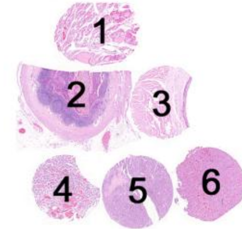
Assessment Run 34 2012

Cytokeratin 19 (CK19)

Material
The slide to be stained for CK19 comprised:



1. Thyroid gland, 2. Appendix, 3. Esophagus, 4. Papillary thyroid carcinoma, 5 & 6. Pancreatic neuroendocrine carcinomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK19 staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of virtually all the appendiceal surface epithelial cells, and at least a weak to moderate staining reaction of the epithelial cells in the basal part of the crypts.
- A strong cytoplasmic staining reaction of the majority of the basal squamous epithelial cells in the esophagus and a weak to moderate staining reaction of scattered intermediate epithelial cells (* some variation in the staining pattern between levels was seen in these cores).
- At maximum a weak to moderate staining reaction in scattered epithelial cells in the thyroid gland.
- A moderate to strong, distinct staining reaction of virtually all the neoplastic cells of the papillary thyroid carcinoma and the pancreatic neuroendocrine carcinoma no. 5, and an at least a weak to moderate dot-like staining reaction of the majority of the neoplastic cells in the pancreatic neuroendocrine carcinoma no. 6.

147 laboratories participated in this assessment. 46 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CK19, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A53-B/A2.26**	6 6 1 1 1	Cell Marque Thermo/NeoMarkers DBS IDLabs Zytomed	4	5	5	1	60 %	86 %
mAb clone **BA17**	3 1	Thermo/NeoMarkers Master Diagnostica	3	1	0	0	-	-
mAb clone **b170**	7	Leica/Novocastra	5	2	0	0	100 %	100 %
mAb clone **K19.2**	1	Thermo/NeoMarkers*	0	0	0	1	-	-
mAb clone **Ks19.1**	4	Biocare	2	2	0	0	-	-
mAb **RCK108**	61 3 1 1 1 1	Dako Biogenex Abcam Eurodiagnostica EuroProxima Thermo/NeoMarkers	7	16	24	21	34 %	57 %
rmAb **EP72**	1	Epitomics	1	0	0	0	-	-
pAb **RB-9021**	1	Thermo/NeoMarkers	0	0	0	1	-	-
Ready-To-Use Abs:								
mAb clone **A53-B/A2.26 760-4281**	17	Ventana/Cell Marque	6	6	3	2	71 %	90 %
mAb clone **A53-B/A2.26 319M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone	1	Zhongshan	0	1	0	0	-	-

A53-B/A2.26 **ZM-0074**								
mAb clone **b170** **PA0799**	3	Leica	0	0	1	2	-	-
mAb clone **Ks19.1** **PM242**	1	Biocare	0	1	0	0	-	-
mAb clone **RCK108** **IS/IR615**	22	Dako	2	3	12	5	23 %	50 %
mAb clone **RCK108** **MS-1902-R7**	1	Thermo/NeoMarkers	0	0	1	0	-	-
Total	147		30	38	46	33	-	
Proportion			22 %	26 %	31 %	23 %	46 %	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below
* Product has been discontinued by the vendor

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **A53-B/A2.26**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (CC1, BenchMark, Ventana) (3/9)* or Tris-EDTA/EGTA pH 9 (1/3)* as the retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **BA17**: The protocols giving an optimal result were all based on HIER using either CC1 (BenchMark, Ventana) (1/1), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **b170**: The protocols giving an optimal result were all based on HIER using either Bond Epitope Retrieval Solution 2 (BERS 2, Bond, Leica) (4/5) or CC1 (BenchMark, Ventana) (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 6 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **Ks19.1**:The protocols giving an optimal result were both based on HIER using either BERS 2 (Bond, Leica) (1/1) or Borg Decloaker pH 9.5 (Biocare) (1/1) as the retrieval buffer. The mAb was diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **RCK108**: The protocols giving an optimal result were all based on HIER using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9, Dako) (2/13), BERS 2 (Bond, Leica) (1/7), Borg Decloaker pH 9.5 (Biocare) (1/1), Tris-EDTA/EGTA pH 9 (2/10) or Citrate pH 6.7 (1/1) as the 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 16 out of 28 (57 %) laboratories produced a sufficient staining (optimal or good).

rmAn clone **EP72**: The protocol giving an optimal result was based on HIER using Citrate pH 6 as the retrieval buffer. The mAb was diluted 1:100.

Ready-To-Use Abs
mAb clone **A53-B/A2.26** (prod. no. 760-4281 Ventana/Cell Marque): The protocols giving an optimal result were typically based on HIER in CC1 (BenchMark, Ventana) mild or standard, an incubation time of 24-32 min in the primary Ab and UltraView (760-500) with or without amplification kit or OptiView as the detection system. Using these protocol settings 9 out of 10 (90 %) laboratories produced a sufficient staining.

mAb clone **RCK108** (prod. no. IS/IR615, Dako): The protocols giving an optimal result were all based on HIER in PT-Link (heating time for 20 min at 97°C) using TRS pH 9 (3-in-1) or TRS pH 9 as the HIER buffer,

an incubation time of 20 min in the primary Ab and EnVision Flex/Flex+(K8000K8002) as the detection system. Using these protocol settings 4 out of 8 (50 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:

- Too low concentration of the primary Ab
- Less successful performance of the primary mAb clone RCK108
- Inappropriate epitope retrieval - use of proteolytic pre-treatment or omission of pre-treatment
- Imprecise data sheets from the main providers of the Abs for CK19

In this assessment and in concordance with the previous NordiQC assessment for CK19, run 29 2010, the prevalent feature of an insufficient staining was a too weak or false negative reaction of the cells expected to be demonstrated. This was seen in 97 % of the insufficient results (77 out of 79). In the remaining 3 % a false positive staining and an impaired morphology was seen.
The majority of laboratories were able to demonstrate CK19 in high antigen expressing structures such as the luminal columnar epithelial cells of the appendix and the neoplastic cells of the pancreatic neuroendocrine carcinoma no. 5, whereas the demonstration of CK19 in the basal columnar epithelial cells of the appendix, the epithelial cells of the esophagus, the pancreatic neuroendocrine carcinoma no. 6 and the papillary thyroid carcinoma expressing less CK19 was much more challenging and required an optimally calibrated protocol.

Several antibodies could be used to obtain an optimal staining result. In general an efficient HIER in an alkaline buffer and a carefully calibrated Ab titre were the main parameters to provide an optimal staining. The use of proteolytic pre-treatment was found to give suboptimal results, as 14 out of 17 protocols based on proteolysis were assessed as insufficient and none gave an optimal result. This was seen for all the antibodies used.
In this context it has to be stressed that the data sheets from many vendors for the Abs for CK19 give misleading and imprecise guidelines concerning the epitope retrieval and protocol set-up for the antibodies. E.g., the protocol recommended by Dako (the most used vendor) for the mAb clone RCK108 as a concentrate is based on proteolytic pre-treatment, whereas HIER is recommended when the clone is sold as a Ready-To-Use (RTU) format from same vendor! The mAb clones B170, Leica/Novocastra and Ks19.1, Biocare could both give an optimal result providing that HIER was performed and not proteolytic pre-treatment as recommended in the data sheets from these vendors. In addition, the data sheets for these two clones suggest skin to be used as positive control for CK19, which cannot be recommended to validate the IHC performance for this antibody. CK19 is not expressed in keratinizing epithelial cells and the content of sweat glands hair follicles expressing Ck19 is highly varying complicating the interpretation.

The most widely used mAb clone RCK108 as a concentrate gave a very low pass rate of 37 % and even with optimal protocol settings - appropriate titre of the mAb and pre-treatment settings, only a slight improvement to 57 % was seen. Use of a 2-step polymer or multimer based detection system as e.g., EnVision Flex, Dako or UltraView, Ventana gave a significantly lower pass rate of 21 %, compared to 50 % when a more sensitive 3-step polymer or multimer based detection system such as, e.g., EnVision Flex+, Bond Refine (Leica) or UltraView + amplification was used. With the same clone as Ready-To-Use format, Dako, an even lower overall pass rate was seen.
These results clearly indicate that the mAb clone RCK108 is less robust for the demonstration of CK19 compared to the mAb clones A53-B/A2.26, BA17, b170 and Ks19.1, all giving a pass rate of 90-100 % when used with optimal protocol settings as described above and similar as applied for the mAb clone RCK108.

As positive control for CK19, the combination of esophagus and appendix was found to be most reliable as critical stain quality indicators for CK19. In the optimal protocols virtually all the basal epithelial cells in these two tissues showed a moderate to strong distinct cytoplasmic staining reaction.
In the insufficient results deemed too weak the basal cells only showed an equivocal or totally negative staining reaction.
In the optimal protocols a distinct positive staining reaction was seen in scattered epithelial cells of the normal thyroid epithelial cells. However, the staining intensity and proportion of positive cells was significantly lower than those of the thyroid carcinoma.

This was the 2nd assessment of CK19 in NordiQC (Table 2), and in this run a significantly lower pass rate was seen compared to the previous run in 2010.

Table 2. **Proportion of sufficient results for CK19 in the two NordiQC runs performed**

	Run 29 2010	Run 34 2012
Participants, n=	109	147
Sufficient results	69 %	46 %

In this assessment for CK19 many new laboratories participated for the first time and for these a lower pass rate was observed compared to the laboratories also participating in the previous run 29, 2010: For the laboratories participating for the first time the pass rate was 35 % (17 out of 48), whereas the pass rate was 52 % (51 out of 99) for the laboratories participating in both runs. This indicate that the combination of many new participants and a more challenging tissue block circulated most likely caused the lower pass rate in this run for CK19.

Conclusion
The mAb clones A53-B/A2.26, b170, BA17 and Ks19.1 were in this assessment the most robust Abs for CK19. For all clones HIER should be used for an optimal performance. Appendix and esophagus are the recommended positive controls for CK19: In the esophagus the basal squamous epithelial cells as well as scattered intermediate cells must show a distinct cytoplasmic staining reaction and in the appendix the basal columnar epithelial cells must show an at least weak to moderate staining reaction.

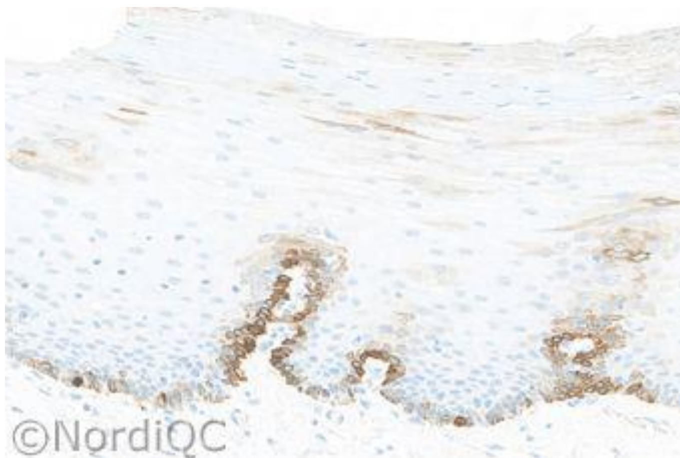


Fig 1a
Optimal staining for CK19 of the esophagus using the mAb clone A53-B/A2.26 optimally calibrated and with HIER in an alkaline buffer. The majority of the basal squamous epithelial cells show a moderate cytoplasmic staining reaction and a weak to moderate staining reaction is seen in scattered intermediate epithelial cells.

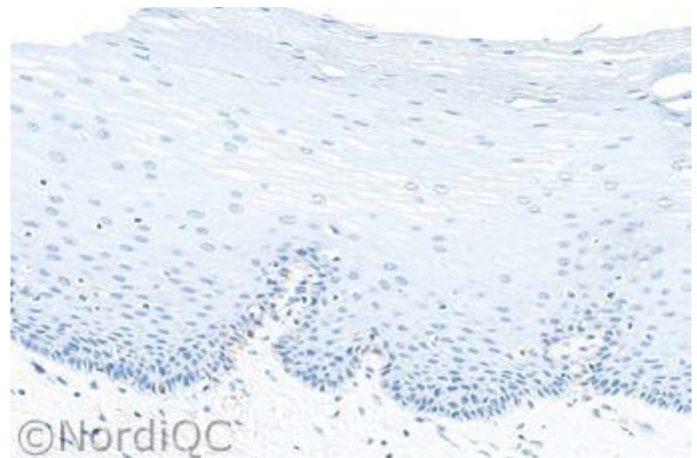


Fig 1b
Insufficient staining for CK of the esophagus the mAb clone A53-B/A2.26 by a protocol giving a too low sensitivity – too low concentration of the primary Ab., same field as in Fig. 1a. No staining reaction is seen in the squamous epithelial cells – also compare with Figs. 2b - 4b same protocol.

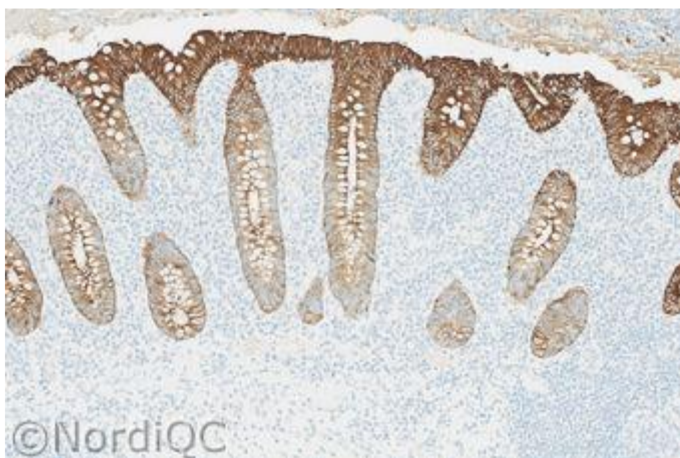


Fig 2a
Optimal staining for CK19 of the appendix using same protocol as in Fig. 1a. The surface columnar epithelial cells show a strong cytoplasmic staining reaction, while the columnar epithelial cells in the basal parts of the crypts show a weak to moderate staining reaction.
No background staining is seen.

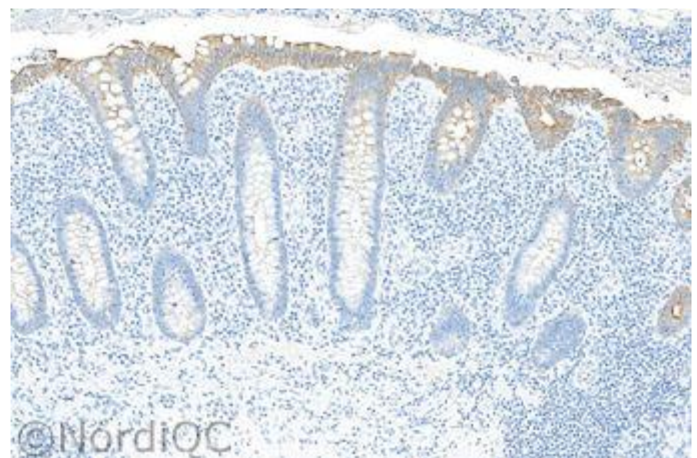


Fig 2b
Insufficient staining for CK19 of the appendix using same protocol as in Fig. 1b., same field as in Fig. 2a. Only the surface columnar epithelial cells show a moderate cytoplasmic staining reaction, while virtually no staining reaction is seen in the basal part of the crypts – also compare with Figs. 3b - 4b same protocol.

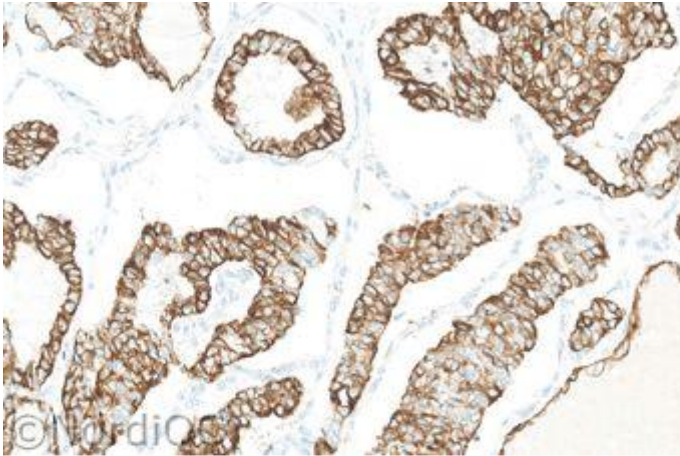


Fig 3a
Optimal staining for CK19 of the papillary thyroid carcinoma using same protocol as in Figs. 1a - 2a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction. No background reaction is seen.

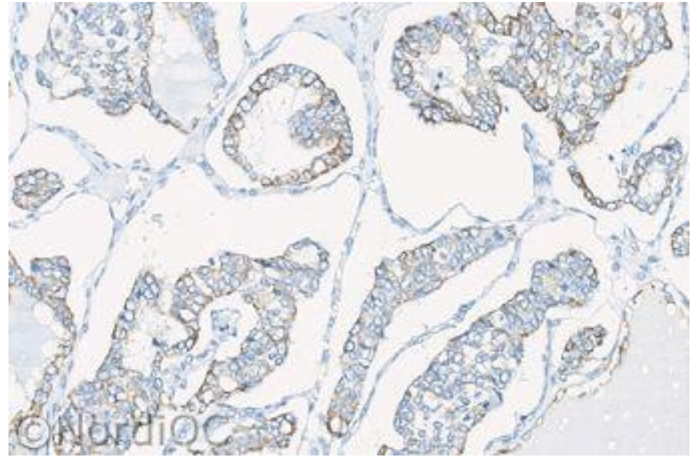


Fig 3b
Insufficient staining for CK19 of the papillary thyroid carcinoma using same protocol as in Figs. 1b & 2b., same field as in Fig. 3a.
Only scattered neoplastic cells show a weak to moderate staining reaction.

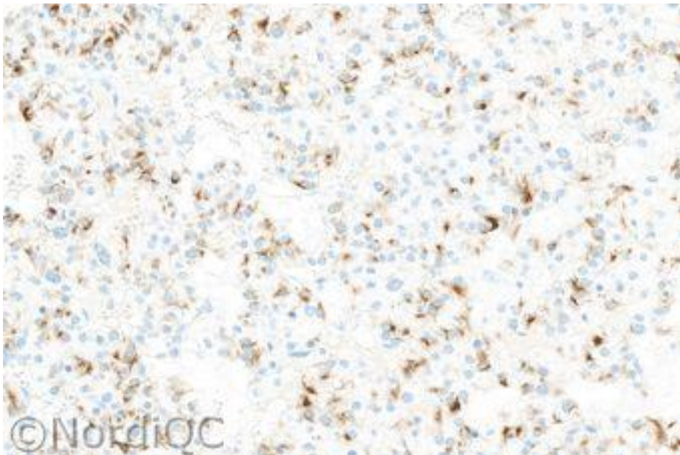


Fig 4a
Optimal staining for CK19 of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a weak to moderate cytoplasmic staining reaction with a dot-like accentuation. No background reaction is seen.

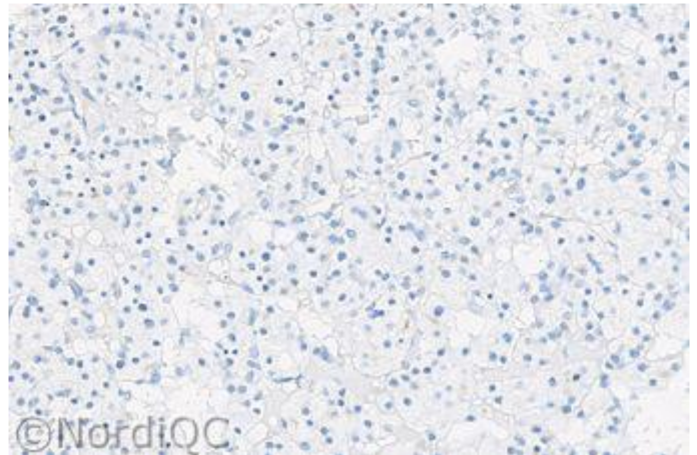


Fig 4b
Insufficient staining for CK19 of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1b – 3b., same field as in Fig. 4a.
The neoplastic cells are false negative.

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