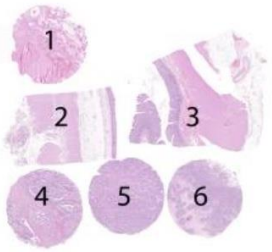
Assessment Run 50 2017

Cytokeratin 19 (CK19)

Material
The slide to be stained for CK19 comprised:



1. Thyroid gland 2. Esophagus 3. Colon 4. Thyroid papillary carcinoma
5. Breast carcinoma 6. Breast neuroendocrine carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CK19 staining as optimal included:

- At maximum a weak to moderate staining reaction in scattered epithelial cells in the thyroid gland.
- A moderate to 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 this staining pattern was seen in the individual cores).
- A strong, distinct cytoplasmic staining reaction of virtually all the surface epithelial cells in colon and at least a weak to moderate staining reaction of the epithelial cells in the basal part of the crypts.
- A moderate to strong, distinct staining reaction of virtually all the neoplastic cells of the papillary thyroid carcinoma and the breast carcinoma.
- An at least weak to moderate staining reaction of the majority of the neoplastic cells in the breast neuroendocrine carcinoma.

Participation

Number of laboratories registered for CK19, run 50	255
Number of laboratories returning slides	245 (96%)

Results
245 laboratories participated in this assessment, 82% 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 were:
- Low sensitivity detection systems
- Too short efficient HIER time
- Inappropriate epitope retrieval - use of proteolytic pre-treatment
- Too low concentration of the primary antibody

Performance history
This was the third NordiQC assessment of CK19. A major increase in pass rate was observed (see Table 2).

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

	Run 29 2010	Run 34 2012	Run 50 2017
Participants, n=	109	147	245
Sufficient results	69 %	46 %	82%

The increased pass rate (82%) in this run may be explained by several factors. Firstly, the proportion of laboratories using the very efficient mAb clone A53-B/A2.26 has increased significantly, from 22% in 2012 to 40% in the present run. Secondly, the proportion of laboratories using sensitive 3-step detection systems has also increased.

Conclusion
The mAb clones **A53-B/A2.26, b170, BA17, BS23, Ks19.1, RCK108** and the rmAb clone **EPR1579Y** can all be used to obtain optimal staining for CK19. The mAb clones A53-B/A2.26 and RCK108 were the most frequently used antibodies for CK19, with clone A53-B/A2.26 being the most successful clone with pass rates close to 100%. The clone RCK108 was much more challenging and less robust. Irrespective of

the Ab applied, efficient HIER, preferable in an alkaline buffer, in combination with a sensitive and specific IHC system (3-step polymer/multimer system) were the main prerequisites for optimal performance. Epitope retrieval based on proteolytic pre-treatment alone, failed to provide optimal staining results and can not be recommended.
In this assessment, the CK19 Ready-To-Use system based on clone A53-B/A2.26 (760-4281) from Ventana/Cell Marque performed very well reaching a pass rate of 98% with 77% of the laboratories achieving optimal staining results. The high number of optimal scores was a direct result of modifications made to the Ventana recommended protocol settings. As such 94% of the laboratories used modified protocols. The remaining 6% of the laboratories followed the recommended protocol, all passed, but none achieved an optimal mark. Similar problems were seen with the Dako/Agilent RTU system IS615/IR615 based on mAb clone RCK108. The Dako/Agilent recommended protocol settings for this challenging clone is based on a 2-step polymer detection system. Consequently, only 23% of the laboratories using these protocol settings received sufficient marks, whereas 89% of the laboratories using modified protocol settings (e.g. the use of a 3-step polymer detection system) received sufficient marks. These data suggest that both Dako/Agilent and Ventana/Cell Marque should revise their recommended protocol settings for these RTU systems.

The combination of esophagus and colon was found to be the most reliable positive tissue controls. In optimal protocols, virtually all basal epithelial cells in these two tissues showed a moderate to strong distinct cytoplasmic staining reaction. In protocols with insufficient results due to weak staining reaction, 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 were significantly lower than those of the thyroid carcinoma.

Table 1. **Antibodies and assessment marks for CK19, Run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A53-B/A2.26**	17 6 5 1 1 1	Cell Marque Thermo/NeoMarkers Zytomed Systems DBS IDLabs Immunologic	20	8	3	0	90 %	93 %
mAb clone **b170**	17	Leica/Novocastra	6	9	1	1	88 %	92 %
mAb clone **BA17**	4 2	Thermo/NeoMarkers Immunologic	4	1	1	0	83 %	100 %
mAb clone **BS23**	4	Nordic Biosite	4	0	0	0	-	-
mAb clone **Ks19.1**	3	Biocare	2	1	0	0	-	-
mAb **RCK108**	58 2 1 1	Dako/Agilent Biogenex Abcam Thermo/NeoMarkers	11	33	15	3	71 %	84 %
rmAb **EPR1579Y**	1	Epitomics	1	0	0	0	-	-
Ready-To-Use Abs:								
mAb clone **A53-B/A2.26 760-4281**	47	Ventana/Cell Marque	36	10	0	1	98 %	100 %
mAb clone **A53-B/A2.26 319M-17 (or -18)**	17	Cell Marque	10	7	0	0	100 %	-
mAb clone **A53-B/A2.26 Kit-0030**	1	maixin	0	1	0	0	-	-
mAb clone **A53-B/A2.26 8303-C010**	1	Sakura Finetek	1	0	0	0	-	-
mAb clone **b170 PA0799**	7	Leica/Novocastra	0	3	4	0	-	-
mAb clone **BA17 MAD-002163QD-7/N**	3	Master Diagnostica SL	2	1	0	0	-	-
mAb clone **Ks19.1**	2	Biocare	1	1	0	0	-	-

PM242AA								
mAb clone **RCK108 IS615/IR615**	27	Dako/Agilent	2	12	10	3	52 %	-
mAb clone **RCK108 GA615**	15	Dako/Agilent	2	12	1	0	94 %	100 %
mAb clone **RCK108 AM246-5M**	1	BioGenex	0	0	1	0	-	-
Total	245		102	99	36	8	-	
Proportion			42 %	40 %	15 %	3 %	82 %	

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

Detailed analysis of CK19, Run 50
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **A53-B/A2.26**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1, Ventana) (15/19)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/2), Target Retrieval Solution (TRS) High pH (Dako/Agilent) (1/1), EDTA pH 8 (1/1) or Citrate pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50–1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 25 of 27 (93%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **b170**: Protocols with optimal results were based on HIER using BERS2 (Leica) (3/9), CC1 (Ventana) (2/3) or TRS High pH (Dako/Agilent, 3-1) (1/3) as retrieval buffer. The mAb was diluted in the range of 1:50–1:200. Using these protocol settings, 12 of 13 (92%) laboratories produced a sufficient staining result.

mAb clone **BA17**: Protocols with optimal results were based on HIER using CC1, Ventana (3/4) or BERS2 (Leica) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:100–1:1600. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.

mAb clone **BS23**: Protocols with optimal results were based on HIER using TRS High pH (Dako/Agilent) (3/3) or Tris-EDTA / EGTA pH 9 (1/1) as retrieval buffer. The mAb was diluted in the range of 1:200–1:250. Using these protocol settings, 4 of 4 (100%) laboratories produced an optimal staining result.

mAb clone **Ks19.1**: Protocols with optimal results were based on HIER using BERS2 (Leica) (2/2) as retrieval buffer. The mAb was diluted in the range of 1:100–1:200. Using these protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result.

mAb clone **RCK108**: Protocols with optimal results were all based on HIER using TRS High pH (Dako/Agilent, 3-in-1) (3/10), CC1 (Ventana) (2/20), Tris-EDTA / EGTA pH 9 (2/7), BERS2 (Leica) (1/7), TRS High pH (Dako/Agilent) (1/6) or a combined pre-treatment using HIER in CC1 (efficient heating time 16 min.) followed by proteolysis in P2 for 8 min. (1/1). The mAb was typically diluted in the range of 1:10–1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 38 of 45 (84%) laboratories produced a sufficient staining result.

rmAb clone **EPR1579Y**: One protocol with an optimal result was based on HIER in using CC1 (Ventana) for 64 min., 60 min. incubation of the primary Ab and OptiView (Ventana 760-700) as detection system.

Table 3. **Proportion of optimal results for CK19 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer Link / Classic**		**Dako/Agilent Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **A53-B/A2.26**	0/1**	-	1/1	-	15/18 (83%)	0/1	2/2	-
mAb clone **b170**	1/3	-	-	-	2/3	-	3/9 (33%)	0/1
mAb clone **RCK108**	3/10 (30%)	-	1/6 (17%)	-	2/20 (10%)	-	1/7 (14%)	-

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

Ready-To-Use antibodies and corresponding systems
mAb clone **A53-B/A2.26, 760-4281**, Ventana/Cell Marque, BenchMark GX / XL / ULTRA:
Protocols with optimal results were typically based on 32-64 min. HIER using Cell Conditioning 1 (CC1, Ventana), 16–60 min. incubation of the primary Ab and UltraView (Ventana 760-500). One lab used a combined pre-treatment using HIER in CC1 16 min. followed by proteolysis in P2 for 8 min. UltraView (Ventana 760-500) with amplification (760-080) or OptiView (Ventana 760-700) were used as detection systems. Using these protocol settings, 46 of 46 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **A53-B/A2.26,** product no. **8303-C010**, Sakura Finetek, Genie:
One protocol with an optimal result was based on HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

mAb clone **RCK108**, product no. **GA615,** Dako/Agilent, Omnis:
Protocols with optimal results were based on HIER using Target Retrieval Solution, High pH (TRS pH 9) (efficient heating time 30 min. at 97°C), 20 min. incubation of the primary Ab and Envision FLEX (GV800/GV823) with mouse linker (GV821) as detection system. Using this and comparable protocol settings, 12 of 12 (100%) laboratories produced a sufficient staining result.

mAb clone **RCK108**, product no. **IS615/IR615,** Dako/Agilent, Autostainer Classic/Link:
One protocol with an optimal result was based on HIER in PT-Link using TRS High pH (3-in-1) (efficient heating time 20 min. at 97°C), 20 min. incubation of the primary Ab and Envision FLEX (K8000/SM802) with mouse linker (K8021) as detection system. Using this and comparable protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

mAb clone **Ks19.1** product no. **PM242AA**, Biocare, intelliPATH:
One protocol with an optimal result was based on HIER using Borg Decloaker pH 9.5 in a Pressure Cooker (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-Polymer (M4U534) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basic protocol settings. Only protocols performed on the specific IHC stainer device were included.

Table 4. **Proportion of sufficient and optimal results for CK19 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb A53-B/A2.26 **760-4281**	100% (3/3)	0% (0/3)	98% (43/44)	82% (36/44)
Dako AS mAb RCK108 **IS615/IR615**	23% (3/13)	0% (0/13)	89% (8/9)	11% (1/9)
Dako Omnis mAb RCK108 **GA615**	100% (13/13)	15% (2/13)	0% (0/1)	0% (0/1)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment and in concordance with the previous NordiQC assessments for CK19, the prevalent feature of an insufficient staining was a general too weak or false negative staining reaction of cells and structures expected to be demonstrated. The majority of laboratories were able to demonstrate CK19 in high-level antigen expressing structures such as the luminal columnar epithelial cells of the colon, the neoplastic cells of the thyroid papillary carcinoma and the breast carcinoma, whereas demonstration of CK19 in the basal columnar epithelial cells of the colon, the epithelial cells of the esophagus and the neoplastic cells in the neuroendocrine carcinoma expressing less CK19 was much more challenging and required an optimally calibrated protocol (see Figs. 1 – 5).

In concordance with the previous NordiQC assessment for CK19, run 34 in 2012, no protocols, irrespectively of clone and format (RTU or concentrate), based on proteolytic pretreatment as single retrieval method could provide an optimal mark. 16 protocols were based on proteolytic pretreatment alone and 8 (50%) were assessed as insufficient. The main problem with proteolytic pretreatment was characterized by too weak staining reactions (see Figs. 7a and 7b), but in addition also critical over-digestion was registered resulting in a very poor morphology (see Figs. 6a and 6b). In contrast to these results, three laboratories used a combination of HIER and a mild proteolytic pretreatment and all achieved optimal staining results. Two of the laboratories used the mAb clone RCK108 and one laboratory used the mAb clone A53-B/A2.26.

51% (124 of 245) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for CK19. The mAb clone RCK108 and mAb clone A53-B/A2.26 were the most widely used antibodies, as seen in Table 1. With mAb clone RCK108, optimal results could be obtained on the four most widely used IHC platforms, whereas mAb A53-B/A2.26 were rarely used on the Dako platforms in general, and no optimal results were registered on the Dako Autostainer. However, in general the performance of mAb clone A53-B/A2.26 was very good, providing a pass rate of 90%. Especially on the Ventana platform, the mAb clone A53-B/A2.26 exhibited an impressive optimal rate of 83% (15 of 18) (see Table 3). The general pass rate for RCK108 was significantly lower, reaching just 71%, and only 17% of the laboratories achieving optimal marks. These data suggest that the mAb clone RCK108 is more difficult to optimise in a LD assay compared to the mAb clone A53-B/A2.26. Although the pass rate for clone RCK108 was not impressive, major improvements were registered in this run compared to the previous CK19 run in 2012. In run 34 in 2012 the pass rate was very low. Only 37% passed at that occasion. The improvement in the current run seems to be related to a higher number of laboratories using a 3-step polymer system instead of less sensitive 2-step polymer systems, and the fact that Dako/Agilent after run 34 in 2012, changed their datasheet for the RCK108 concentrate. In 2012, many vendors for the Abs for CK19 gave misleading and imprecise guidelines concerning the epitope retrieval and protocol set-up for the antibodies. E.g., the protocol recommended by Dako/Agilent (the most used vendor) for the mAb clone RCK108 as a concentrate was based on proteolytic pre-treatment, whereas HIER was recommended when the clone was sold as a Ready-To-Use (RTU) format from same vendor. Dako/Agilent has now eliminated that inconsistency and recommends HIER at high pH for both products based on mAb clone RCK108. Unfortunately, Novocastra/Leica have not made the same necessary correction in their data sheets for the products based on the mAb clone b170. For the concentrated format, HIER at high pH is recommended, whereas proteolytic pre-treatment is recommended for the RTU format. Consequently, a high pass rate of 88% (with 35% optimal) is seen for the concentrate (because of correct retrieval recommendation in the datasheet for this product), contrasting a very low pass rate (43%) with no optimal for the RTU (because of incorrect retrieval recommendation for this product).

The recently introduced mAb clone BS23 showed promising results. Four laboratories used this new clone on the Dako Omnis or Thermo Autostainer and all obtained optimal results.

49% (121 of 245) of the laboratories used Abs in Ready-To-Use formats. The most widely used RTU systems for CK19 were Ventana/Cell Marque **760-4281,** Dako/Agilent **IS615/IR615** and Dako/Agilent **GA615.** The Ventana RTU system 760-4281 based on the mAb clone A53-B/A2.26 had a very high pass rate of 98% with a corresponding high rate of optimal marks (77%). However, the data revealed, that only 6% of laboratories (3 of 47) followed the Ventana/Roche recommended protocol settings for 760-4281 (see Table 4). All passed, but none achieved optimal mark. In contrast, 94% of the laboratories (44 of 47) used laboratory modified protocol settings resulting in a pass rate of 98% and an impressive optimal rate of 82%. The predominant successful modifications of the recommended protocol settings were use of a 3-step detection system opposed to the recommended 2-step system and use of longer incubation time of the primary Ab than the recommended 16 min.
The Dako/Agilent RTU system GA615 is based on the mAb clone RCK108 and designed for the Omnis stainer. The recommended protocol settings including HIER at high pH for 30 min., incubation of the primary Ab for 20 min. and use of 3-step polymer detection system. This resulted in a pass rate of 100%, but a surprisingly low rate of optimal marks – only 15% (2 of 13) achieved an optimal mark (see Table 4).
The Dako/Agilent RTU system IS615/IR615 is also based on the mAb clone RCK108 but designed for the Autostainer. In contrast to the Omnis protocol (GA615), the Autostainer protocol is based on a 2-step polymer detection system and a shorter HIER time (20 min.) in high pH retrieval buffer. These differences might explain the low pass rate seen when the recommended protocol settings were used for Dako/Agilent IS615/IR615. Only 23% (3 of 13) passed and none achieved optimal mark. Laboratories that used laboratory modified protocol settings for IS615/IR615, typically using the 3-step polymer based detection system FLEX+ (and not FLEX) performed much better. A pass rate of 89% (8 of 9) was seen with the modified protocol settings.

Controls
In concordance with previous CK19 assessments, the combination of esophagus and colon was found to be the most reliable positive tissue controls. In the optimal protocols, virtually all the basal epithelial cells in these 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.
Normal thyroid gland has in literature been suggested as negative tissue control for CK19. In this assessment, optimal protocols provided a distinct positive staining reaction 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.

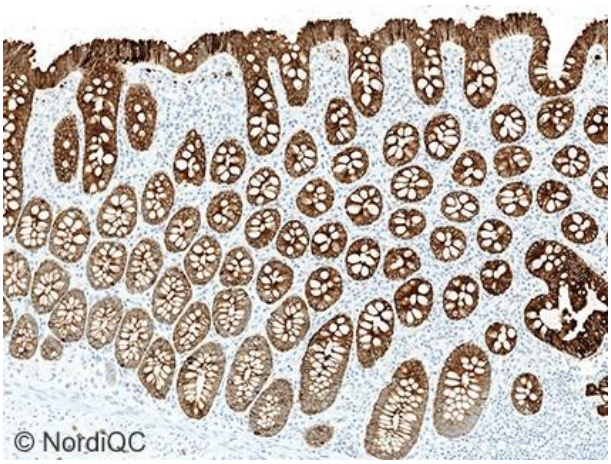


Fig. 1a (x100)
Optimal staining for CK19 of colon using the mAb clone A53-B/A2.26 optimally calibrated with HIER in an alkaline buffer. Surface columnar epithelial cells show a strong cytoplasmic staining reaction, while the columnar epithelial cells in the basal parts of the crypts show a weak to moderate staining reaction.
No background staining is seen. Also compare with Figs. 2a – 5a, same protocol.

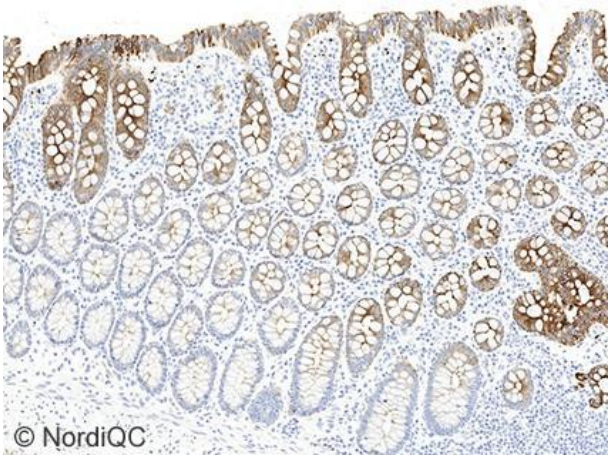


Fig. 1b (x100)
Insufficient staining for CK19 of colon using the mAb clone A53-B/A2.26 by a protocol giving a too low sensitivity – too low concentration of the primary Ab., same field as in Fig. 1a. Only the surface columnar epithelial cells show a moderate cytoplasmic staining reaction, while virtually no staining reaction is seen in the basal part of the crypts. Compare with fig. 1a. – same field. Also, compare with Figs. 2b - 5b – same protocol.

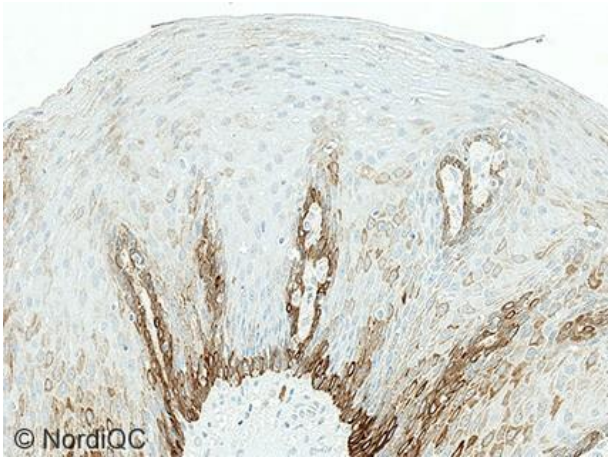


Fig. 2a (x200)
Optimal staining for CK19 of the esophagus using same protocol as in Fig. 1a. The majority of the basal squamous epithelial cells show a moderate to strong cytoplasmic staining reaction and a weak to moderate staining reaction is seen in scattered intermediate epithelial cells. No background reaction is seen.

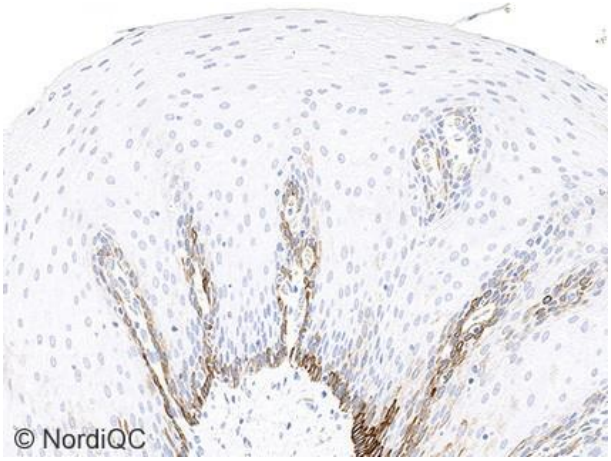


Fig. 2b (x200)
Insufficient staining for CK19 of the esophagus using same protocol as in Fig. 1b., same field as in Fig. 2a. Only a weak reaction is seen in the basal squamous epithelial cells and no reaction is registered in the intermediate epithelial cells. Also, compare with Figs. 3b – 5b, same protocol.

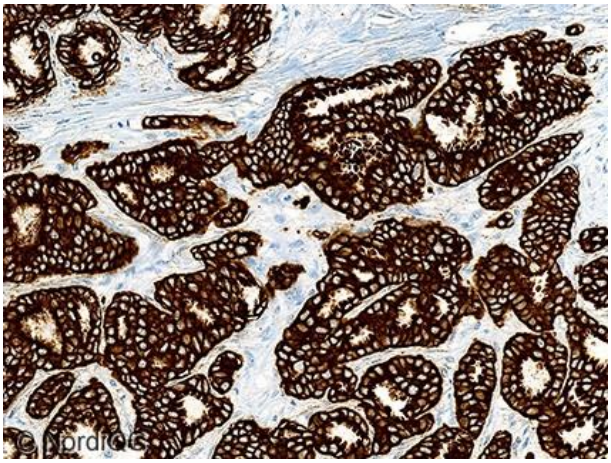


Fig. 3a (x200)
Optimal CK19 staining of the breast carcinoma (high-level expressor) using same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a strong cytoplasmic staining reaction. No background reaction is seen.

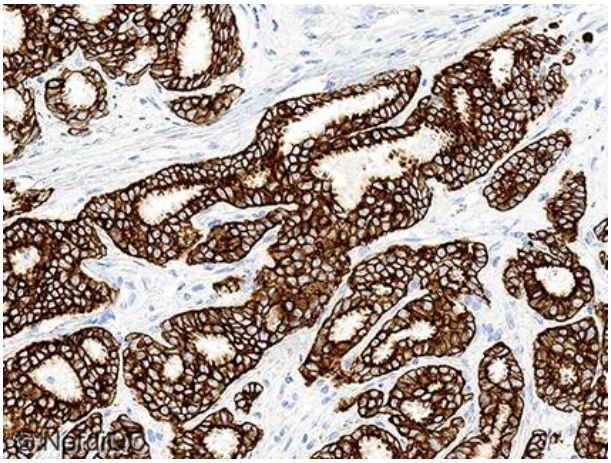


Fig. 3b (x200)
CK19 staining of the breast carcinoma (high expressor) using the same insufficient protocol as in Figs. 1b and 2b. Due to the high-level expression of CK19, virtually all neoplastic cells show a moderate to strong cytoplasmic staining reaction. Compare with Fig. 3a – same field.

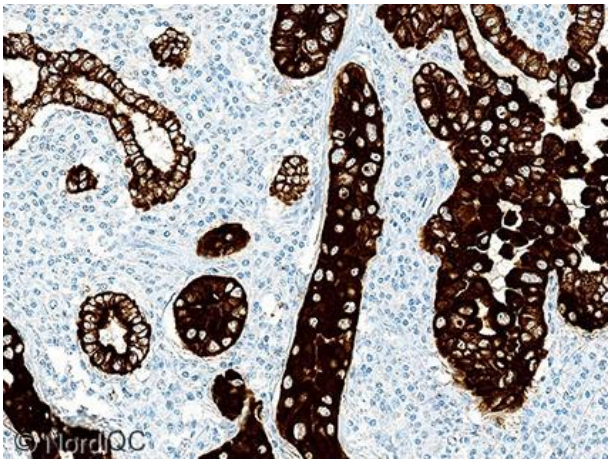


Fig. 4a (x200)
Optimal CK19 staining of the thyroid papillary carcinoma (high-level expressor) using same protocol as in Figs. 1a - 3a. Virtually all neoplastic cells show a strong cytoplasmic staining reaction. No background reaction is seen.

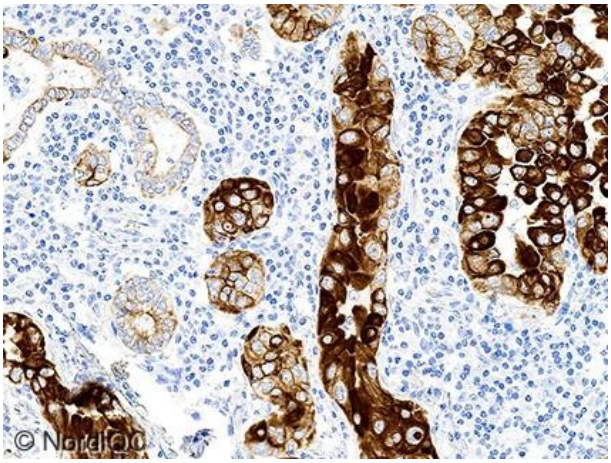


Fig. 4b (x200)
Insufficient CK19 staining of the thyroid papillary carcinoma (high-level expressor) using the same protocol as in Figs. 1b - 3b. Due to the high-level expression of CK19, the majority of neoplastic cells show a weak to moderate cytoplasmic staining reaction. Compare with Fig. 4a – same field.

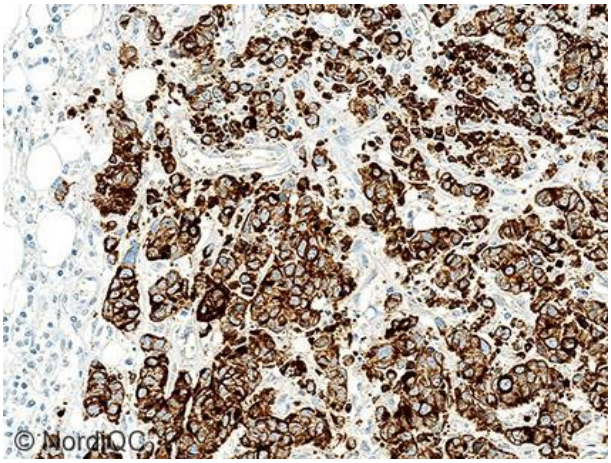


Fig. 5a (x100)
Optimal CK19 staining of the neuroendocrine carcinoma (low expressor) using same protocol as in Figs. 1a - 4a. Virtually all neoplastic cells show a moderate to strong cytoplasmic staining reaction. No background reaction is seen.

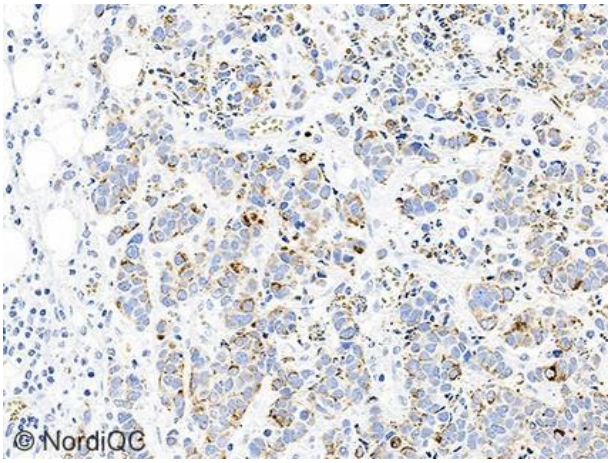


Fig. 5b (x100)
Insufficient CK19 staining of the neuroendocrine carcinoma (low expressor) using the same protocol as in Figs. 1b - 4b. Only a few scattered neoplastic cells show a very weak cytoplasmic staining reaction. Compare with Fig. 5a – same field.

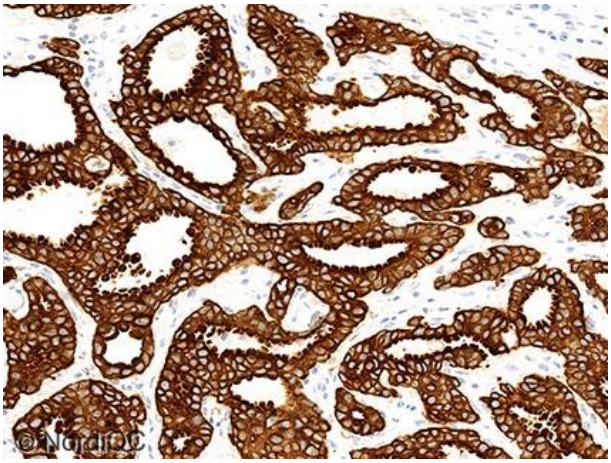


Fig. 6a (x200)
Optimal staining for CK19 of the breast carcinoma using the mAb clone b170 optimally calibrated and with HIER in an alkaline buffer. Virtually all neoplastic cells show a strong cytoplasmic staining reaction. No background reaction is seen. Notice the well-preserved morphology and compare to Fig. 6b

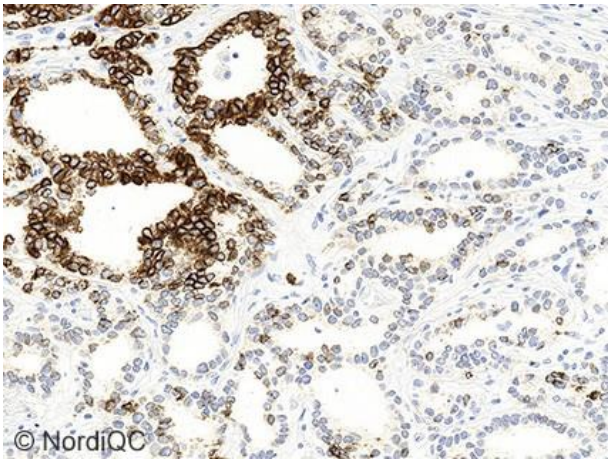


Fig. 6b (x200)
Insufficient CK19 staining of the breast carcinoma using the mAb clone b170 in a protocol using proteolytic pretreatment. In this case, too strong pretreatment was performed using the Bond Enzyme Pretreatment Kit, resulting in impaired morphology and weak staining reaction due to degradation of the cytoplasm of the neoplastic cells. Compare with Fig. 6a – same field.

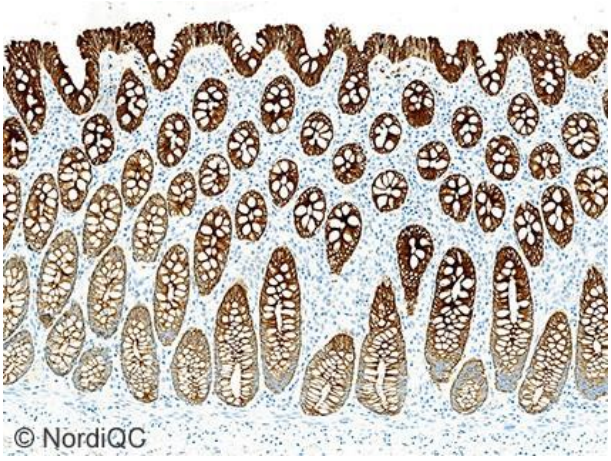


Fig. 7a (x100)
Optimal staining for CK19 of the colon using the mAb clone RCK108 optimally calibrated and with HIER in an alkaline buffer. Surface columnar epithelial cells show a strong cytoplasmic staining reaction, while the columnar epithelial cells in the basal parts of the crypts show a weak to moderate staining reaction. Compare with Fig. 7b. (same field)

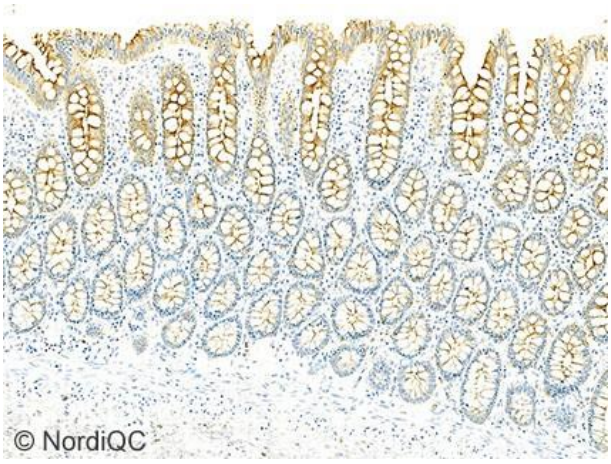


Fig. 7b (x100)
Insufficient staining for CK19 of the colon using the mAb clone RCK108 in a protocol with similar settings as in Fig. 7a but with the use of mild proteolytic pretreatment. This morphology preserving proteolytic pretreatment results in a too low sensitivity of the protocol. Only the surface columnar epithelial cells show a moderate cytoplasmic staining reaction, while virtually no staining reaction is seen in the basal part of the crypts. Compare with Fig. 7a. – same field.

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