Assessment Run 55 2019

Cytokeratin 5 (CK5)

Material

The slide to be stained for cytokeratin 5 (CK5) comprised:

1. Tonsil, 2. Liver, 3. Pancreas, 4. Prostate hyperplasia, 5. Lung adenocarcinoma, 6-7. Lung squamous cell carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CK5 staining as optimal included:

- A moderate to strong and distinct, cytoplasmic staining reaction in virtually all squamous epithelial cells in the tonsil.
- A weak to moderate, predominantly membranous staining reaction of scattered cuboidal epithelial cells in the pancreatic intercalated ducts.
- A strong and distinct cytoplasmic staining reaction in the majority of basal cells in the hyperplastic prostate glands.
- A moderate to strong cytoplasmic staining reaction of virtually all neoplastic cells in the lung squamous cell carcinomas, tissue cores no. 6 and 7.
- No staining of neoplastic cells in the lung adenocarcinoma.
- No staining reaction in the liver.

Participation

Number of laboratories registered for CK5, run 55	270
Number of laboratories returning slides using appropriate antibodies*	263 (97%)

*1 laboratory used an inappropriate antibody.

Results

263 laboratories participated in this assessment. 116 (44%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of mouse monoclonal Ab (mAb) clone D5/16 B4 – both as concentrate and in Ready-To-Use (RTU) systems
- Too low concentration of the primary Ab
- Use of less sensitive detection systems
- Insufficient Heat Induced Epitope Retrieval (HIER) – too short efficient HIER time

Performance history

This was the third NordiQC assessment of CK5. A significant decrease in the pass rate was observed compared to the latest run (Run 46 in 2016) as listed in Table 2. This seems to be related to a more challenging material circulated in the present run.

Table 2. **Proportion of sufficient results for CK5 in the three NordiQC runs performed**

	Run 12 2004	Run 46 2016	Run 55 2019
Participants, n=	74	266	263
Sufficient results	47%	68%	44%

Conclusion

Optimal staining results could be obtained with the mAb clone **XM26**, and the rmAbs clones **BSR55**, **EP24** and **SP27** used as concentrates, and these clones are all recommendable antibodies for the demonstration of CK5. However, SP27 has in NordiQC studies (unpublished) shown positive reaction in lung adenocarcinomas being negative for other CK5 antibodies as well as p40. The significance of this is uncertain. The ascites produced mAb clone D5/16 B4 used as a concentrate was less successful (although a few optimal staining results were seen). Low analytical sensitivity in combination with the risk of false positive staining reactions due to "Mouse Ascites Golgi" (MAG) [1] reaction makes this antibody very difficult

to optimize on most platforms. Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer), careful calibration of the primary antibody and use of a sensitive detection system (preferable a 3-step polymer/multimer system) were the most important prerequisites for an optimal staining result.
The Ventana RTU system based on rmAb clone SP27, 760-4935, was the most successful RTU system providing a pass rate of 100%, followed by an 86% pass rate obtained with the mAb clone XM26 based Leica system. The RTU systems based on mAb clone D5/16 B4 all performed poorly.
Tonsil and pancreas can be recommended as positive tissue controls. In tonsil, virtually all squamous epithelial cells throughout all cell layers must show a moderate to strong cytoplasmic staining reaction. In pancreas, scattered cuboidal epithelial cells of intercalated ducts must show a weak to moderate predominantly membranous staining reaction. Liver can be recommended as negative tissue control; no staining reaction must be seen in hepatocytes and bile ducts.

Table 1. **Antibodies and assessment marks for CK5, run 55**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CK5/6.007**	1	Biocare	0	1	0	0	-	-
mAb clone **D5/16 B4**	45 7 1 1 1	Dako/Agilent Cell Marque Millipore Thermo Scientific Zytomed	4	10	31	10	25%	26%
mAb clone **XM26**	49 1 1 1 1	Leica/Novocastra Biocare Diagnostic BioSystems Histols Reagents Monosan	32	9	10	2	77%	81%
mAb clone **XM26/LL002**	1 1 1	Biocare Diagnostic BioSystems Zytomed	1	1	1	0	-	-
rmAb clone **BSR55**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **EP1601Y**	5 1	Cell Marque Biocare	0	1	5	0	-	-
rmAb clone **EP24**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP27**	1	Immunologic	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **D5/16 B4 790-4554**	56	Ventana/Cell Marque	4	14	34	4	32%	82%
mAb **D5/16 B4 GA780**	21	Dako/Agilent	0	1	20	0	5%	-
mAb **D5/16 B4 GA780** [3]	1	Dako/Agilent	0	0	0	1	-	-
mAb clone **D5/16 B4 IR/IS780**	16	Dako/Agilent	0	0	12	4	0%	-
mAb clone **D5/16 B4 IR/IS780** [4]	9	Dako/Agilent	1	2	4	2	-	-
mAb clone **D5/16 B4 356M-10** [5]	2	Cell Marque	0	0	2	0	-	-
mAb clone **GM028 8294**	1	Sakura	0	0	1	0	-	-
mAb clone **XM26 PA0468**	7	Leica/Novocastra	4	2	1	0	-	-
mAb clone **XM26 PA0468** [6]	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **XM26 PM234**	1	Biocare	0	1	0	0	-	-
mAb clone **XM26/LL002 MSG106**	1	Zytomed	0	1	0	0	-	-
rmAb/mAb clone **EP1601Y/LL002 905H-8**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **EP1601Y**	4	Cell Marque	0	3	1	0	-	-

305R-18								
rmAb clone **EP24 RMA-0846**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP24/EP67 MAD-000651QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **SP27 760-4935**	18	Ventana /Cell Marque	15	3	0	0	100%	100%
Total	263		65	51	124	23	-	
Proportion			25%	19%	47%	9%	44%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent full-automatic system (Dako Omnis), but used by a laboratorium on the Leica full-automatic platform (Leica Bond)
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on different full-automatic platforms (e.g. Ventana Benchmark, Leica Bond and Dako Omnis).
5) RTU system not developed for a specific platform, but used by laboratories on the Ventana Benchmark platform.
6) RTU system developed for the Leica Bond system, but used on BioCare Intellipath platform.

Detailed analysis of CK5, Run 55
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **D5/16 B4**: Protocols with optimal results were typically based on HIER using Bond Epitope Retrieval Solution 2 (BERS2, Leica) (3/9) or Cell Conditioning 1 (CC1, Ventana) (1/35). The mAb was typically diluted in the range of 1:25-1:200. Using these protocol settings, 11 of 43 (26%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **XM26**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS) pH 9 (Dako) (4/8), Target Retrieval Solution (TRS) pH 9 (3-in-1)(Dako) (8/8), CC1 (Ventana) (11/23), BERS2 (Leica) (7/10), Tris-EDTA pH 9 (1/1) or DBS Montage Citrate pH (Diagnostic Biosystem) 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:200. Using these protocol settings, 39 of 48 (81%) laboratories produced a sufficient staining result.

mAb clone **XM26/LL002**: One protocol with an optimal result was based on HIER using Citrate pH 6 as retrieval buffer (efficient heating time 7 min. at 110°C in a pressure cooker), 30 min. incubation of the primary Ab, diluted 1:50 and Zytomed HRP Polymer kit (POLHRP-100, Zytomed) as detection system.

rmAb clone **BSR55**: One protocol with an optimal result was based on HIER using Tris-EGTA/EDTA pH 9 (efficient heating time 20 min. at 98°C), 30 min. incubation of the primary Ab, diluted 1:150 and BioSiteHisto Plus HRP Polymer anti-Rabbit kit (KDB-10046, Nordic Biosite) as detection system.

rmAb clone **EP24**: One protocol with an optimal result was based on HIER using TRS pH 9 (3-in-1) (Dako) (efficient heating time 30 min. at 97°C), 40 min. incubation of the primary Ab, diluted 1:100 and 3-step EnVision FLEX+ (K8002/SM802, Dako) as detection system.

rmAb clone **SP27**: One protocol with an optimal result was based on HIER using CC1 (Ventana) (efficient heating time 24 min. at 95°C), 32 min. incubation of the primary Ab, diluted 1:50 and OptiView (760-700, Ventana) as detection system.

Table 3. **Proportion of optimal results for CK5 for the most commonly used antibodies as concentrates on the four main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako 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	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **D5/16 B4**	0/3**	-	0/4	-	1/34 (3%)	0/1	-	3/9 (33%)	-
mAb clone **XM26**	5/6 (83%)	-	3/7 (43%)	-	9/20 (45%)	2/3	-	7/10 (70%)	0/1

* 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 **D5/16 B4**, product no. **790-4554**, Ventana/Cell Marque, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1, efficient heating time 32-64 min. and 24-32 min. incubation of the primary Ab. OptiView (760-700) +/- Tyramide amplification kit (760-099 / 860-099) were used as detection systems. Using these protocol settings, 9 of 11 (82%) laboratories produced a sufficient staining result.

mAb clone **XM26**, product no. **PA0468**, Leica/Novocastra, Leica Bond-III/Bond-Max:
Protocols with optimal results were typically based on HIER using BERS 2 pH 9 (efficient heating time 10-20 min. at 95-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 6 of 7 (86%) laboratories produced an optimal staining result.

rmAb **clone EP24**, product no. **RMA-0846**, Maixin, manual:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (Waterbath) for 20 min., 60 min. incubation of the primary Ab and MaxVision III DAB (Maixin) as detection system.

rmAb clone **SP27**, product no. **760-4935**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1, efficient heating time 24-64 min. and 16-32 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 17 of 17 (100%) laboratories produced a sufficient staining result.

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 basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for CK5 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone **D5/16 B4, 790-4554**	0% (0/14)	0% (0/14)	43% (18/42)	10% (4/42)
Dako Omnis mAb clone **D5/16 B4, GA780**	6% (1/17)	0% (0/17)	0% (0/4)	0% (0/4)
Dako Autostainer mAb clone **D5/16 B4, IR/IS780**	0% (0/5)	0% (0/5)	0% (0/11)	0% (0/11)
Leica Bond mAb clone **XM26, PA0468**	86% (6/7)	57% (4/7)	(0/0)	(0/0)
Ventana Benchmark rmAb clone **SP27, 760-4935**	100% (7/7)	100% (7/7)	100% (11/11)	73% (8/11)

* 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 of CK5, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 87% of the insufficient results (128 of 147). The remaining 13% (19 of 147) insufficient results were characterized by a false positive staining reaction, alone or in combination with a too weak or false negative staining reaction. Virtually all laboratories were able to demonstrate CK5 in high-level antigen expressing structures such as neoplastic cells of the lung squamous cell carcinomas and the squamous epithelial cells of tonsil. Demonstration of CK5 in low-level antigen expressing structures as the basal cells in the hyperplastic prostate glands and especially the cuboidal epithelial cells of intercalated ducts in pancreas was significantly more challenging and required a carefully calibrated protocol. Compared to the previous CK5 assessment (Run 46 in 2016), a significant decrease in the pass rate was seen (see Table 2). The reason could be the introduction of a "new" low-level CK5 expressor, namely the normal pancreas in the tissue microarray for the current Run 55. The use of pancreas as positive tissue control for CK5 is recommended in the guidelines published by the

International Ad Hoc Expert Committee[2]. In pancreas, scattered cuboidal epithelial cells of intercalated ducts must show a weak to moderate predominantly membranous staining reaction.

46% (121 of 263) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for CK5. The well-established mAb clones D5/16 B4 and XM26 for CK5/6 and CK5, respectively, were the two most widely used Abs. 89% (108 of 121) of the LD-assays were based on one of these two clones. Within a LD assay, mAb clone XM26 was by far the most successful of the two, and optimal results could be obtained on all four main IHC platforms from Dako, Leica and Ventana (see Table 3). In concordance with previous assessment the main prerequisites for a sufficient staining with mAb clone XM26 were efficient HIER typically in an alkaline buffer (alone or in combination with weak proteolysis, Protease 3, Ventana), careful calibration of the titre of the primary Ab and the use of a sensitive detection system, preferably a 3-step polymer/multimer system. The proportion of sufficient staining results for the use of 3-step polymer/multimer based detection systems and 2-step polymer/multimer based systems was 88% and 57%, respectively. The mAb clone D5/16 B4 in a LD assay had a very low pass rate on all IHC platforms. The overall proportion of sufficient staining results was only 25% (14 of 55), and optimal staining results were only obtained by 4 laboratories (7%). These were based on HIER in an alkaline buffer and 3-step polymer/multimer based detection systems. Staining of the basal cells in the hyperplastic prostate glands and especially the low-level CK5 expressing cuboidal epithelial cells of intercalated ducts in pancreas was challenging for the mAb clone D5/16 B4 and required a carefully calibrated protocol. These data indicate that mAb clone D5/16 B4 has a significantly lower analytical sensitivity than the mAb clone XM26 (see Fig 1-Fig. 4). Furthermore, it was observed that mAb clone D5/16 B4 occasionally showed an inferior performance due to a distinct, aberrant (false positive) cytoplasmic staining reaction in pancreas, most likely caused by MAG reaction[1] (see Fig. 8a and Fig 8b). Dako provides the mAb as an ascites format and it is well-known that this aberrant MAG reaction can be seen in tissues of blood type A patients.

Three laboratories using LD assays based on one of the three recently launched rmAbs for CK5, clones BSR45, EP24 and SP27 all obtained optimal staining results. In contrast, the rmAb clone EP1601Y that provided a pass rate of 100% (9 of 9) in the previous assessment (run 46 in 2016), now has a pass rate of only 17% (1 of 6). Again, the more challenging tissue material circulated in this run seems to reveal a to low analytical sensitivity of the submitted EP1601Y protocols.

Ready-To-Use (RTU) antibodies was used by 54% (142 of 263) of the laboratories. The Ventana RTU system based on rmAb clone SP27, 760-4935, was the most successful and provided a proportion of sufficient staining results of 100% (18 of 18) of which 83% (15 of 18) were assessed as optimal. Optimal results could both be obtained using the protocol recommendations given by Ventana but also by laboratory modified protocol settings (typically adjusting HIER time and/or incubation time of the primary Ab). However, SP27 has in NordiQC studies (unpublished) shown positive reaction in lung adenocarcinomas being negative for other CK5 antibodies as well as p40. The significance of this is uncertain. The Leica RTU system based on mAb XM26, PA0468, also provided a high pass rate. Using the recommended protocol settings given by Leica, the proportion of sufficient staining results was 86% (6 of 7) of which 57% (4 of 7) were assessed as optimal.

The Dako RTU systems based on mAb clone D5/16 B4, IR/IS780 and GA780, both provided an alarming low proportion of sufficient staining results. Compared to the disappointing staining results with the corresponding LD assays based on the same clone, the Dako RTUs performed even worse. Including results from both recommended protocol settings given by Dako and laboratory modified protocol settings, the Dako Omnis RTU system, GA780, had a pass rate of 5% (1 of 21), whereas the Dako Autostainer RTU system, IR/IS780 had a pass rate of 0% (0 of 16).

The Ventana RTU system based on mAb clone D5/16 B4, 790-4554, performed somewhat better than the corresponding Dako RTU systems. 75% (42 of 56) of the laboratories used laboratory modified protocol settings and 43% (18/42) obtained sufficient staining results of which 10% (4/42) were assessed as optimal. Noteworthy, only 25% (14 of 42) used the 790-4554 RTU system with the recommended protocol settings given by Ventana and none of these laboratories were assessed as sufficient. The protocols producing optimal staining results were all based on a sensitive 3-step multimer system, OptiView, typically with tyramide signal amplification.

Controls

Tonsil and pancreas can be recommended as positive tissue control[2]. In tonsil, virtually all squamous epithelial cells throughout all cell layers must show a moderate to strong cytoplasmic staining reaction. In pancreas, scattered cuboidal epithelial cells of intercalated ducts must show a weak to moderate predominantly membranous staining reaction. Liver can be recommended as negative tissue control; no staining reaction must be seen in hepatocytes and bile ducts.

[1]Kliman HJ, Feinberg RF, Schwartz LB, Feinman MA, Lavi E, Meaddough EL. A mucin-like glycoprotein identified by MAG (mouse ascites Golgi) antibodies. Menstrual cycle-dependent localization in human endometrium. Am J Pathol. 1995;146(1):166–81.

[2]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

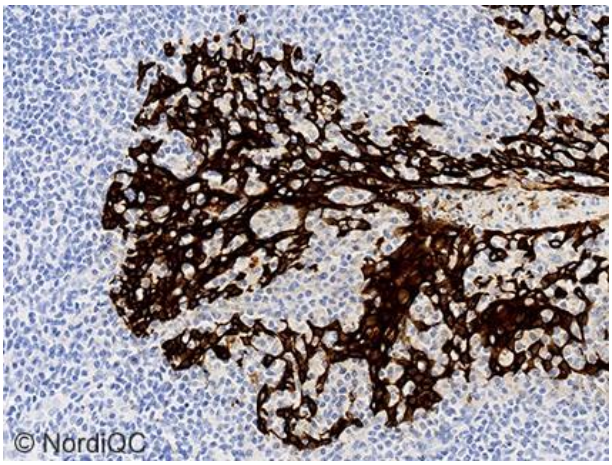

Fig. 1a (x200)
Optimal CK5 staining of the tonsil, tissue core no. 1, using the **mAb clone XM26** in 1:50 dilution with an incubation time of 30 min. after HIER in TRS pH 9 for 30 min. Staining was performed on the Dako Omnis using a 3-step polymer system (EnVision+). A strong staining reaction is seen in virtually all squamous epithelial cells in the tonsillar crypt. No background staining is seen. Also compare with Figs. 2a-4a, same protocol.

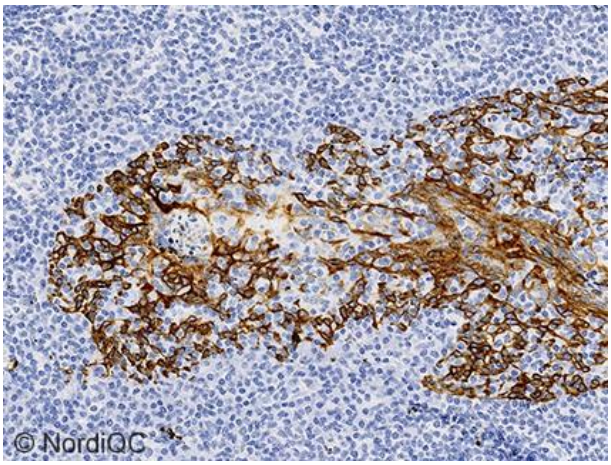

Fig. 1b (x200)
CK5 staining of the tonsil, tissue core no. 1, using the **mAb clone D5/16 B4** in an RTU format (Dako, GA780) using the vendors recommended protocol settings on the Dako Omnis. Using this insufficient staining protocol, the intensity of the epithelial cells demonstrated is reduced compared to the level expected and obtained in Fig. 1a, but because of high-level CK5 expression the majority of epithelial cells in the tonsillar crypt are clearly demonstrated - same field as in Fig. 1a. Also compare with Figs. 2b-4b, same protocol.

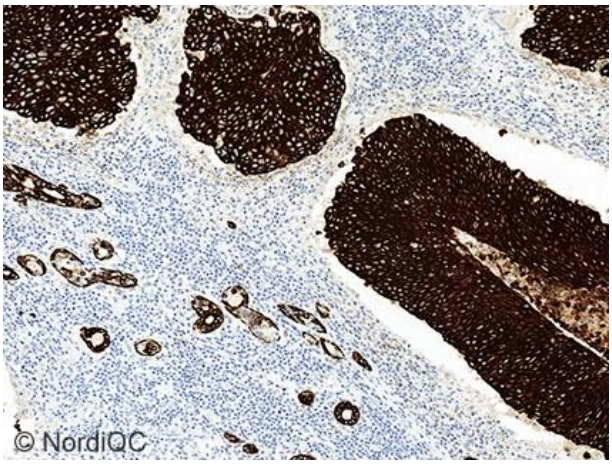

Fig. 2a (x100)
Optimal CK5 staining of the lung squamous cell carcinoma, tissue core no. 7, with high-level CK5 expression using same protocol as in Fig. 1a. All the neoplastic cells show a strong and distinct cytoplasmic staining reaction.

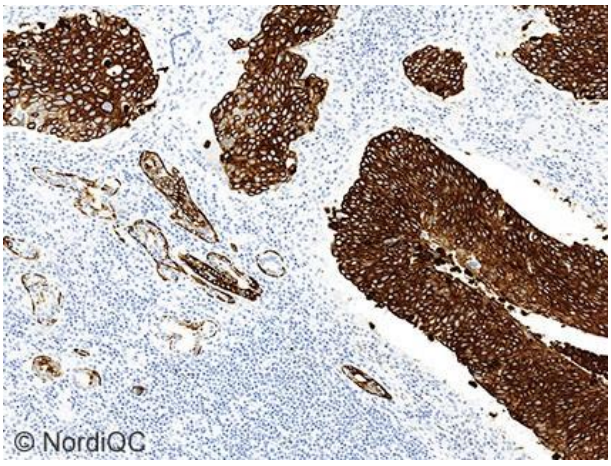

Fig. 2b (x100)
CK5 staining of the lung squamous cell carcinoma, tissue core no. 7, with high-level CK5 expression using the same insufficient protocol as in Fig. 1b. – same field as in Fig. 2a. The intensity of the neoplastic cells demonstrated is reduced compared to the level expected and obtained in Fig. 2a, but because of high-level CK5 expression all neoplastic cells are clearly demonstrated.

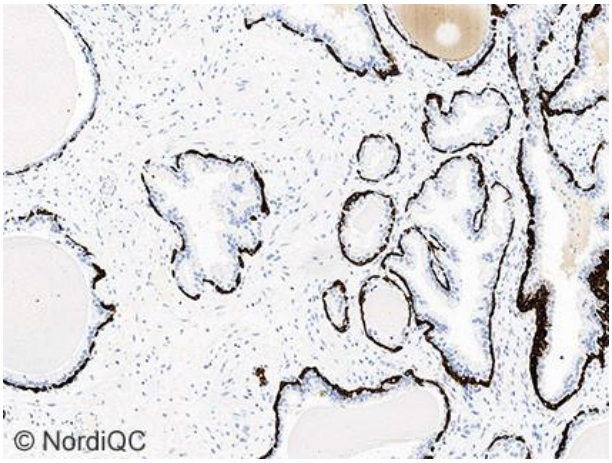

Fig. 3a (x100)
Optimal CK5 staining of the prostate hyperplasia, tissue core no. 4, using same protocol as in Figs. 1a and 2a. A strong and distinct cytoplasmic staining reaction is seen in the majority of basal cells in the hyperplastic prostate glands. No background staining is seen.

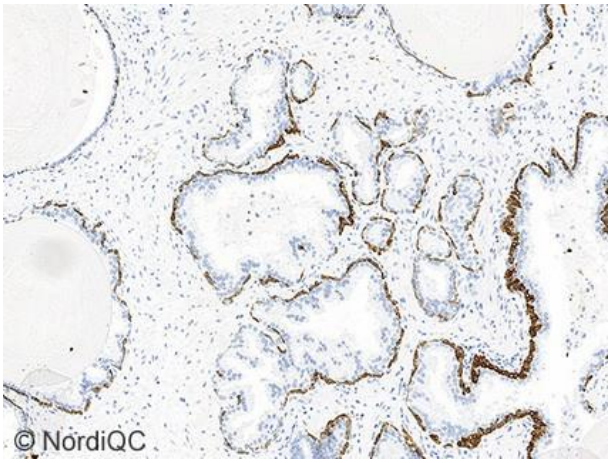

Fig. 3b (x100)
Insufficient CK5 staining of the prostate hyperplasia, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. Only weak to moderate staining reaction is seen in the basal cells in the hyperplastic prostate glands, making interpretation difficult.

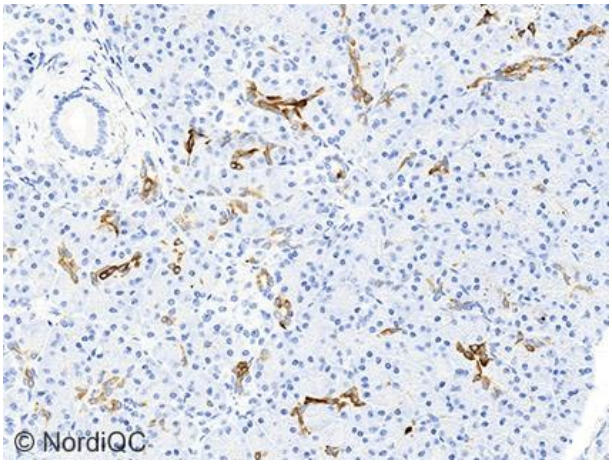

Fig. 4a (x200)
Optimal CK5 staining of pancreas, tissue core no. 3, with low-level CK5 expression using same protocol as in Figs. 1a-3a. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining is seen.

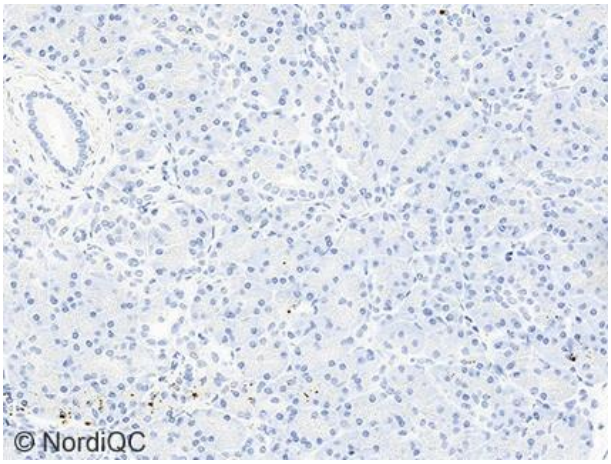

Fig. 4b (x200)
Insufficient CK5 staining of the pancreas, tissue core no. 3, with low-level CK5 expression using same protocol as in Figs. 1b-3b – same field as in Fig. 4a. No staining reaction is seen in the epithelial cell of the intercalated ducts. Staining gives a false negative result – compare with Fig. 4a.

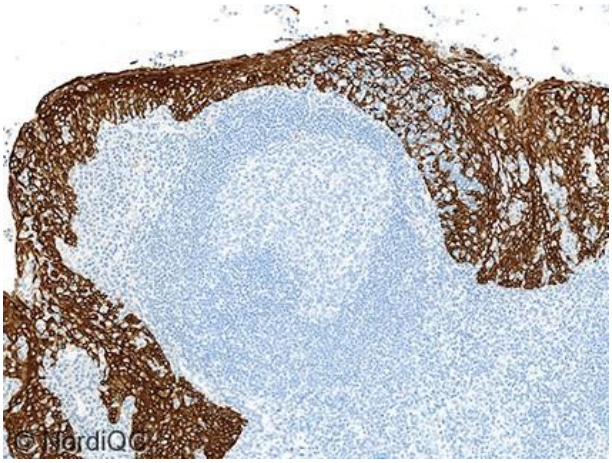

Fig. 5a (x100)
Optimal CK5 staining of the tonsil, tissue core no. 1, using the **rmAb clone SP27** in an RTU format (Ventana, 760-4935) using the vendors recommended protocol settings on the Ventana BenchMark. A strong staining reaction is seen in virtually all squamous epithelial cells in the tonsil. No background staining is seen. Also compare with Figs. 6a-7a, same protocol.

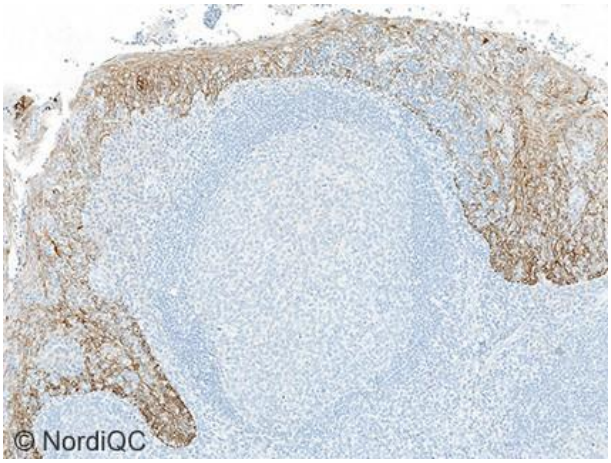

Fig. 5b (x100)
CK5 staining of the tonsil, tissue core no. 1, using the **mAb clone D5/16 B4** in an RTU format (Ventana, 790-4554) using the vendors recommended protocol settings on the Ventana BenchMark. Using this insufficient staining protocol, a weak to moderate staining reaction is seen in the majority of squamous epithelial cells in the tonsil – same field as in Fig. 5a. Also compare with Figs. 6b-7b, same protocol.

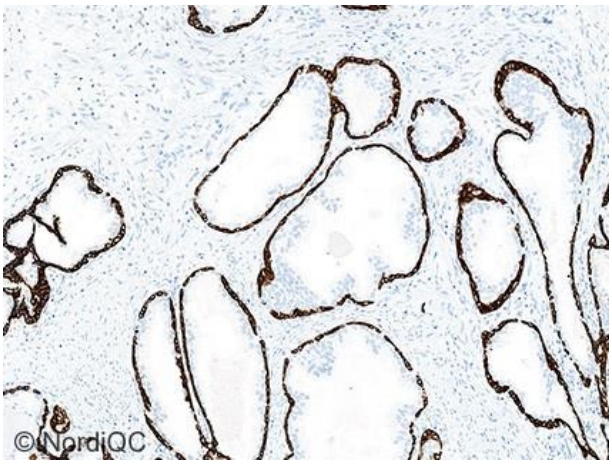

Fig. 6a (x100)
Optimal CK5 staining of the prostate hyperplasia, tissue core no. 4, using same protocol as in Figs. 5a.
A strong and distinct cytoplasmic staining reaction is seen in the majority of basal cells in the hyperplastic prostate glands. No background staining is seen.

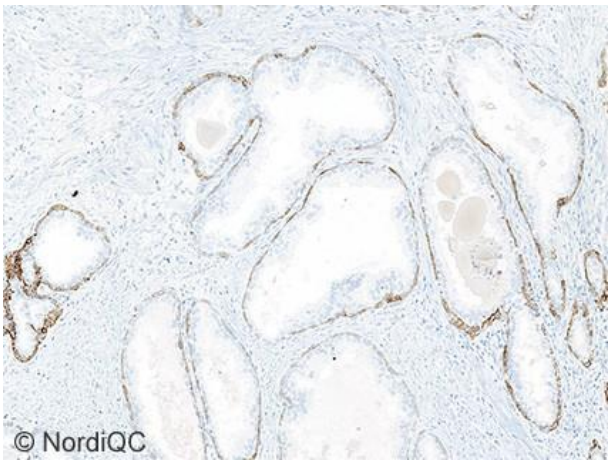

Fig. 6b (x100)
Insufficient CK5 staining of the prostate hyperplasia, tissue core no. 4, using same protocol as in Figs. 5b – same field as in Fig. 6a. Only weak to moderate staining reaction is seen in the basal cells in the hyperplastic prostate glands, making interpretation difficult.

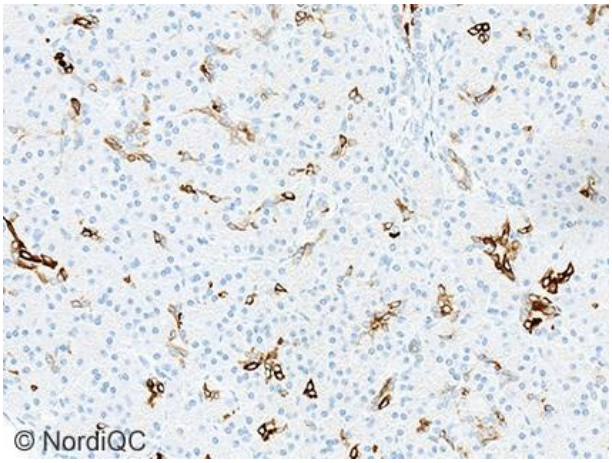

Fig. 7a (x200)
Optimal CK5 staining of pancreas, tissue core no. 3, with low-level CK5 expression using same protocol as in Figs. 5a-6a. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining is seen.

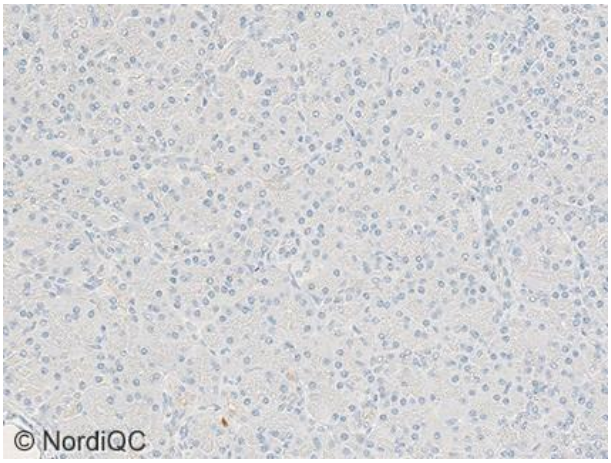

Fig. 7b (x200)
Insufficient CK5 staining of the pancreas, tissue core no. 3, with low-level CK5 expression using same protocol as in Figs. 5b-6b – same field as in Fig. 7a. No staining reaction is seen in the epithelial cell of the intercalated ducts. Staining gives a false negative result – compare with Fig. 7a.

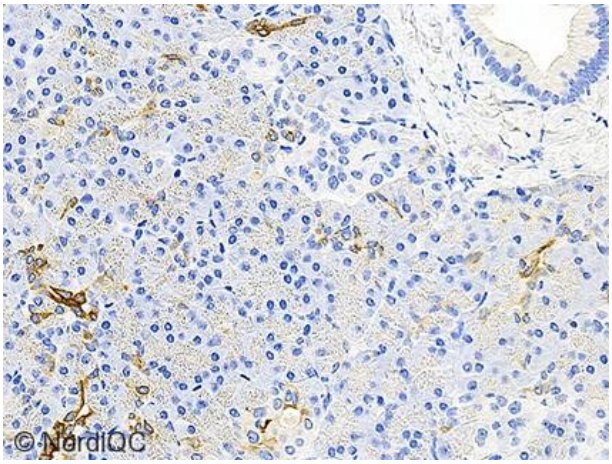

Fig. 8a (x300)
Optimal CK5 staining of pancreas, tissue core no. 3, using the **mAb clone XM26** in an optimally calibrated protocol on the Leica Bond. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining is seen.
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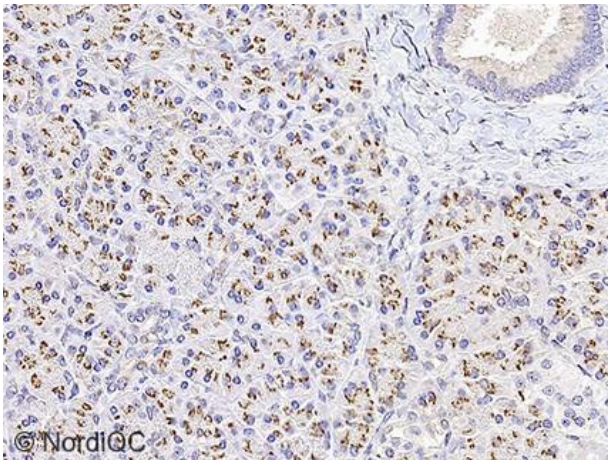

Fig. 8b (x300)
Insufficient CK5 staining of the pancreas, tissue core no. 3, using the **mAb clone D5/16 B4 (Dako, M7237)** at relative high concentration in an UltraView protocol with amplification on the Ventana BenchMark – same field as in Fig. 8a. No staining reaction is seen in the epithelial cell of the intercalated ducts. Instead, perinuclear MAG reaction (Mouse Ascites Golgi) is seen in the majority of acinar cells. In other words, this protocol produces both a false negative and false positive staining result – compare with Fig. 8a.

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