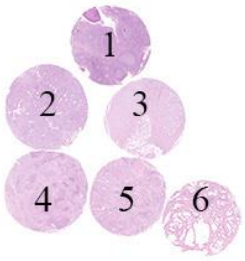
Assessment Run 63 2021

Cytokeratin 5 (CK5)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC assays among the NordiQC participants for CK5, typically used in the diagnostic work-up of prostate samples differentiating hyperplasia, prostate interepithelial neoplasia and carcinoma and also in lung samples to identify mesothelioma and to differentiate squamous cell carcinoma and adenocarcinoma. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for CK5 (see below).

Material

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



1: Tonsil, 2: Pancreas, 3: Malignant mesothelioma epithelial subtype, 4: NSCLC – squamous cell type, 5: NSCLC – adenocarcinoma, 6: Prostate hyperplasia

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 carcinoma.
- An at least weak to moderate staining reaction in the majority of neoplastic cells in the malignant epithelioid mesothelioma.
- No staining of neoplastic cells in the lung adenocarcinoma.

Participation

Number of laboratories registered for CK5, run 63	307
Number of laboratories returning slides using appropriate antibodies	283 (92%)

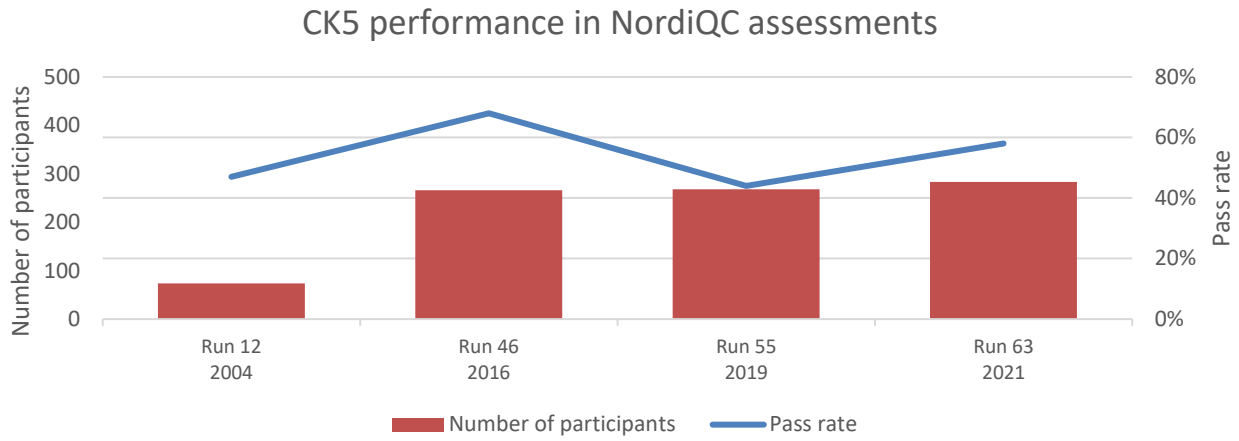
Results
At the date of assessment, 92% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

283 laboratories participated in this assessment. 164 (58%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 3).

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
- Use of less sensitive detection systems

Performance history
This was the fourth NordiQC assessment of CK5. An improvement in the pass rate was observed compared to the latest run (Run 55 in 2019) as seen in Graph 1. The improved pass rate might be related to an extended use of the robust Abs as mAb clone XM26 and rmAb clone SP27 on the expense of the less successful mAb clone D5/16 B4 being used by 61% of the participants in run 55 and 46% in this run 63.

Graph 1. **Proportion of sufficient results for CK5 in the NordiQC runs performed**



Conclusion

The mAbs clones **XM26**, **D5/16 B4** and the rmAb clones **BSR55** and **EP24/EP67** could all be used as concentrate within a laboratory developed assay for the demonstration of CK5. The widely used mAb clone XM26 was significantly more successful compared to mAb clone D5/16 B14 with pass rates of 86% and 21%, respectively. The mAb clone D5/16 B4 typically provided a too low analytical sensitivity and as most frequently being produced as an ascites format with the risk of false positive staining reactions due to "Mouse Ascites Golgi" (MAG)[1] reaction, this complicates the optimization process for the antibody.
Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer), careful calibration of the primary antibody and use of a sensitive 3-step polymer/multimer detection system were the most important prerequisites for an optimal staining result.
In this assessment, the Ventana/Roche RTU system based on rmAb clone SP27, 760-4935, was the most successful RTU system providing a pass rate of 100% of which 100% were optimal. However, SP27 has in NordiQC studies (ref; Thomsen et al[3]) shown positive reaction in lung adenocarcinomas being negative for other CK5 antibodies as well as p40, which has to be taken in account when used for subclassification of NSCLC.
The RTU systems based on mAb clone D5/16 B4 all performed poorly.

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.

Table 1. **Antibodies and assessment marks for CK5, run 63**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff[1]	OR[2]
mAb clone **CK5/6.007***	1	Biocare	0	0	0	1	-	-
mAb clone **D5/16 B4***	29 6 1 1 1 1	Dako/Agilent Cell Marque Millipore LifeTechnologies Thermo Scientific Zytomed	4	4	22	9	21%	10%
mAb clone **XM26**	2 1 3 1 68 2	Abcam Biocare Medical Diagnostic BioSystems Histols Leica Biosystems Monosan	45	21	10	1	86%	58%
mAb clone **IHC556***	1	GenomeMe	0	1	0	0	-	-
mAb clone **ZM186**	1	Zeta Corporation	0	0	0	1	-	-
rmAb clone **BSR55**	2	Nordic Biosite	2	0	0	0	-	-
rmAb clone **EP1601Y**	3 1	Cell Marque Biocare Medical	1	1	1	1	-	-
rmAb clone **EP24/EP67***	2	Cell Marque	2	0	0	0	-	-
rmAb clone **BP6021**	1	Biolynx Biotechnology Co., Ltd	0	0	1	0	-	-
rmAb clone **QR027**	1	Quartett	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **D5/16 B4* 790-4554[3]**	3	Ventana/Roche	0	0	2	1	-	-
mAb clone **D5/16 B4* 790-4554[4]**	40	Ventana/Roche	4	11	22	3	38%	10%
mAb **D5/16 B4* GA780[3]**	14	Dako/Agilent	0	1	10	3	7%	0%
mAb **D5/16 B4* GA780[4]**	18	Dako/Agilent	1	4	12	1	28%	6%
mAb clone **D5/16 B4* IR/IS780[3]**	2	Dako/Agilent	0	0	0	2	-	-
mAb clone **D5/16 B4* IR/IS780[4]**	14	Dako/Agilent	3	1	7	3	29%	21%
mAb clone **D5/16 B4* 8295-C010**	1	Sakura Finetek	0	1	0	0	-	-
rmAb clone **RM226 8408-C010**	1	Sakura Finetek	0	1	0	0	-	-
mAb clone **XM26 PA0468[3]**	8	Leica Biosystems	3	5	0	0	100%	38%
mAb clone **XM26 PA0468[4]**	7	Leica Biosystems	5	1	0	1	86%	71%
mAb clone **XM26 PM234**	2	Biocare Medical	1	0	1	0	-	-
rmAb/mAb clone **EP1601Y/LL002** 905H-08**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **EP1601Y 305R-17/18**	3	Cell Marque	1	1	1	0	-	-
rmAb clone **EP42 AN853-10M**	1	BioGenex	0	0	1	0	-	-
rmAb clone **EP24/EP67* MAD-000651QD**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **SP27 760-4935[3]**	19	Ventana/Roche	19	0	0	0	100%	100%
rmAb clone **SP27 760-4935[4]**	18	Ventana/Roche	18	0	0	0	100%	100%

rmAb clone **C9E33 CCR-0973**	1	Celnovte	0	0	1	0	-	-
Total	283		110	54	92	27		
Proportion			39%	19%	33%	9%	58%	

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).
*) Cytokeratin 5 and 6.
**) Cytokeratin 5 and 14.

Detailed analysis of CK5, Run 63
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **D5/16 B4**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/6) or Cell Conditioning 1 (CC1, Ventana/Roche) (2/24). The mAb was typically diluted in the range of 1:25-1:200. Using these protocol settings, 6 of 29 (21%) 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 based on HIER using Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (12/21), TRS pH 9 (3-in-1)(Dako/Agilent) (3/5), CC1 (18/31), BERS2 (10/14), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/1) or Tris-EDTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:200. Using these protocol settings, 63 of 71 (89%) laboratories produced a sufficient staining result.

Table 2. **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	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **D5/16 B4**	-	-	-	-	1/23** (4%)	1/1	-	2/5 (40%)	-
mAb clone **XM26**	3/5 (60%)	-	12/20 (60%)	-	17/30 (57%)	1/1	-	10/13 (77%)	1/1

* 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/Roche, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using CC1, efficient heating time 48-64 min. and 20-40 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, 10 of 14 (71%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **D5/16 B4**, product no. **GA780**, Dako/Agilent, Dako Omnis:
One protocol with an optimal result was based on HIER using TRS High pH, efficient heating time 30 min., 13 min. incubation of the primary Ab and EnVision Flex+ as detection system. Using these protocol settings, 2 of 3 laboratories produced ad sufficient staining result.

mAb clone **XM26**, product no. **PA0468**, Leica Biosystems, Leica Bond-III/Bond-Max:
Protocols with optimal results were based on HIER using BERS2 pH 9 (efficient heating time 10-30 min. at 95-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 13 of 13 (100%) laboratories produced an optimal staining result.

rmAb clone **SP27**, product no. **760-4935**, Ventana/Roche, 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, 36 of 36 (100%) laboratories produced a sufficient staining result.

Table 3 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 3. **Proportion of sufficient and optimal results for CK5 for the most commonly used RTU IHC systems**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone **D5/16 B4, 790-4554**	(0/3)	(0/3)	40% (15/38)	11% (4/38)
Dako Omnis mAb clone **D5/16 B4, GA780**	7% (1/14)	0% (0/14)	31% (5/16)	6% (1/16)
Dako Autostainer mAb clone **D5/16 B4, IR/IS780**	(0/2)	(0/2)	0% (0/7)	0% (0/7)
Leica Bond mAb clone **XM26, PA0468**	100% (8/8)	38% (3/8)	100% (5/5)	80% (4/5)
Ventana Benchmark rmAb clone **SP27, 760-4935**	100% (19/19)	100% (19/19)	100% (17/17)	100% (17/17)

* 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 94% of the insufficient results (112 of 119). The remaining 6% (7 of 119) insufficient results were characterized by either a false positive staining reaction (n=3) or poor signal-to-noise ratio/excessive background (n=4). Virtually all laboratories were able to demonstrate CK5 in high-level antigen expressing structures such as neoplastic cells of the lung squamous cell carcinoma and the squamous epithelial cells of tonsil. Demonstration of CK5 in low-level antigen expressing structures as the neoplastic cells in the malignant mesothelioma and especially the cuboidal epithelial cells of intercalated ducts in pancreas was significantly more challenging and required a carefully calibrated protocol. The pass rate has increased in this run from 44% in run 55 till 58% in this run, however the pass rate is still relatively low compared to the CK5 assessment in run 46 with a pass rate at 68%. The lower pass rate could be caused by the introduction of a "new" low-level CK5 expressor, namely the normal pancreas in the tissue microarray for the 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]. The reason for an increased pass rate in this current run 63 could be related to laboratories using more successful and robust antibodies/RTU assays for CK5. In the previous run 55, 61% of the participants used the less successful clone, mAb D5/16 B4 compared to only 46% in this current run 63.

46% (129 of 283) 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. 30% (39 of 129) of the LD-assays were based on the mAb clone D5/16 B4 whereas 60% (77 of 129) were based on the mAb clone XM26.
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/Agilent, Leica Biosystems and Ventana/Roche (see Table 2). In concordance with previous assessments 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/Roche), careful calibration of the titre of the primary Ab and the use of a sensitive detection system, preferably a 3-step polymer/multimer based detection 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 95% and 58%, 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 21% (8 of 39), and optimal staining results were only obtained by 4 laboratories (10%). These were based on HIER in an alkaline buffer and 3-step polymer/multimer based detection systems. It was observed that mAb clone D5/16 B4 in addition to the

low analytical sensitivity provided occasionally also showed an inferior performance due to a distinct, aberrant (false positive) cytoplasmic staining reaction in pancreas, most likely caused by MAG reaction[1]. The two main vendors, Dako/Agilent and Cell Marque of the mAb provides the product as an ascites format and it is well-known that this aberrant MAG reaction can be seen in tissues of blood type A patients. As CK5 is localized in the cytoplasmic compartment similar to the MAG reaction a genuine risk of false positivity and hereby misclassification of e.g. NSCLC is induced.

Four laboratories using LD assays based on one of the rmAbs for CK5 or CK5/6, clones BSR45 and EP24/EP67 all obtained optimal staining results. The protocols were based on HIER in an alkaline buffer and 3-step polymer/multimer based detection systems.

Ready-To-Use (RTU) antibodies was used by 54% (154 of 238) of the laboratories. The Ventana/Roche RTU system based on rmAb clone SP27, 760-4935, was the most successful and provided a proportion of sufficient staining results of 100% (37 of 37), all assessed as optimal. Optimal results could both be obtained using the protocol recommendations given by Ventana but also by laboratory modified protocol settings (typically minor adjustments of HIER time and/or incubation time of the primary Ab).
The RTU system based on SP27 gave a very high qualitative result in this assessment and test performed as expected in all tissue samples included.
However, SP27 has in NordiQC studies[3] shown positive reaction in few lung adenocarcinomas being negative for other CK5 antibodies as well as p40. The significance of this is uncertain but must be taken into account in the subclassification of NSCLC and emphasizes that a panel of markers must be applied to secure a correct diagnosis.

The Leica Biosystems RTU system based on mAb clone XM26, PA0468, also provided a high pass rate. Using the recommended protocol settings given by Leica, the proportion of sufficient staining results was 100% (8 of 8) of which 38% (3 of 8) were assessed as optimal. As seen in Table 3, a pass rate of 100% (5 of 5), 80% optimal, was seen if modifying the protocol settings. Only minor changes for HIER time and/or incubation time of the primary Ab was made.

The Dako/Agilent RTU systems based on mAb clone D5/16 B4, IR/IS780 and GA780 for Autostainer and Omins, respectively, both provided a low proportion of sufficient and optimal staining results. For both platforms the performance of the RTU systems used as "plug-and-play" was inferior to the performance obtained by laboratory modified protocol settings as shown in Table 1. The insufficient results were characterized by too weak or false negative test results. The most successful modification was based on use of FLEX+ as detection system and not FLEX as recommended. Surprisingly, no MAG reaction was seen for the Dako/Agilent RTU systems, as observed for the conc. format of the mAb clone D5/16 B4 despite adding a linker (FLEX+) to the protocols and hereby increasing the sensitivity for the ascites-based antibody. Cumulated data for the two RTU systems gave a pass rate of 6%, 0% optimal using the vendor recommended protocol settings, compared to a pass rate of 22% and 4% optimal using modified protocol settings.
The Ventana/Roche RTU system based on mAb clone D5/16 B4, 790-4554, performed slightly better than the corresponding Dako/Agilent RTU systems with a pass rate of 37% if including both vendor recommended and laboratory modified protocol settings, but still inferior to the other Ventana/Roche RTU system based on rmAb clone SP27. 93% (38 of 41) of the laboratories used laboratory modified protocol settings and 40% (15 of 38) obtained sufficient staining results of which 11% (4 of 38) were assessed as optimal. The protocols producing optimal staining results were all based on a sensitive 3-step multimer system, OptiView, giving a pass rate of 60% (15 of 25). If using iView or UltraView (as recommended in package insert), only insufficient results were obtained.

[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.

[3]Thomsen, C., Nielsen, O., Nielsen, S., Røge, R., & Vyberg, M. (2020). NordiQC Assessments of Keratin 5 Immunoassays. Applied Immunohistochemistry & Molecular Morphology, 28(7), 566-570. https://doi.org/10.1097/PAI.0000000000000855

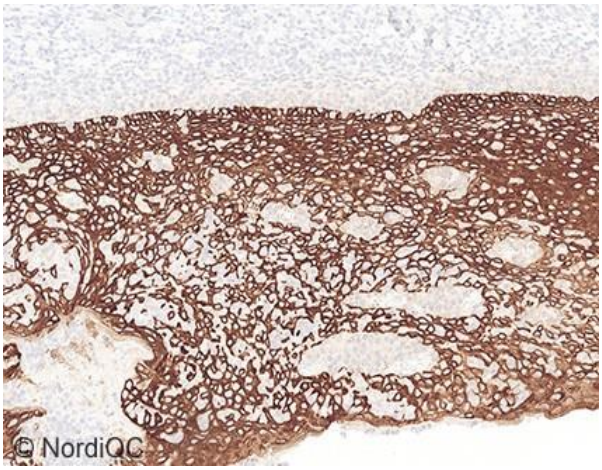


Fig. 1a
Optimal CK5 staining of the tonsil, tissue core no. 1, using the **rmAb clone SP27** in an RTU format (760-4935, Ventana/Roche) using the vendor recommended protocol settings on the BenchMark Ultra. A strong cytoplasmic staining reaction is seen in virtually all squamous epithelial cells in the tonsil. Also compare with Figs. 2a-5a, same protocol.

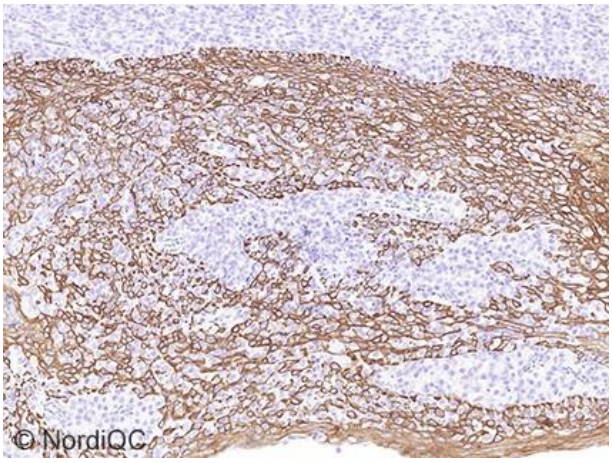


Fig. 1b
CK5 staining of the tonsil, tissue core no. 1, using the **mAb clone D5/16 B4** in an RTU format (IR780, Dako/Agilent) using the vendor recommended protocol settings on the Dako Autostainer. Using this staining protocol, the intensity of the epithelial cells demonstrated is reduced compared to the level expected and obtained in Fig. 1a, but overall, all cells are demonstrated as these have a high-level CK5 expression - same field as in Fig. 1a. However also compare with Figs. 2b-5b, same protocol.

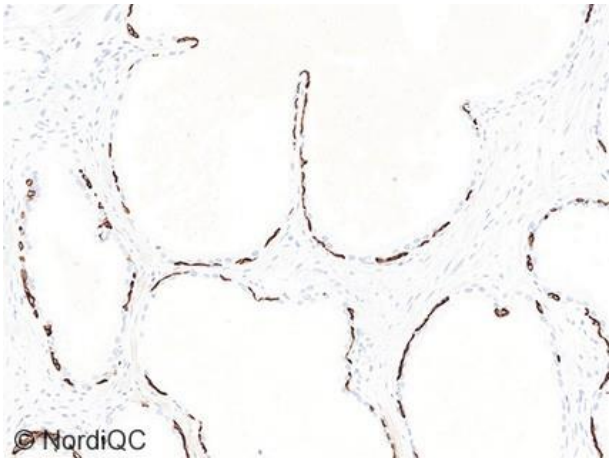


Fig. 2a
Optimal CK5 staining of the prostate hyperplasia, tissue core no. 6, using same protocol as in Fig. 1a.
A strong and distinct cytoplasmic staining reaction is seen in the majority of basal cells in the hyperplastic prostate glands. No background staining is seen.

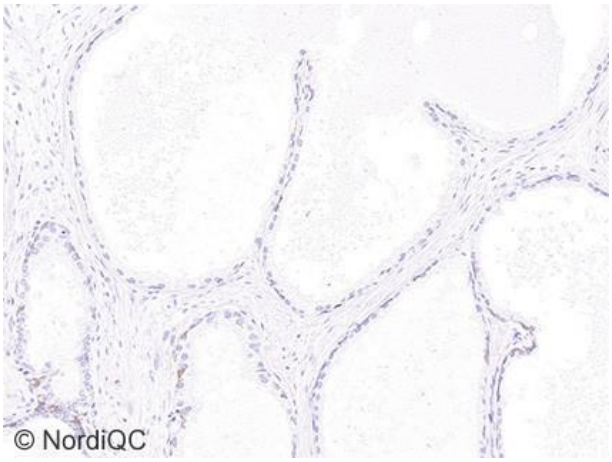


Fig. 2b
Insufficient CK5 staining of the prostate hyperplasia, tissue core no. 6, using same protocol as in Fig. 1b – same field as in Fig. 2a. Only scattered basal cells in the hyperplastic prostate glands show a weak staining reaction, compromising the diagnostic utility of the test in prostate samples.

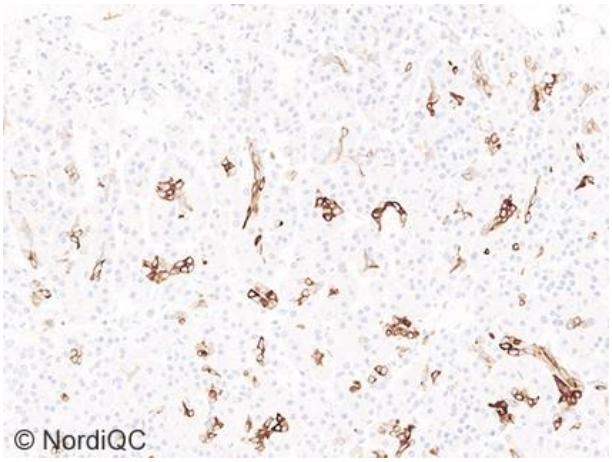


Fig. 3a
Optimal CK5 staining of pancreas, tissue core no. 2, with low-level CK5 expression using same protocol as in Figs. 1a-2a. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining is seen.

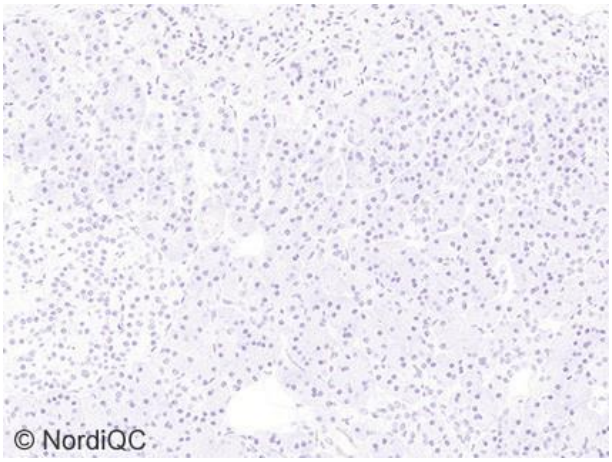


Fig. 3b
Insufficient CK5 staining of the pancreas, tissue core no. 2, with low-level CK5 expression using same protocol as in Figs. 1b-2b – same field as in Fig. 3a. No staining reaction is seen in the epithelial cell of the intercalated ducts giving a false negative result – compare with Fig. 3a.

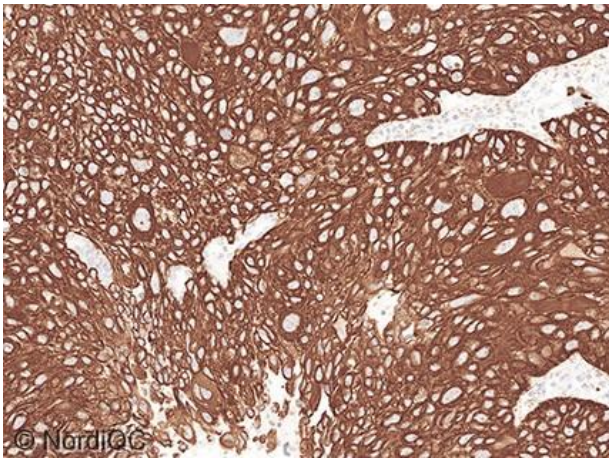


Fig. 4a
Optimal CK5 staining of the lung squamous cell carcinoma, tissue core no. 4, with high-level CK5 expression using same protocol as in Figs. 1a-3a. All the neoplastic cells show a strong and distinct cytoplasmic staining reaction.

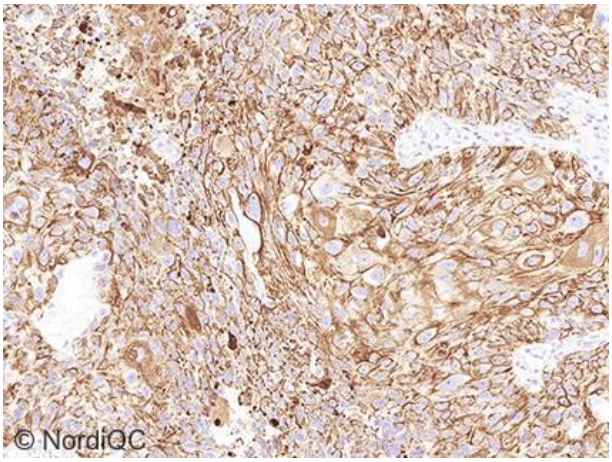


Fig. 4b
CK5 staining of the lung squamous cell carcinoma, tissue core no. 4, with high-level CK5 expression using the same insufficient protocol as in Figs. 1b-3b – same field as in Fig. 4a. The intensity of the neoplastic cells demonstrated is reduced compared to the level expected and obtained in Fig. 4a, but because of high-level CK5 expression all neoplastic cells are clearly demonstrated.

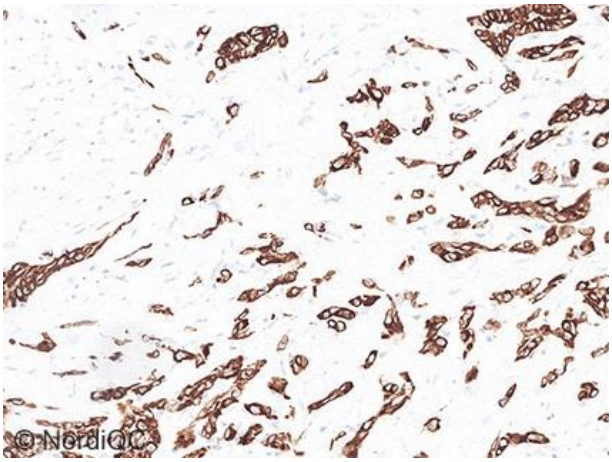


Fig. 5a
Optimal CK5 staining of the malignant mesothelioma, tissue core no. 3, with a low to medium level of CK5 expression using same protocol as in Figs. 1a-4a. All the neoplastic cells show a moderate to strong, distinct cytoplasmic staining reaction.

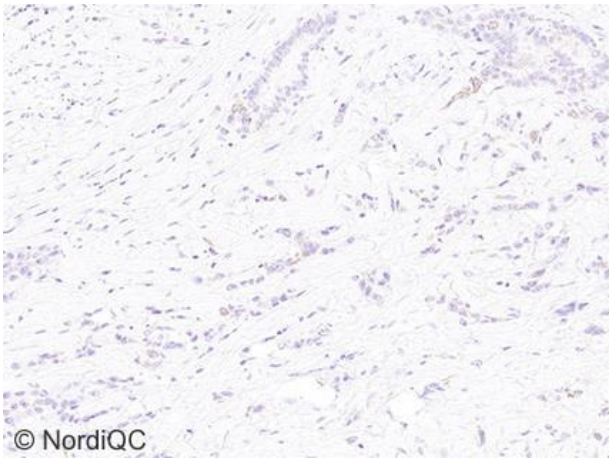


Fig. 5b
CK5 staining of the malignant mesothelioma, tissue core no. 3, using the same insufficient protocol as in Figs. 1b-4b – same field as in Fig. 5a. Virtually no staining reaction is seen in the neoplastic cells, giving a false negative staining reaction.

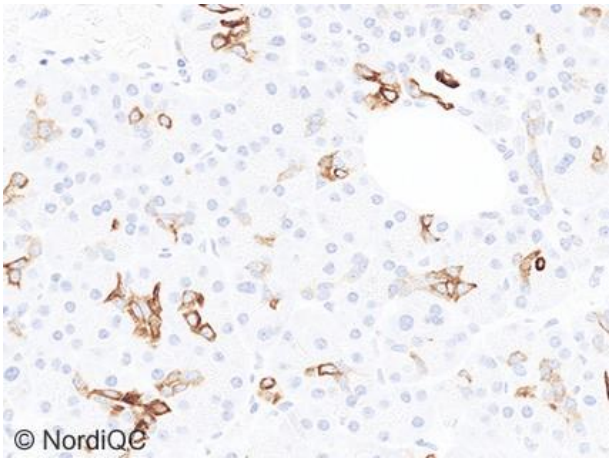


Fig. 6a
High magnification of the optimal CK5 staining of pancreas, tissue core no. 2, with low-level CK5 expression using same protocol as in Figs. 1a-5a. Scattered cuboidal epithelial cells of intercalated ducts display a weak to moderate predominantly membranous staining reaction. No background staining is seen.

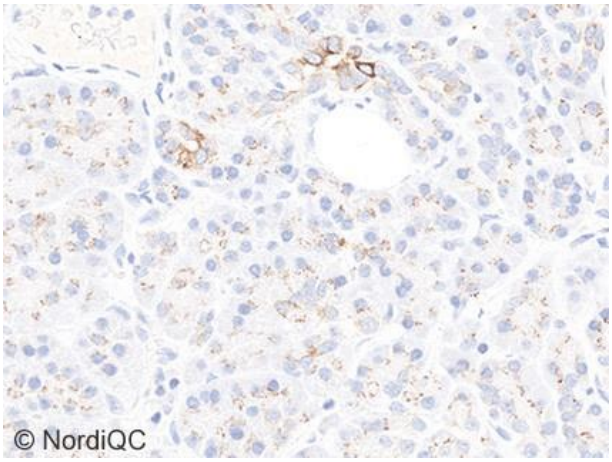


Fig. 6b
Insufficient CK5 staining of pancreas, tissue core no. 2, using the **mAb clone D5/16 B4** in a concentrated format, using a relatively high concentration (1:25), HIER in an alkaline buffer and a 3-layer detection system - same field as in Fig. 6a. No staining reaction is seen in the epithelial cells of the intercalated ducts. Instead, a cytoplasmic MAG reaction (Mouse Ascites Golgi) is seen in the majority of acinar cells. The staining result is thus both false negative and false positive.

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