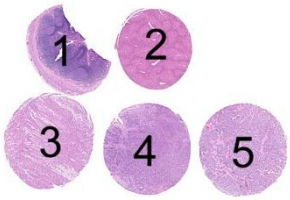
Assessment Run 27 2009
CEA

The slide to be stained for CEA comprised:
1. Appendix, 2. Liver, 3 - 4. Colon adenocarcinoma, 5. Medullary carcinoma of the thyroid.
Erratum: In the accompany letter tissue specimen no. 5 was erroneously listed as a colon adenocarcinoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CEA staining as optimal included:

- An at least weak to moderate, distinct cytoplasmic reaction of the majority of the surface epithelial cells of the appendix enhanced in the glycokalyx.
- A moderate to strong, distinct cytoplasmic reaction in the majority of the neoplastic cells of the two colon adenocarcinomas and the medullary carcinoma of the thyroid.
- No staining reaction in any other cells – in particular no reaction due to non-specific cross-reacting antigen, (NCA = CEACAM6) in leukocytes, and biliary glycoprotein (BGP = CEACAM1) in bile canaliculi.

136 laboratories participated in this assessment. 13 used a pAb "CEA" antibody considered to be inappropriate due to cross reaction with NCA and BGP and, hence, not assessed. 123 laboratories were assessed, of which 75 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CEA, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **II-7**	78	Dako	37	34	6	1	91 %	96 %
mAb clone **COL-1**	5 2 1 1 1	NeoMarkers Zymed Biocare Master Diagnostica Zytomed	6	2	2	0	80 %	100 %
mAb clone **12-140-10**	3 1	Novocastra Vector	0	0	1	3	-	-
mAb clone **PARLAM 4**	1 1	Bio-Science AG Euro Diagnostica	0	0	0	2	-	-
mAb clone **B01-94-11M-P**	1	BioGenex	0	0	0	1	-	-
mAb clone **TF3H8-1**	1	BioGenex	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **II-7, IS622/IR622**	11	Dako	11	0	0	0	100 %	100 %
mAb clone **TF3H8-1, 760-2507**	13	Ventana	0	0	0	13	0 %	-
mAb clone **COL-1, IP058**	1	Biocare	1	0	0	0	-	-
mAb clone **CEA31, ZM0062**	1	Zhongshan Bio	1	0	0	0	-	-
mAb clone **85A12, E056**	1	Linaris	0	0	0	1	-	-
Total	123		56	36	9	22	-	-
Proportion			46 %	29 %	7 %	18 %	75 %	-

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 **II-7**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (12/21)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako), (9/17), Bond Epitope Retrieval Solution 2 (Bond, Leica) (5/6), Bond Epitope Retrieval Solution 1 (Bond, Leica) (2/2), Cell Conditioning 1 (BenchMark, Ventana) (3/15), Target Retrieval Solution pH 6.1 (S1699/S1700, Dako) (2/4), Diva Decloaker, Biocare, (1/1) or Citrate pH 6 (3/8) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 68 out of 71 (96 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **COL-1**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/1), Cell Conditioning 1 (BenchMark, Ventana) (1/1), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:50–1:2.400 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 out of 8 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **II-7** (prod. no IS622/IR622, Dako): 10 of the protocols giving an optimal result were based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, 10 or 20min), an incubation time of 20 or 30 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. 1 optimal protocol was based on HIER using Tris-EDTA/EGTA pH9 (MWO) and an incubation time of 30 min in the primary Ab and Envision (K5007) as the detection system. Using these protocol settings 11 out of 11 (100 %) laboratories produced a sufficient staining.

mAb clone **CEA31** (prod.no. ZM0062, Zhongshan Bio): The protocol giving an optimal result was based on HIER using Citrate pH 6 in a pressure cooker, an incubation time of 60 min in the primary Ab and Zhongshan Bio B10 D12-110 as the detection system.

The most frequent causes of insufficient stains were:
- Less successful primary Ab (e.g., all of 14 protocols based on the mAb clone TF3H8-1 gave an insufficient result)
- Inappropriate epitope retrieval: Omission of HIER or proteolysis
- Too low concentration of the primary antibody.

In this assessment the prevalent features of an insufficient staining were either a false positive reaction or a generally too weak staining in the specimens tested. A false positive reaction was seen in 71 % of the insufficient results (22/31). This was related to the primary Ab clones: The mAb clone 12-140-10 gave a cross reaction with non-specific cross-reacting antigen, (NCA, CEACAM6) in leukocytes, the mAb clone B01-94-11M-P gave a cross reaction with biliary glycoprotein (BGP; CEACAM1) in bile canaliculi, and the mAb clones PARLAM 4 and TF3H8-1 gave a cross reaction with both NCA and BGP. All slides showing this positive reaction in either leukocytes and/or bile canaliculi were assessed as insufficient. With the four Abs mentioned, the staining pattern was seen for all 22 protocols, irrespective of protocol settings and sensitivity levels.
In the remaining 9/31 insufficient stains (29 %), a generally too weak staining was seen - mainly when the mAb clone II-7 was used without HIER and/or too diluted.
In accordance with the previous assessments of CEA, appendix was a reliable positive control, provided that the epithelial cells showed a distinct cytoplasmic staining, as all laboratories demonstrating CEA in this cellular compartment also could demonstrate CEA in the two colon adenocarcinomas and the medullary thyroid carcinoma. If only the glycocalyx was demonstrated, a too weak staining, especially in one of the colon adenocarcinomas (specimen no 4) was seen. In order to validate the specificity of the mAb, liver should be used as a negative control (both bile canaliculi and leukocytes must be negative).
The mAb clone COL-1 seemed to give a more pronounced cytoplasmic staining in the epithelial cells of the appendix and labelled a higher proportion of the neoplastic cells in the colon adenocarcinoma no. 4 compared to the staining obtained with the mAb clone II-7.

This was the second assessment of CEA in NordiQC. The proportion of sufficient results declined from 86 % in run 12, 2004 to 75 % in the current run – see table 2. The lower pass rate is mainly due to the high proportion of mAbs giving a cross reaction. These mAbs were in previous assessment classified as inappropriate and therefore not influencing the pass rate.

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

	Run 12 2004	Run 27 2009
Participants, n=	60	123
Sufficient results	86 %	75 %

Conclusion

The mAb clones II-7 and COL-1 are robust and recommendable mAbs for CEA. HIER is mandatory to obtain an optimal result. Appendix is a reliable positive control, in which the epithelial cells must show a distinct cytoplasmic staining. Liver is a recommendable control to validate the specificity of the mAb clone: no reaction should be seen in bile canaliculi or leucocytes.

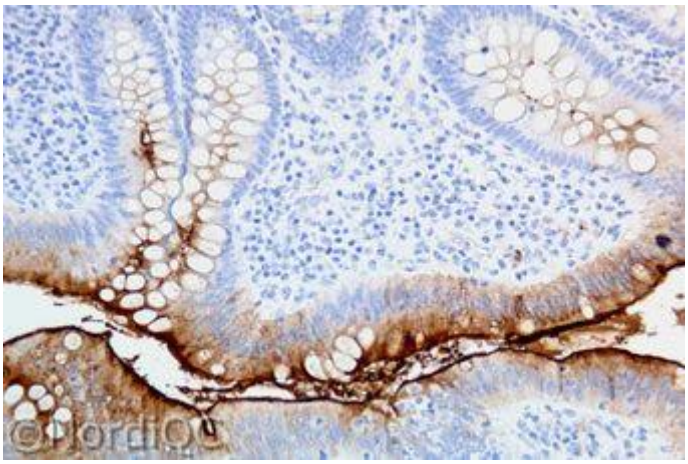


Fig. 1a
Optimal CEA staining using the mAb clone II-7, optimally calibrated and with HIER in an alkaline buffer. A weak to moderate staining reaction is seen in the majority of the luminal epithelial cells of the appendix, whereas the glycokalyx show an intense staining reaction.

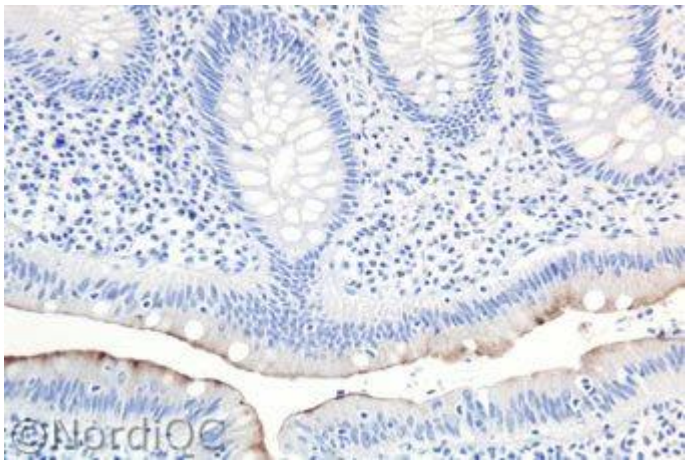


Fig. 1b
Insufficient CEA staining using the mAb clone II-7 too diluted. Only the glycokalyx show a weak staining, while the cytoplasmic compartment of the epithelial cells is totally negative. Same field as in Fig. 1a. Also compare with Figs. 2b left & right – same protocol.

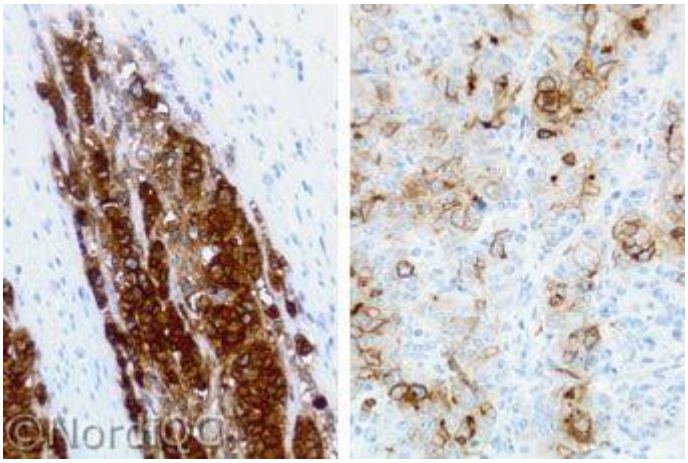


Fig. 2a
Optimal staining for CEA using same protocol as in Fig. 1a
Left: Colon adenocarcinoma tissue no. 3 in the multitissue block: Virtually all the neoplastic cells show a strong cytoplasmic staining reaction.
Right: Colon adenocarcinoma tissue no. 4 in the multitissue block: The majority of the neoplastic cells show a moderate to strong cytoplasmic staining reaction.

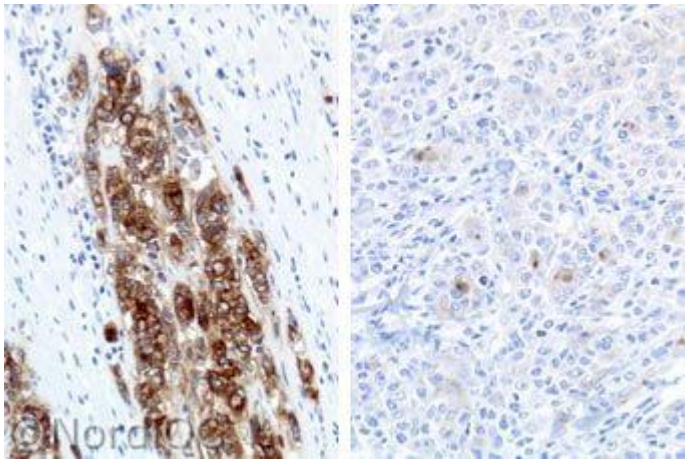


Fig. 2b
Insufficient CAE staining using same protocol as in Fig. 1b – same fields as in Figs. 2a left & right.
Left: Colon adenocarcinoma tissue no. 3 in the multitissue block: Virtually all the neoplastic cells show a strong cytoplasmic staining reaction.
Right: Colon adenocarcinoma tissue no. 4 in the multitissue block: Only scattered neoplastic cells show a weak and dubious cytoplasmic staining reaction.

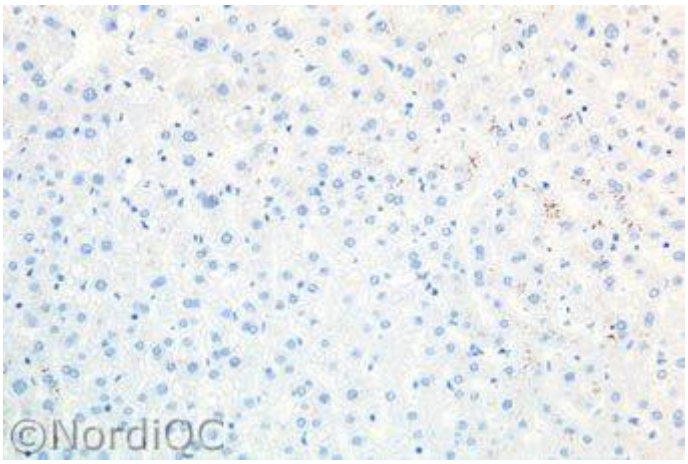


Fig. 3a
Optimal CEA staining of the liver using same protocol as in Figs. 1a & 2a based on the mAb clone II-7. No staining reaction is seen in the Kupffer cells or in the bile canaliculi.

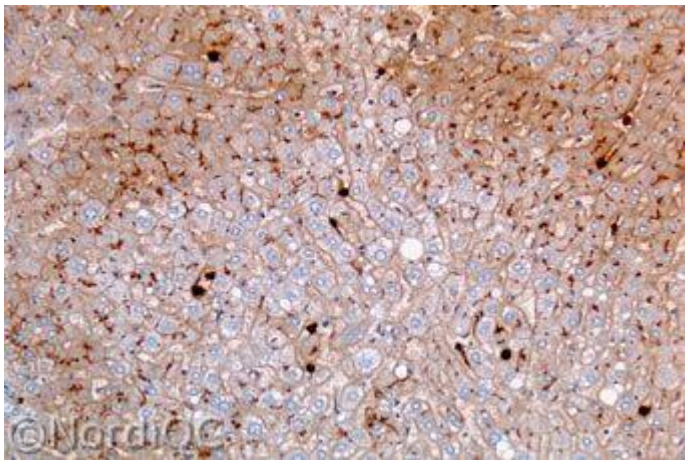


Fig. 3b
Insufficient CEA staining of the liver using mAb clone TF3H8-1. Both the Kupffer cells, leucocytes and bile canaliculi are stained due to a cross reaction of the Ab to NCA (CEACAM6) and BGP (CEACAM1).

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