Assessment Run 35 2012

Cytokeratin 20 (CK20)

Material
The slide to be stained for CK20 comprised:

1. Colon adenocarcinoma, 2. Merkel cell carcinoma, 3. Colon adenocarcinoma, 4. Appendix
5. Gastric body and 6. Urothelial carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK20 staining as optimal included:

- A strong, distinct cytoplasmic staining reaction of all the surface epithelial cells of the appendix and at least a weak to moderate staining reaction in most crypt cells.
- An at least moderate, distinct cytoplasmic staining reaction of the majority of the foveolar epithelial cells of the stomach.
- A moderate to strong, distinct cytoplasmic staining reaction in virtually all the neoplastic cells of the colon adenocarcinoma no. 3 and focally in the colon adenocarcinoma no. 1
- A moderate to strong, distinct dot-like intracytoplasmic staining reaction in virtually all the neoplastic cells of the Merkel cell carcinoma
- An at least weak to moderate, distinct cytoplasmic staining reaction in the majority of the neoplastic cells of the urothelial carcinoma

196 laboratories participated in this assessment. One lab submitted an inappropriate antibody (CK-LMW). Out of the remaining 195 labs 85 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CK20, run 35**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ks20.8**	92 10 2 2 2 1 1 1 1 1	Dako Leica/Novocastra Cell Marque Eurodiagnostics Thermo/NeoMarkers Biocare DBS Europroxima Master Diagnostica Progen	56	42	12	3	87 %	91%
mAb clone **PW31**	1	Leica/Novocastra	0	0	1	0	-	-
rmAb clone **EP23**	1	Epitomics	0	1	0	0	-	-
pAb **E16444**	7	Spring Bioscience	4	3	0	0	100 %	100 %
Unknown	1	Unknown	0	1	0	0		
Ready-To-Use Abs:								
mAb clone **Ks20.8 IR/IS777**	25	Dako	13	12	0	0	100 %	100 %
mAb clone **Ks20.8 PM062**	1	Biocare	0	0	1	0	-	-
mAb clone **Ks20.8 320M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **Ks20.8 RTU-CK20**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **Ks20.8 E062**	1	Linaris	1	0	0	0	-	-
mAb clone **Ks20.8**	1	Monosan	0	0	1	0	-	-

mon-rtu1083								
mAb clone **Ks20.8 ZM-0075**	1	Zhongshan	0	1	0	0	-	-
mAb clone **PW31 PA0918**	4	Leica/Novocastra	0	0	3	0	-	-
rmAb clone **SP33 790-4431**	37	Ventana	20	10	6	1	81 %	100 %
Total	195		94	72	25	4	-	
Proportion			48%	37 %	13 %	2%	85 %	

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 **Ks20.8**: the protocols giving an optimal result were based on either heat induced epitope retrieval (HIER), enzymatic pre-treatment or a combination of HIER and enzymatic pre-treatment. In total 56 optimal stainings were seen by the use of one of these three pre-treatment procedures.

50 out of the 56 laboratories obtaining an optimal staining result used HIER in either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (8/20)*, TRS pH 9 (Dako) (3/9), Cell Conditioning 1 (CC1; BenchMark, Ventana) (17/29), Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (13/17),Borg Decloaker pH 9.5 (Biocare) (1/2), Tris-EDTA/EGTA pH 9 (8/16) or EDTA/EGTA pH 8 (1/1) as the retrieval buffer.
The mAb was typically diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 87 out of 96 (91 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this reagent)

3 laboratories obtaining an optimal staining result used enzymatic pre-treatment as Protease 1 (Benchmark, Ventana) (2/11) or Bond Enzyme 1 (1/2).
The mAb was typically diluted in the range of 1:50-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 out of 8 (88 %) laboratories produced a sufficient staining (optimal or good).

2 laboratories obtaining an optimal staining result used a combined pre-treatment using Protease 3 (Benchmark, Ventana) and HIER in CC1 (Benchmark, Ventana) (2/2). The mAb was diluted 1:50-80. Using these protocol settings 2 out of 2 laboratories produced an optimal staining.

pAb **E16444**: The protocols giving an optimal result were all based HIER using either CC1 (BenchMark, Ventana) (2/4), Tris-EDTA/EGTA pH 9 (1/1) or Citrate pH 6 (1/1) as the retrieval buffer.
The pAb was diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 out of 7 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **Ks20.8** (prod. no. IS/IR777, Dako): The protocols giving an optimal result were all based on HIER in PT-Link (heating time for 10-20 min at 97°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20 min in the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as the detection system. Using these protocol settings 20 out of 20 (100 %) laboratories produced a sufficient staining.

rmAb clone **SP33** (prod. no. 790-4431, Ventana): The protocols giving an optimal result were typically based on HIER using mild or standard CC1, an incubation time of 16-32 min in the primary Ab and UltraView (760-500) +/- amplification or OptiView (760-700) as the detection system. Using these protocol settings 27 out of 27 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary Ab
- Less successful primary Ab

- Use of biotin based detection systems giving a false positive staining due to endogenous biotin

In this assessment and in concordance to the previous NordiQC runs for CK20 the prevalent feature of an insufficient staining reaction was a general too weak staining or a false negative staining of the structures expected to be demonstrated. Virtually all laboratories could demonstrate CK20 in the luminal epithelial cells of the appendix and in the neoplastic cells of the colon adenocarcinomas and the Merkel cell carcinoma, whereas the demonstration of CK20 in the foveolar epithelial cells of the stomach and in the neoplastic cells in the urothelial carcinoma was more challenging and required a correctly calibrated protocol.
A too weak or false negative staining reaction was seen in 83 % of the insufficient results and was mainly caused by a too low concentration of the primary Ab or using a less successful clone as the mAb clone PW31, Leica, which gave an insufficient result in all 5 out 5 protocols based on this clone.
In 17 % both a too weak and a false positive staining reaction was observed. The false positive staining reaction was observed when a biotin based detection system was used in combination with efficient HIER and was mainly seen as a granular intracytoplasmic staining reaction in the epithelial cells of the gastric crypt epithelium.
In the previous NordiQC assessment for CK20, run 25, 2009 it was shown that HIER gave a significantly higher pass rate compared to enzymatic pre-treatment for the most widely used mAb clone Ks20.8. If HIER was used a pass rate of 76 % was seen (76 out of 100 labs), compared to 19 % if enzymatic pre-treatment was used (5 out of 26 labs). In this assessment only 12 laboratories used enzymatic pre-treatment for the mAb clone Ks20.8 and for these laboratories a pass rate of 58 % was seen, whereas a pass rate of 91 % was seen if HIER was performed (114 out of 126 labs).

In this assessment stomach was the most appropriate control for CK20, as both the sensitivity and specificity (regarding endogenous biotin) could be evaluated in this tissue. The majority of the foveolar epithelial cells must show an at least weak to moderate cytoplasmic staining, while other epithelial cells shall be negative (neuroendocrine cells in the basal part will show a strong staining reaction).

This was the 3rd assessment of CK20 in NordiQC (Table 2) and a significant increase in the pass rate was seen compared to run 25.

Table 2. **Proportion of sufficient results for CK20 in the three NordiQC runs performed**

	Run 8 2003	Run 25 2009	Run 35 2012
Participants, n=	71	130	195
Sufficient results	90 %	64%	85 %

The significant improvement of the pass rate for CK20 can by influenced by many parameters including new and less challenging material circulated. However as the most challenging tissues in the previous run and this run being the gastric body and the urothelial carcinoma both were incorporated in the multi-blocks constructed for these two runs, this clearly indicates and supports that an improvement was achieved. The improvement most likely was related to the reduced use of enzymatic pre-treatment, which in both this run and the previous run has shown to be less successful compared to HIER for the mAb clone Ks20.8. In run 25, 21 % of the laboratories used enzymatic pre-treatment compared to 8 % in this run for the mAb clone Ks20.8. It was also related to the high quality and extended use of the Ready-To-Use (RTU) systems for CK20 from the two main providers Dako & Ventana, as the RTU systems from these two companies in this assessment showed a pass-rate of 100 % thus being superior to the pass-rates for the in-house validated protocols for CK20. The recently launched RTU system from Ventana based on the rmAb clone SP33 showed a superior performance in this run compared to the previous Ventana RTU system based on the mAb clone Ks20.8 used by the laboratories in run 25, where a pass rate of only 50 % was seen.

Conclusion
The concentrates of the mAb clone **Ks20.8** and the pAb **E16444** are both recommendable antibodies for CK20. For both Abs, HIER is preferred to obtain an optimal staining.
The RTU systems from Dako and Ventana, based on the mAb clone **Ks20.8** and the rmAb clone **SP33** respectively, gave a pass rate of 100 % and thus superior to the in-house validated assays.
Stomach is recommended as positive control: The majority of the foveolar cells must show an at least weak to moderate cytoplasmic staining reaction. Alternatively appendix can be used: Virtually all the luminal epithelial cells must show a strong cytoplasmic staining reaction, while the majority of the crypt epithelial cells an at least weak cytoplasmic staining reaction.

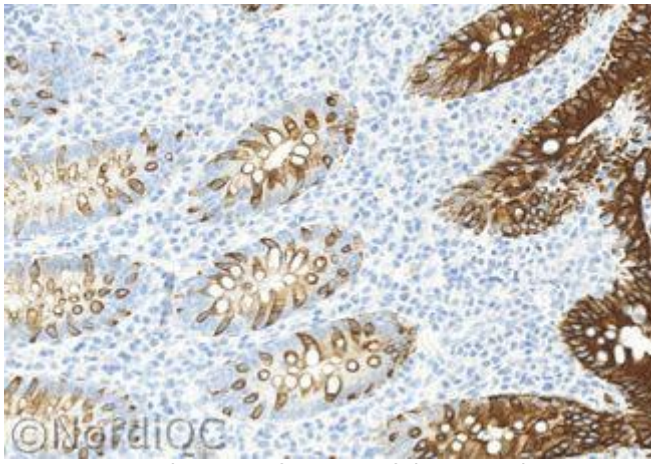

Fig. 1a. Optimal staining for CK20 of the appendix using the mAb clone Ks20.8 optimally calibrated and with HIER.
The luminal epithelial cells show a strong cytoplasmic staining reaction, while the majority of the epithelial cells of the crypts show a weak to moderate cytoplasmic staining reaction.
No background staining seen.
Also compare with Figs. 2a - 4a – same protocol.

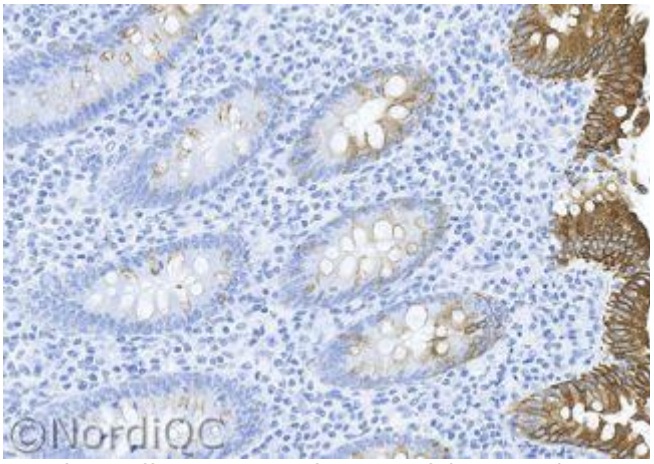

Fig. 1b. Insufficient staining for CK20 of the appendix using the mAb clone Ks20.8 too diluted – same field as in Fig. 1a.
The luminal epithelial cells show a moderate to strong cytoplasmic staining reaction, while the epithelial cells of the crypts show a reduced intensity and proportion of positive cells compared to the result in Fig. 1a.
Also compare with Figs. 2b - 4b – same protocol.

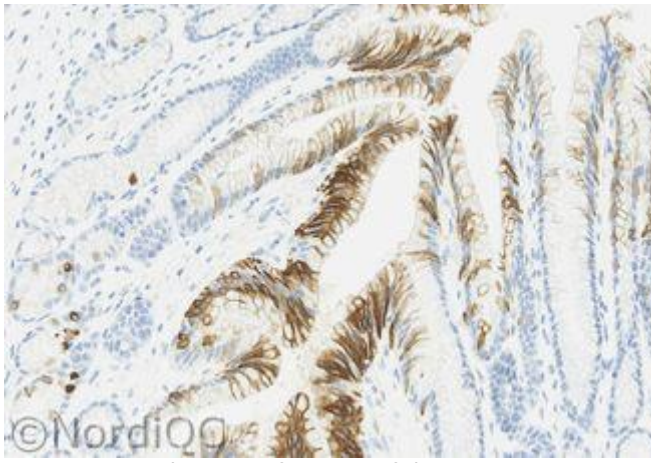

Fig. 2a. Optimal staining for CK20 of the gastric mucosa using the same protocol as in Fig. 1a.
The majority of the foveolar epithelial cells show a distinct cytoplasmic staning reaction.

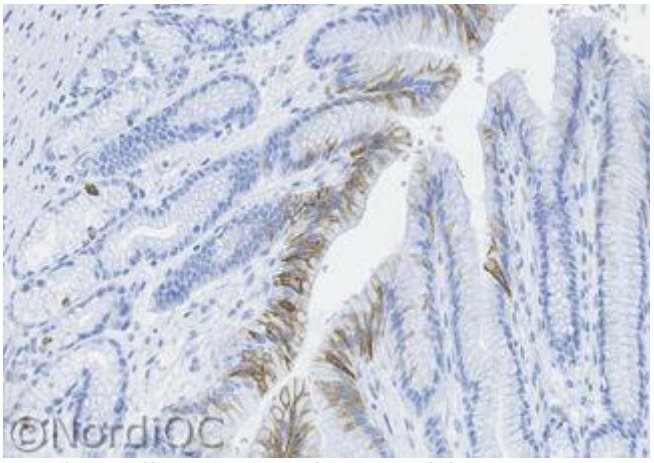

Fig. 2b. Insufficient staining for CK20 of the gastric mucosa using the same protocol as in Fig. 1b - same field as in Fig. 2a.
Only scattered foveolar epithelial cells show a weak and diffuse cytoplasmic staining reaction.

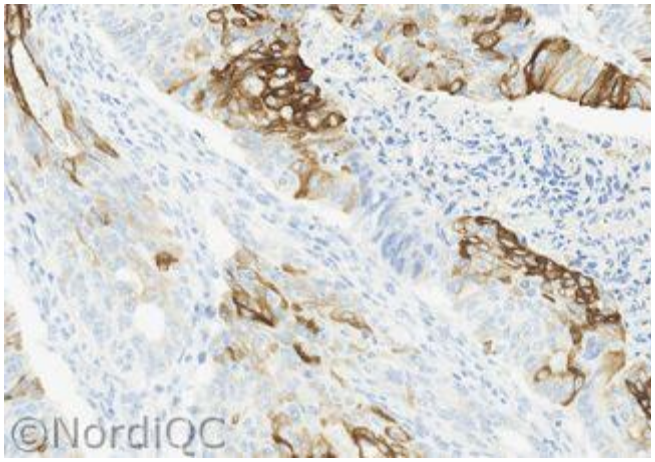

Fig. 3a. Optimal staining for CK20 of the colon adenocarcinoma no. 1 using the same protocol as in Figs. 1a & 2a.
Focally the neoplastic cells show a weak to strong cytoplasmic staining reaction.

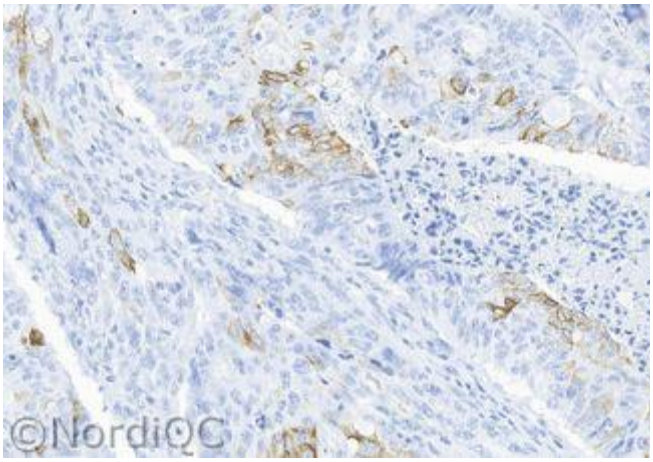

Fig. 3b. Insufficient staining for CK20 of the colon adenocarcinoma no. 1 using the same protocol as in Figs. 1b & 2b - same field as Fig. 3a.
A significantly reduced intensity and proportion of positive cells is seen compared to the result in Fig. 3a.

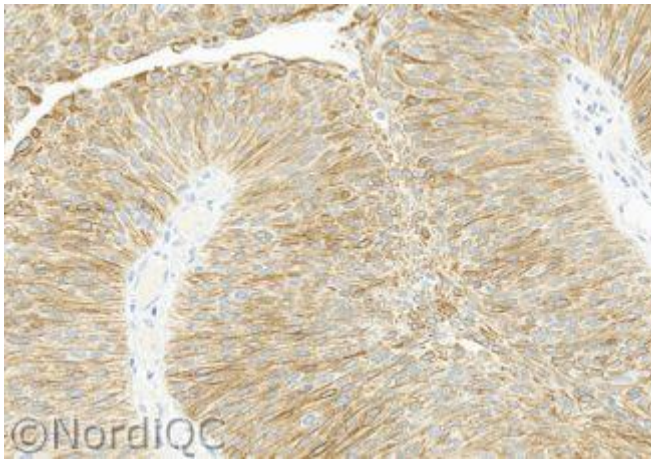

Fig. 4a. Optimal staining for CK20 of the urothelial carcinoma using same protocol as in Figs. 1a - 3a.
The majority of the neoplastic cells show a moderate and distinct cytoplasmic staining reaction.

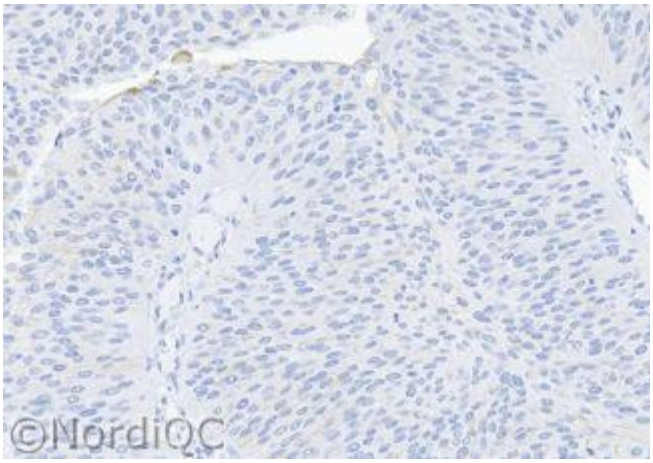

Fig. 4b. Insufficient staining for CK20 of the urothelial carcinoma using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a.
Only scattered neoplastic cells show a weak and diffuse staining reaction.

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