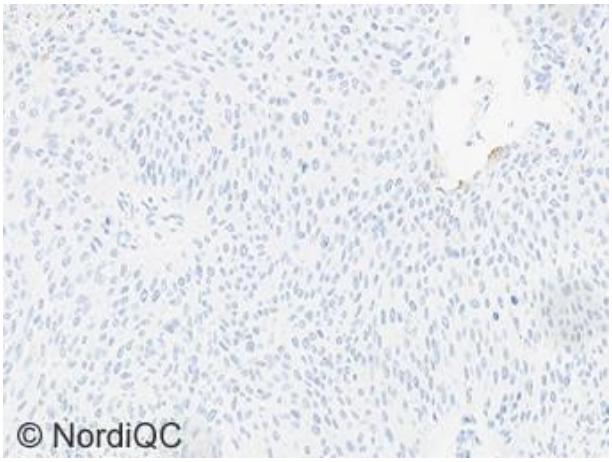



Fig. 4b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells show no staining reaction and a false negative result of the tumour is seen.

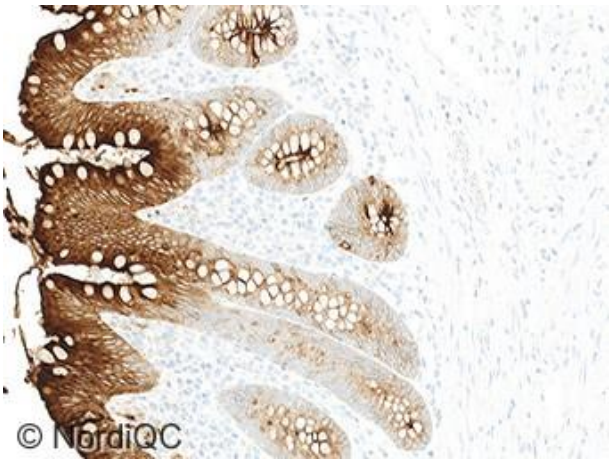



Fig. 5a (x200)
Optimal CEA staining of the appendix using the mAb clone CEA31 diluted 1:400 and with an incubation time of 30 min. after **HIER** in an alkaline buffer (CC1, Ventana). Staining was performed on the Ventana BenchMark using a 3-step multimer system (OptiView)
A weak to moderate staining reaction is seen in the vast majority of the luminal epithelial cells of the appendix, whereas the glycocalyx show an intense staining reaction. Compare also to Fig. 6a, same protocol.

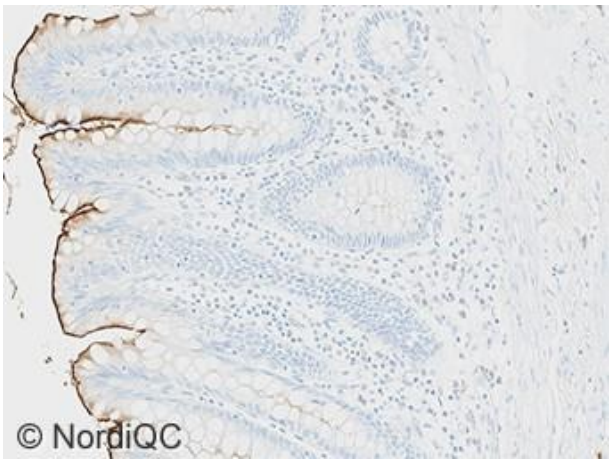



Fig. 5b (x200)
Insufficient CEA staining of the appendix using the mAb clone CEA31 with similar protocol settings as used in Fig. 5a. Only difference was the use of proteolytic pretreatment (Protease 1, Ventana for 8 min.) instead of HIER. Proteolytic pre-treatment results in a drastic reduction in staining intensity. Only the glycocalyx is distinctively demonstrated, while the cytoplasmic compartment of the epithelial cells is unstained - same field as in Fig. 5a. Compare also to Fig. 6b, same protocol.

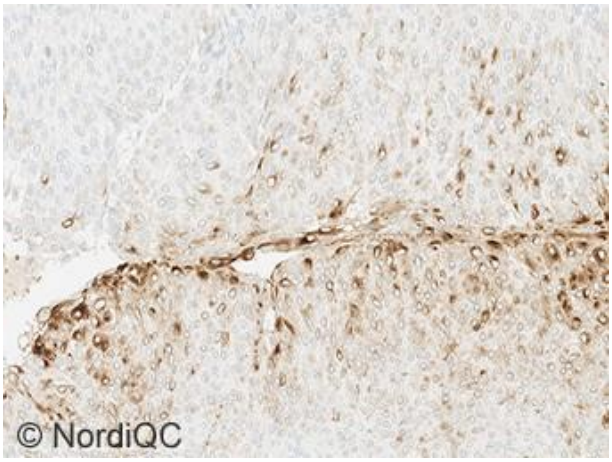



Fig. 6a (x200)
Optimal CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Fig 5a. Focally the neoplastic cells show a moderate to strong and distinct staining reaction.

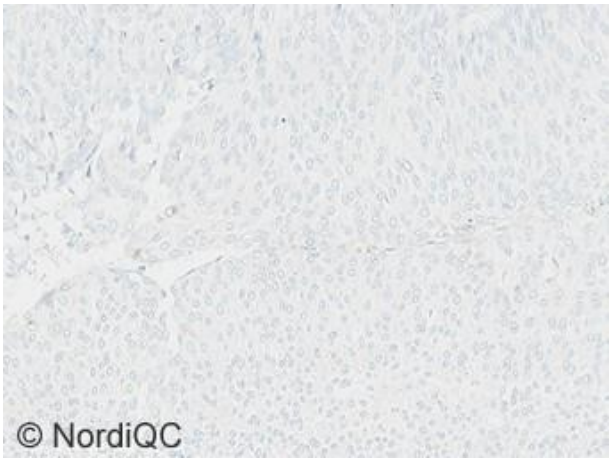



Fig. 6b (x200)
Insufficient CEA staining of the urothelial carcinoma, tissue core no. 5, with low level CEA expression using same protocol as in Fig. 5b – same field as in Fig. 6a. The neoplastic cells show no staining reaction and a false negative result in this tumour is seen.

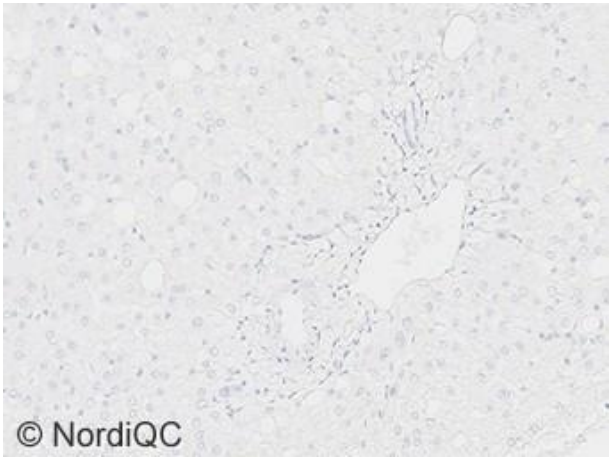



Fig. 7a (x200)
Optimal CEA staining of the liver using same protocol as in Figs. 5a and 6a based on the **mAb clone CEA31**. No staining reaction is seen in the Kupffer cells, leucocytes and the bile canaliculi. No background staining is seen.

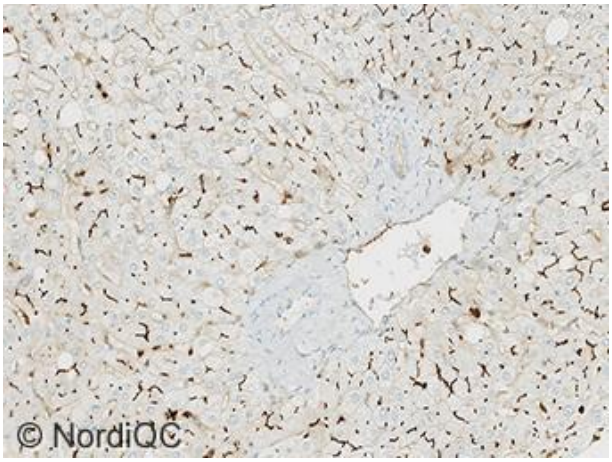



Fig. 7b (x200)
Insufficient CEA staining of the liver using the **mAb clone TF3H8-1.** Both the Kupffer cells, leucocytes and bile canaliculi are stained due to a cross reaction of the Ab to NCA (CEACAM6) and BGP (CEACAM1) – same field as in Fig. 7a.

ON/SN/LE/MV/RR 11.06.2016