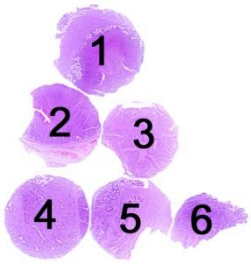
Assessment Run 25 2009

Cytokeratin 20 (CK20)

The slide to be stained for CK20 comprised:
1. Appendix, 2. Gastric body, 3. Transitional cell carcinoma, 4. Pancreas adenocarcinoma, 5-6. Colon adenocarcinoma.
All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing a CK20 staining as optimal included:

- A strong, distinct cytoplasmic reaction of all the surface epithelial cells of the appendix and at least a moderate reaction in most crypt cells, also in the basis.
- An at least moderate, distinct cytoplasmic reaction of the majority of the foveolar epithelial cells of the stomach.
- A moderate to strong, distinct cytoplasmic reaction in the majority of the neoplastic cells of the transitional cell carcinoma and the two colon adenocarcinomas
- A moderate to strong, distinct cytoplasmic reaction in scattered cells of the pancreas adenocarcinoma.

130 laboratories participated in the assessment. The results are summarized in Table 1.

Table 1. **Abs and scores for CK20, run 25**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ks20.8**	89 4 4 3 2 1 1	Dako NeoMarkers Novocastra Progen Euro Diagnostica Cymbus Innovex Biosciences	27	42	27	8	66 %	83 %
mAb clone **Q2**	1	NeoMarkers	0	0	0	1	-	-
pAb **E16444**	3	Spring Bioscience	2	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **Ks20.8**	14	Ventana, 760-2635	1	6	7	0	50 %	-
mAb clone **Ks20.8**	4	Dako, IR777	3	1	0	0	-	-
mAb clone **Ks20.8**	2	Dako, N1627	0	0	1	1	-	-
mAb clone **Ks20.8**	2	Linaris	0	1	1	0	-	-
Total	130		33	51	36	10	-	-
Proportion			25 %	39 %	28 %	8 %	64 %	84 %

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

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

Concentrated Abs
mAb clone **Ks20.8**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (16/46), Bond Epitope Retrieval Solution 2 (Bond, Leica) (7/8) or Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (4/15) as retrieval buffer. The mAb was typically diluted in the range of 1:25 – 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 50 out of 60 (83 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

pAb **E16444**: The protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (1/1) or Citrate pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:80 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **Ks20.8**, prod. no. 760-2635, Ventana: The protocols giving an optimal result were based on HIER, Cell Conditioning 1, standard, as retrieval buffer and the incubation time for the primary Ab was 32 min and UltraView was used as detection system. Using these protocol settings 1 out of 2 laboratories produced a sufficient staining.

mAb clone **Ks20.8**, IR777, Dako: The protocols giving an optimal result were all based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) and an incubation time for 20 min in the primary Ab and EnVision Flex as the detection system. Using these protocol settings 4 out of 4 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Enzymatic pre-treatment - 21/26 protocols using enzymatic pre-treatment gave an insufficient result
- Use of biotin based detection systems combined with HIER
- Less successful Abs.

In this assessment the prevalent feature of an insufficient staining was a general too weak staining or a false negative reaction of the structures expected to stain. This was seen in 65 % of the insufficient results and was mainly caused by a too low concentration of the primary Ab. Virtually all laboratories could demonstrate CK20 in the luminal epithelial cells of the appendix and in the two colon adenocarcinomas, whereas the demonstration of CK20 in the foveolar epithelial cells of the stomach and in the neoplastic cells in the transitional cell carcinoma was more challenging and required a correctly calibrated protocol. In 35 % a false positive reaction was observed. The false positive reaction was observed when a biotin based detection system was used in combination with HIER and was mainly seen in the cytoplasm of the epithelial cells of the gastric crypt epithelium but also in the normal and neoplastic pancreatic cells. Enzymatic pre-treatment combined with the mAb clone Ks20.8 used in a relatively high concentration frequently gave a false positive reaction in the normal pancreatic ducts. The proportion of sufficient results was significantly lower when enzymatic pre-treatment was used compared to HIER, which is illustrated in table 2.

Table 2. **Pass rate for laboratories using mAb clone Ks20.8 with HIER and enzymatic pre-treatment**

	HIER	**Enzymatic pre-treatment**
Sufficient[1]	76/100 (76 %)	5/26 (19 %)

1) Proportion of sufficient stains (optimal or good),

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 shall show a moderate to strong cytoplasmic staining, while other epithelial cells shall be negative.

This was the 2nd assessment of CK20 in NordiQC, as CK20 also was assessed in run 8, 2003. The proportion of sufficient results declined from 90 % in run 8, 2003, to 64 % in the current run. The lower pass rate is probably due to more challenging tissue material circulated.

Table 3. **Sufficient results with CK20 in two runs**

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

Conclusion
The mAb clone Ks20.8 and the pAb E16444 are recommendable antibodies for CK20. HIER should be used to obtain an optimal staining. Stomach is recommended as positive control: The foveolar cells shall show a moderate to strong cytoplasmic reaction. Alternatively appendix can be used: The majority of the epithelial cells even in the crypt bottom shall show a moderate to strong cytoplasmic reaction.

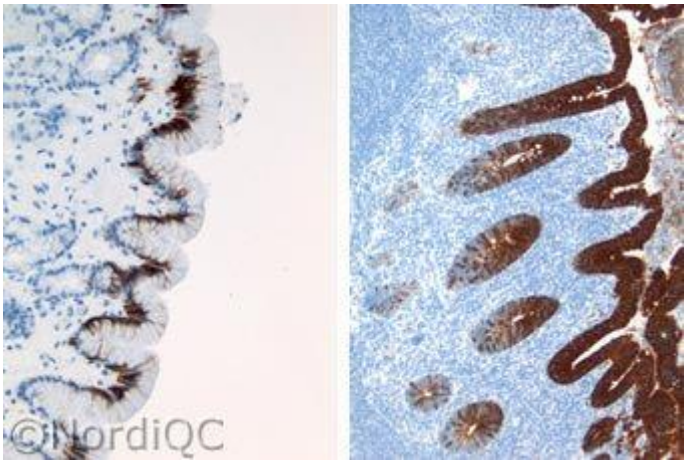


Fig. 1a
Optimal staining for CK20 using the mAb clone Ks20.8 optimally calibrated and with HIER.
Left: Gastric mucosa: The majority of the foveolar epithelial cells show a distinct cytoplasmic reaction.
Right: Appendix: The luminal epithelial cells and the majority of the epithelial cells of the crypts show a strong cytoplasmic reaction. No background reaction is seen.
Also compare with Figs. 2a & 3a – same protocol.

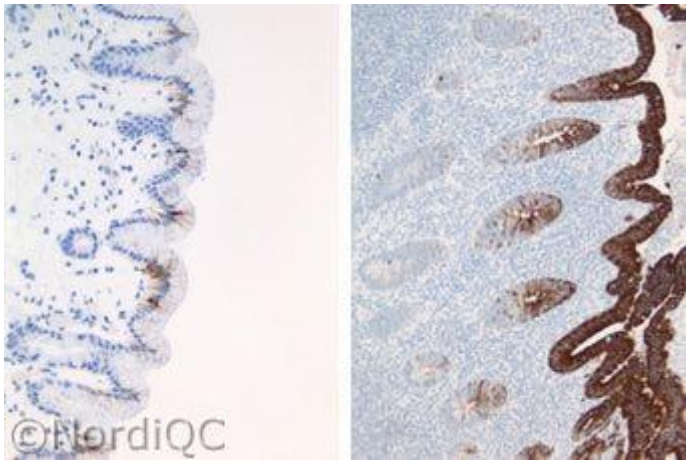


Fig. 1b
Insufficient staining for CK20 using the mAb clone Ks20.8 too diluted – same fields as in Fig. 1a.
Left: Gastric mucosa: Only scattered foveolar epithelial cells show a weak cytoplasmic reaction.
Right: Appendix: The luminal epithelial cells show a strong cytoplasmic reaction, while the epithelial cells of the crypts show a reduced intensity and proportion of positive cells compared to Fig. 1a. Also compare with Figs. 2b & 3b – same protocol.

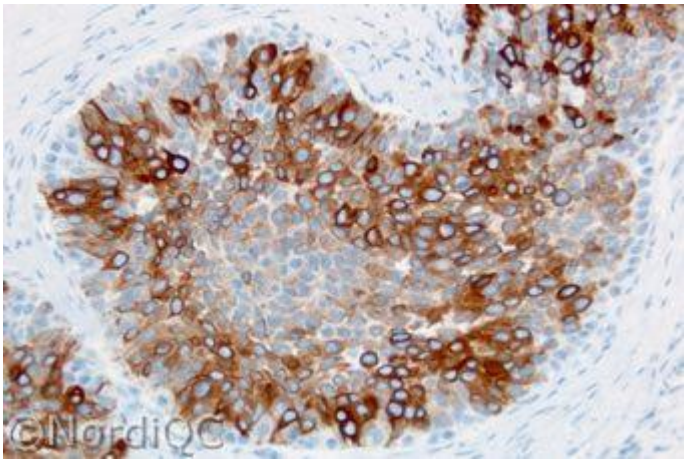


Fig. 2a
Optimal staining for CK20 of the transitional cell carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate and distinct cytoplasmic reaction.

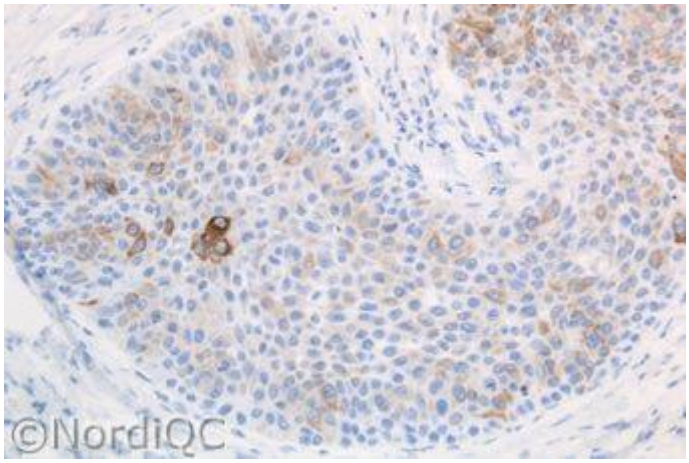


Fig. 2b
Insufficient staining for CK20 of the transitional cell carcinoma using same protocol as in Fig. 1b. Only scattered neoplastic cells show a diffuse reaction - same field as in Fig. 2a.

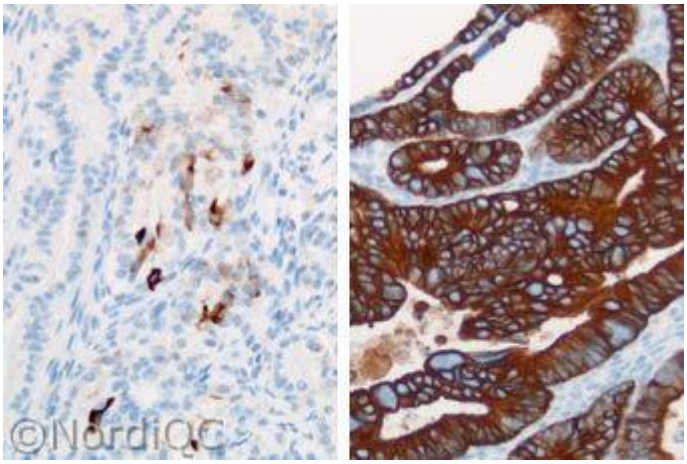


Fig. 3a
Optimal staining for CK20 using same protocol as in Fig. 1a & 2a.
Left: Pancreas adenocarcinoma: Scattered neoplastic cells show a moderate and distinct cytoplasmic reaction.
Right: Colon adenocarcinoma: Virtually all the neoplastic cells show a strong cytoplasmic reaction.

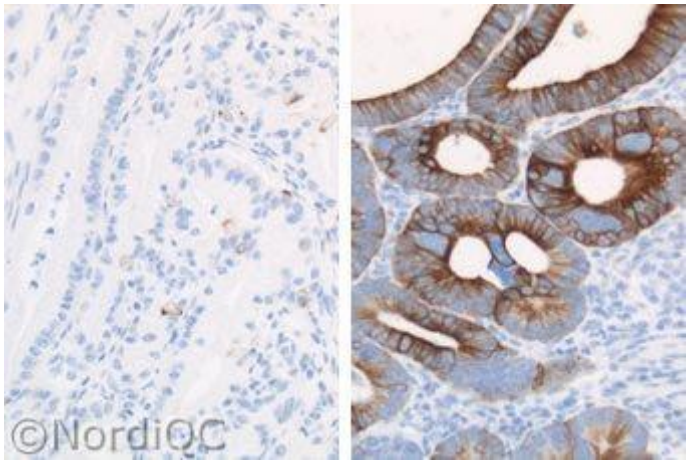


Fig. 3b
Insufficient staining for CK20 using same protocol as in Fig. 1b & 2b.
Left: Pancreas adenocarcinoma: Only few neoplastic cells show a weak and dubious reaction – compare with Fig. 3a left – same field.
Right: Colon adenocarcinoma: The majority of the neoplastic cells show a moderate cytoplasmic reaction.

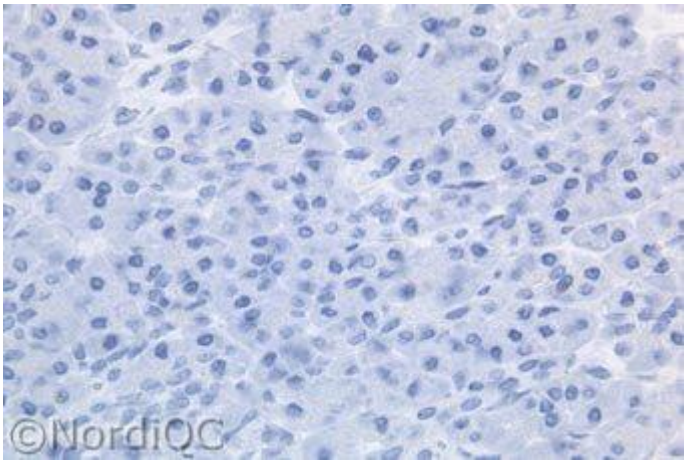


Fig. 4a
Optimal staining for CK20 using the mAb clone Ks20.8 with HIER. No staining is seen in any of the pancreatic cells.

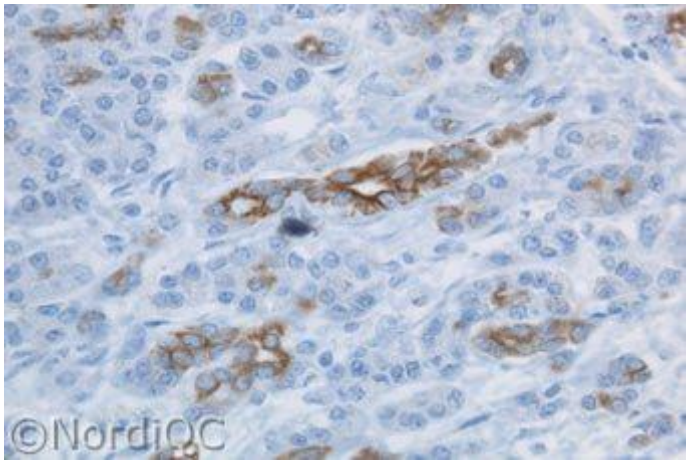


Fig. 4b
Insufficient staining for CK20 using the mAb clone Ks20.8 with enzymatic pretreatment. The epithelial cells of the intercalated ducts show a false positive reaction. This reaction pattern was seen in 21/26 protocols based on enzymatic pre-treatment.

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