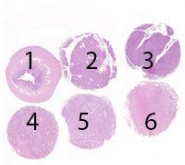
Assessment Run 61 2021
CDX2

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CDX2, typically identifying intestinal differentiation and especially colorectal adenocarcinomas in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CDX2 (see below).

Material
The slide to be stained for CDX2 comprised:



1. Appendix, 2. Pancreas, 3. Tonsil, 4. Lung adenocarcinoma, 5-6. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CDX2 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all epithelial cells in the appendix.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all ductal and intercalated duct epithelial cells in the pancreas.
- An at least moderate, predominantly nuclear staining reaction of the majority of the neoplastic cells in the colon adenocarcinoma, tissue core no. 5.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells in the colon adenocarcinoma, tissue core no. 6. Among circulated slides, the tumor displayed heterogeneity showing completely negative reaction in a minor area of the tumour but still being strongly positive in the majority of the neoplastic cells.
- No staining reaction in the lung adenocarcinoma and tonsil*.

A weak to moderate cytoplasmic reaction in cells with strong nuclear staining was accepted.

* In tonsil, dispersed lymphatic cells displayed a weak to moderate nuclear staining reaction.

Participation

Number of laboratories registered for CDX2, run 61	369
Number of laboratories returning slides	325 (88%)

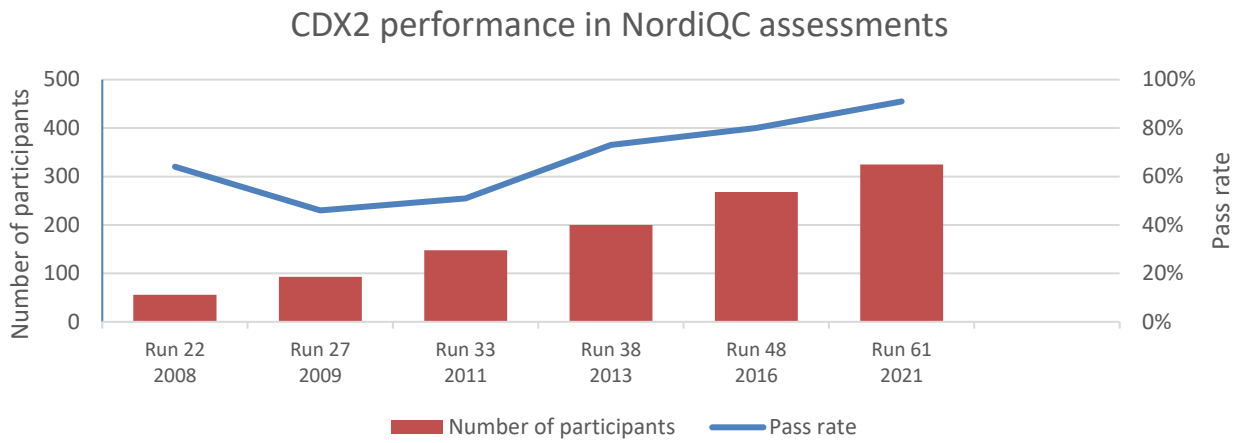
Results
At the date of assessment, only 88% of the participants had returned the circulated NordiQC slides, in particular subscribed to the Covid-19 pandemic and associated postal delays. All slides returned after the assessment were assessed, and laboratories received advice if assessment score was insufficient, but primary data was not included in this report.

325 laboratories participated in this assessment and 91% 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 reactions were:
- Too low concentration of the primary antibody.
- Inefficient HIER (too short HIER time or use of citric based buffer).
- Use of less sensitive detection systems.

Performance history
This was the sixth NordiQC assessment of CDX2. The pass rate increased compared to the previous run and a consistent improvement has been observed in the four latest assessments (see Figure 1).

Figure 1. **Proportion of sufficient results for CDX2 in the last six NordiQC runs performed**



Conclusion

The mouse monoclonal antibody (mAb) clone **DAK-CDX2** and the rabbit monoclonal antibodies (rmAb) clones **EPR2764Y** and **EP25** are all recommendable for demonstration of CDX2. Optimal results were obtained using efficient HIER, preferably in an alkaline buffer in combination with careful calibration of the antibody titer adjusted to the total sensitivity of the protocol applied. Assays based on the mAb clone DAK-CDX2, required use of a sensitive 3-step detection system (e.g., Envision Flex+) for optimal performance. The RTU systems from Dako/Agilent (GA080), Leica (PA0375) and Ventana/Roche (760-4380) provided superior results for demonstration of CDX2, and using vendor recommended protocol settings, all results submitted were assessed as sufficient.

Pancreas is an appropriate positive tissue control for CDX2: The majority of ductal and intercalated duct epithelial cells must display an at least weak to moderate, distinct nuclear staining reaction. Appendix and colon cannot be recommended as primary positive tissue control, due to the high level of CDX2 expression. Tonsil is recommended as negative tissue control and virtually all cells must be negative except for few dispersed lymphatic cells.

Table 1. **Antibodies and assessment marks for CDX2, Run 61**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **DAK-CDX2**	11	Dako/Agilent	4	4	1	2	72%	36%
mAb clone **CDX2-88**	1 1	Biocare Medical Biogenex	0	0	1	1	-	-
rmAb clone **EPR2764Y**	41 4 3 2 2 2 1 1 1	Cell Marque Thermo Scientific Zytomed Systems Abcam Monosan Zeta Corporation Epitomics Nordic Biosite Chemie Brunschwig	43	11	2	1	95%	75%
rmAb clone **EP25**	1 1	Epitomics Diagnostic Biosystems	1	0	1	0	-	-
rmAb clone **ZR215**	1	Zeta Corporation	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **DAK-CDX2, IR080/IS080[3]**	5	Dako/Agilent	2	1	2	0	60%	20%
mAb clone **DAK-CDX2, IR080/IS080[4]**	25	Dako/Agilent	14	5	2	4	76%	56%
mAb clone **DAK-CDX2, GA080[3]**	30	Dako/Agilent	26	4	0	0	100%	87%
mAb clone **DAK-CDX2, GA080[4]**	26	Dako/Agilent	17	2	4	3	73%	65%
mAb clone **CDX2-88, AM392**	1	BioGenex	1	0	0	0	-	-
mAb clone **MX024, MAB-0713**	2	Fuzhou Maixin Biotech	2	0	0	0	-	-

rmAb clone **IHC302, IHC302**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **EPR2764Y, 760-4380**[3]	20	Ventana/Roche	19	1	0	0	100%	95%
rmAb clone **EPR2764Y, 760-4380**[4]	95	Ventana/Roche	90	2	3	0	97%	95%
rmAb clone **EPR2764Y, 235R-17/18**	19	Cell Marque	17	2	0	0	100%	89%
rmAb clone **EPR2764Y, RM-2116**	2	Immunologic	2	0	0	0	-	-
rmAb clone **EPR2764Y, CCR-0821**	1	Celnovte Biotechnology	0	0	1	0	-	-
rmAb clone **EP25, PA0375**[3]	9	Leica Biosystems	9	0	0	0	100%	100%
rmAb clone **EP25, PA0375**[4]	8	Leica Biosystems	8	0	0	0	100%	100%
rmAb clone **EP25, 8285-C010**	4	Sakura Finetek	0	4	0	0	-	-
rmAb clone **EP25, MAD-000645QD**	2	Master Diagnostica	1	0	0	1	-	-
rmAb clone **EP25, API3144**	2	Biocare Medical	1	1	0	0	-	-
Total	325		257	38	17	13	-	
Proportion			79%	12%	5%	4%	91%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
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 applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of CDX2, Run 61
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **DAK-CDX2**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/5)* or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (3/4) as retrieval buffer. The mAb was diluted in the range of 1:10-1:50. Using these protocol settings, 8 of 8 (100%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EPR2764Y**: Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1, Ventana) (29/34), TRS pH 9 (3-in-1) (Dako/Agilent) (3/5), BERS2 (Leica) (9/12) or Tris-EDTA pH 9 (2/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:500. Using these protocol settings, 44 of 46 (96%) laboratories produced a sufficient staining result.

rmAb clone **EP25**: One protocol with an optimal result was based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted 1:25 and Envision Flex+ (Dako/Agilent) was used as the detection system.

Table 2. **Proportion of optimal results for CDX2 for the two most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche 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 **DAK-CDX2**	1/1**	-	0/3	-	0/2	-	3/4	-
rmAb clone **EPR2764Y**	0/1	-	3/3	-	26/30 (87%)	-	8/11 (73%)	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 **DAK-CDX2**, product no. **IR/IS080**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 97-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 15 of 18 (83%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **DAK-CDX2**, product no. **GA080**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (efficient heating time 24-30 min. at 97°C), 15-30 min. incubation of the primary Ab and Envision FLEX+ (GV800+GV821) as detection system. Using these protocol settings, 43 of 43 (100%) laboratories produced a sufficient staining result.

mAb clone **MX024**, product no. **MAB-0713**, Fuzhou Maixin Biotech, Titan S:
Protocols with optimal results were based on HIER using High pH buffer (DNS-0811) (efficient heating time 20 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super Detection Kit (TT-0805) as detection system.

rmAb clone **EP25** product no. **PA0375**, Leica, Bond III/MAX:
Protocols with optimal results were typical based on HIER using BERS2 (efficient heating time 20 min. at 99-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 9 of 9 (100%) produced a sufficient staining result.

rmAb clone **EPR2764Y**, product no. **760-4380**, Ventana/Roche, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-72 min.) and 16-48 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 103 of 103 (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 according 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 CDX2 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb DAK-CDX2 **IR/IS080**	60% (3/5)	20% (1/5)	79% (15/19)	58% (11/19)
Dako Omnis mAb DAK-CDX2 **GA080**	100% (30/30)	87% (26/30)	73% (17/23)	65% (15/23)
Leica Bond III/MAX rmAb EP25 **PA0375**	100% (9/9)	100% (9/9)	100% (8/8)	100% (8/8)
VMS Ultra/XT/GX rmAb EPR2764Y **760-4380**	100% (20/20)	95% (19/20)	97% (91/94)	95% (89/94)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In this assessment and in concordance with the previous NordiQC runs for CDX2, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This staining pattern was seen in 97% (29/30) of the insufficient results. Virtually all laboratories were able to demonstrate CDX2 in high-level antigen expressing cells of the appendix and the colon adenocarcinoma tissue core no. 6, whereas low-level CDX2 expressing cells of the colon adenocarcinoma tissue core no. 5 and the epithelial cells of the intercalated pancreatic ducts was more challenging and could only be demonstrated with an optimally calibrated protocol.

The rmAb clones EP25, EPR2764Y and the mAb clone DAK-CDX2 were the most widely used antibodies for demonstration of CDX2 and applied by 98% (318/325) of the laboratories (see Table 1). Assays based on these robust primary Abs contributed to the overall high pass rate of 91% obtained in this assessment and emphasize the importance of selecting high performance antibodies in regard of analytical sensitivity and specificity.

Used as concentrated format within laboratory developed (LD) assays, the rmAb clone EPR2764Y provided 95% (54/57) sufficient results of which 75% (43/57) were assessed as optimal. As described in the previous report (Run 48), efficient HIER in an alkaline buffer and careful calibration of the primary Ab were the two most central parameters for optimal results. The vast majority of laboratories (70%, 30/43) obtaining an optimal mark applied a 3-step multimer/polymer based detection system for the demonstration of CDX2. However, and due to the robustness of the primary Ab, the use of sensitive detection systems seems of less importance, although recommendable as general backbone for LD assays, as a significant proportion of protocols (30%, 13/43) based on a 2-step multimer/polymer system, also provided an optimal result as long as the primary ab was carefully calibrated and adjusted to the total sensitivity of the protocol employed. Nine of the thirteen protocols were based on Bond Refine (Leica) as detection system, that by nature only enhances the reaction of mouse primary antibodies as the linker step (post primary molecule) in between primary Ab and polymer is a rabbit anti-mouse antibody and basically acts as a 2-step polymer detection system when the primary Ab is produced from a rabbit.

The mAb clone DAK-CDX2 used within a LD assay could also produce optimal results. The clone was less successful compared to rmAb clone EPR2764Y as a reduced proportion of sufficient and optimal results was observed, despite using similar protocol settings as for rmAb clone EPR2764Y. The prevalent feature of an optimal result was use of a high concentration of the primary Ab (dilution range 1:10-1:50), HIER in an alkaline buffer (efficient heating time for at least 20 min. at 95-100°C) and use of a 3-step polymer detection system Bond Refine (Leica) or Envision Flex+ (Dako/Agilent).
As mentioned in the last report (Run 48), the performance of the mAb clone DAK-CDX2 is influenced by the applied platform and especially challenged on BenchMark (Ventana/Roche). In this assessment, two laboratories used the mAb clone DAK-CDX2 within a LD assay on the BenchMark, both assessed as poor. For this particular platform and adding data from the two latest assessment, only 13% (2/16) of the assays based on DAK-CDX2 provided a sufficient result of which one protocol was optimal. These observations clearly indicate that it is advisable to substitute the mAb clone DAK-CDX2 with e.g., EPR2764Y or EP25, when performed on the BenchMark platform.

In total, 78% (252/325) of the laboratories used a RTU format. The most widely used RTU systems for CDX2 were the Ventana/Roche 760-4380, Leica PA0375, Dako/Agilent IR/IS080 or Dako GA080, based on the rmAb clones EPR2764Y, EP25 and the mAb cloneDAK-CDX2, respectively. Applied on the fully automated platforms, Benchmark (Ventana/Roche); Bond (Leica) or Omnis (Dako/Agilent), these products provided superior performance and following vendor recommended protocol settings, all (59/59) produced a sufficient result (see Table 3). A high proportion of protocols, based on laboratory modified protocol settings, could also provide a significant proportion of sufficient and optimal results, typical adjusting incubation time in primary ab, HIER time/temperature and the choice of the detection system.

The Ventana RTU system 760-4380 (Benchmark) based on the rmAb clone EPR2764Y, was in this assessment used by 35% (114/325) of the participants, giving an overall pass rate of 97% (111/114). Applying vendor recommended protocol settings (32 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView as detection kit), a significant proportion of the results were assessed as optimal (see Table 3). This observation and supported by the data seen for the concentrated formats of the same Ab, emphasize that the rmAb clone EPR2764Y is very robust, as the use of the less sensitive detection system (UltraView) demonstrated excellent performance. Three laboratories obtained an insufficient result, primarily caused by reduced HIER and/or incubation time of the primary Ab or use of the detection system OptiView with amplification - compromising the interpretation.
This product has been developed by Cell Marque for the Ventana Benchmark platforms, and Cell Marque have their own product line (235R-17/18) of the same Ab, providing 100% (19/19) sufficient results of which 89% (17/19) were optimal (see Table 1). All assays were performed on the BenchMark platforms, using similar protocol settings as described above.

The Dako/Agilent RTU system IR080/IS080 (Autostainer) based on the mAb clone DAK-CDX2, provided a relative low pass rate of 75% (18/24) compared to RTU systems performed on the fully automated platforms e.g., Omnis (Dako). Both vendor recommended (20 min. incubation of the primary Ab, HIER in TRS High for 20 min. and EnVision FLEX as detection kit) and laboratory modified protocol settings could produce optimal results. Reduced incubation time of the primary Ab or inefficient HIER in TRS Low pH buffer used in combination with the less sensitive detection system EnVision Flex, were the main reasons for insufficient results. For results based on EnVision Flex+, 100% (6/6) were assessed as optimal, demonstrating that this Ab may benefit or require highly sensitive protocol settings.

The Dako/Agilent RTU system GA080 (Omnis) based on mAb clone DAK-CDX2 provided an overall pass rate of 89% (34/53). Applying vendor recommended protocol settings (25 min. incubation of the primary Ab, HIER in TRS High for 30 min. and EnVision FLEX+ as detection kit), 100% (30/30) of the submitted results were assessed as sufficient and 87% (26/30) were optimal. Especially, the choice of the detection system impacted the overall performance of the RTU system and all results (6/6) assessed as insufficient

were based on EnVision Flex omitting the linker step. In comparison, all protocols (44/44) based on EnVision Flex+ as the detection system provided a sufficient result of which 89% (39/44) were optimal.

The Leica RTU system PA0375 based on the rmAb clone EP25 (Bond) provided superior results and all (17/17) protocols, both vendor recommended (15 min. incubation of the primary Ab, HIER in BERS2 for 20 min. and Refine as detection system) and laboratory modified protocol settings, gave an optimal result.

This was the sixth NordiQC assessment of CDX2. The pass rate has consistently increased over the last three runs (see Figure 1) despite a consistent increased number of new participants, in total 60% new laboratories from run 38 till this present run 61. Several parameters contributed to the high proportion of sufficient results: 1) The extended use of robust primary Abs (e.g. EPR2467Y), 2) The superior performance of the RTU systems developed for fully automated platforms from the three major vendors, e.g. PA0375 (Bond, Leica), GA080 (Omnis, Dako/Agilent) and 760-4380 (BenchMark, Ventana/Roche), and in total applied by 57% (184/325) of the laboratories, 3) Participants following information giving by the NordiQC organization in past runs, typical recommendations to perform HIER in an alkaline buffer, careful calibration of the primary Ab and the use of a 3-step multimer/polymer detection system.
Importantly, protocols must stain accordingly to the expected antigen level and pancreas is the central immunohistochemical critical assay performance control (ICAPC) to guide the level of analytical sensitivity (see below).

Controls
Pancreas is recommended as positive tissue control displaying Low Level of Detection (LLOD). Virtually all ductal and intercalated duct epithelial cells must show an at least weak to moderate, distinct nuclear staining reaction. Appendix and colon are not recommended as primary positive tissue controls, since the epithelial cells express high levels of CDX2 and thus, not an ideal indicator for the appropriate level of analytical sensitivity being crucial both in the validation phase and as routine control to monitor the reproducibility of the CDX2 test.
Tonsil can be used as negative tissue control for CDX2. In order to monitor the specificity, no nuclear or cytoplasmic staining must be seen in endothelial cells and smooth muscle cells. The vast majority of lymphocytes should be negative, although weak nuclear staining reaction may be observed in scattered lymphatic cells, as seen in this assessment. The recommendations of the mentioned tissue controls for IHC are concordant with the guidelines published by the International Ad Hoc Expert Committee[1].

[1]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.

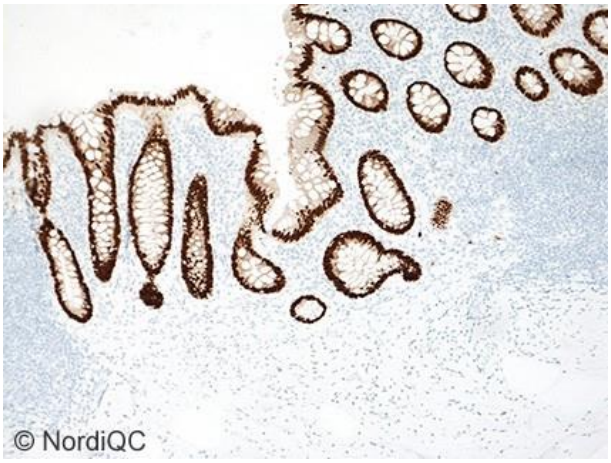


Fig. 1a (x100)
Optimal staining for CDX2 of the appendix using the rmAb clone EPR2764Y as RTU format (Ventana, 760-4380) on BenchMark Ultra following the recommendations given by the vendor (see description above) - same protocol used in Figs. 2a - 6a. Virtually all epithelial cells show a strong nuclear staining reaction. A weak cytoplasmic reaction in cells with nuclear staining rection was accepted.

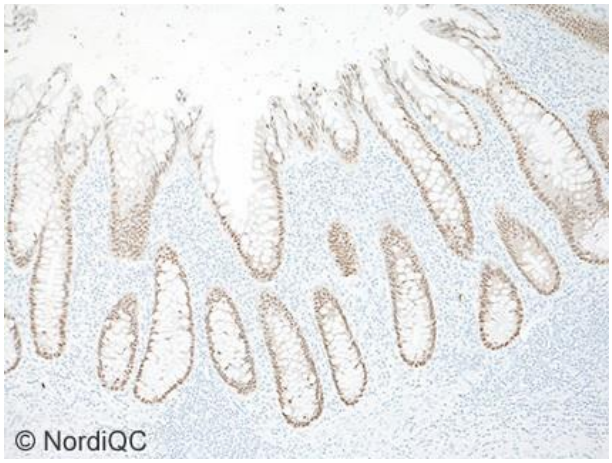


Fig. 1b (x100)
Insufficient staining for CDX2 of the appendix using the rmAb clone EPR2764Y as RTU format (Ventana, 760-4380) within a LD assay on BenchMark Ultra, applying too short HIER time in CC1 (20 min.) in combination with reduced and too short incubation time in primary Ab (20 min.) - same protocol used in Figs. 2b – 6b. Although the nuclei of epithelial cells are demonstrated, the intensity is significantly reduced - compare with Fig. 1a.

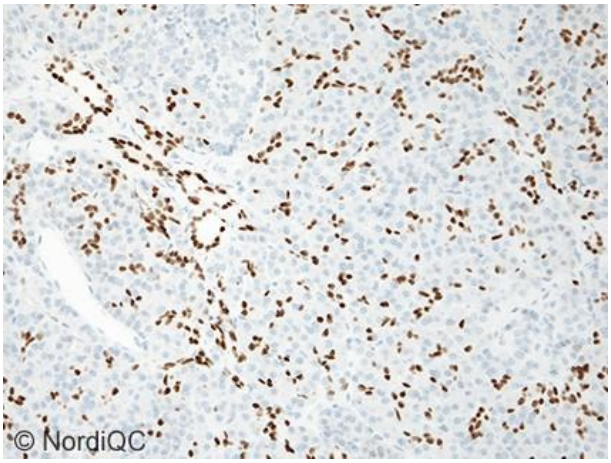


Fig. 2a (x200)
Optimal CDX2 staining of the pancreas using same protocol as in Fig. 1a. Virtually all the ductal and intercalated epithelial cells display a moderate and distinct nuclear staining reaction.

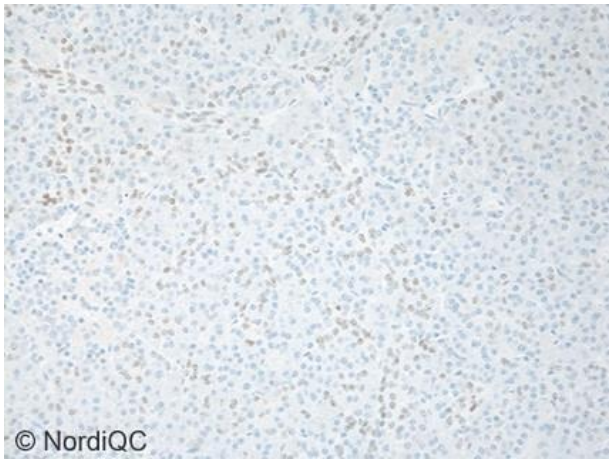


Fig. 2b (x200)
Insufficient CDX2 staining of the pancreas using same protocol as in Fig. 1b. The proportion and staining intensity of the ductal/intercalated duct epithelial cells is significantly reduced, and nuclei only demonstrate faint staining reaction - compare with Fig. 2a.

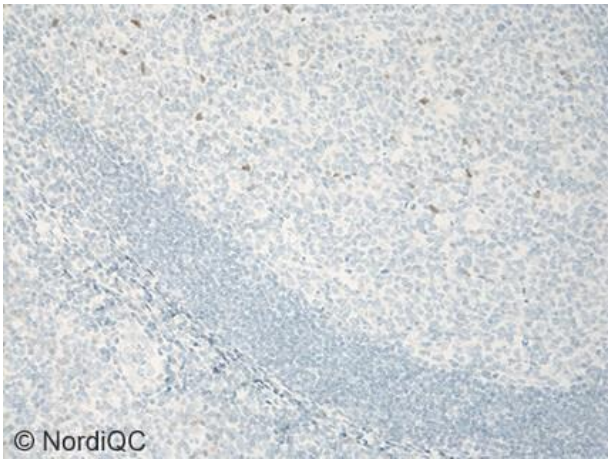


Fig. 3a (x200)
Optimal CDX2 staining of the tonsil, using same protocol as in Figs. 1a and 2a. Virtually all lymphocytes display the expected negative staining reaction. Scattered lymphatic cells showed a weak nuclear staining reaction.

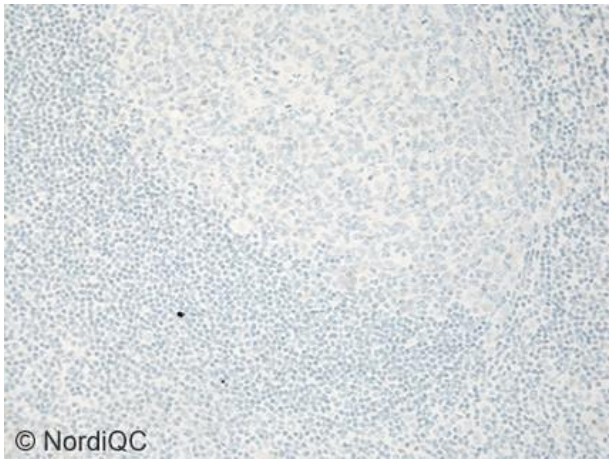


Fig. 3b (x200)
CDX2 staining of the tonsil using the same protocol as in Figs. 1b and 2b. Virtually all cells are as expected negative. The intensity and proportion of the dispersed lymphatic cells is reduced and barely visible - compare with Figs. 3a.

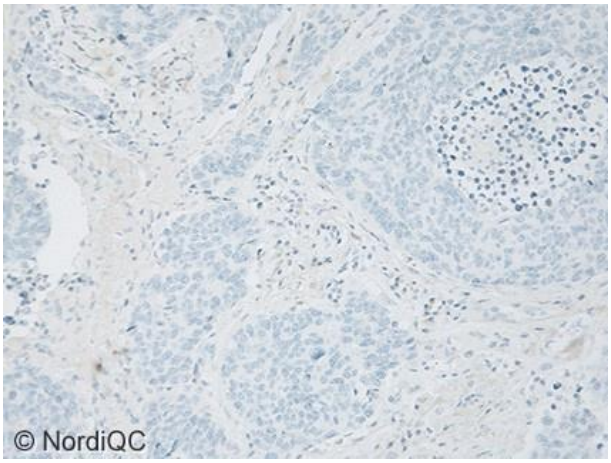


Fig. 4a (x200)
Optimal CDX2 staining of the lung adenocarcinoma, using same protocol as in Figs. 1a - 3a. All neoplastic cells are as expected negative.

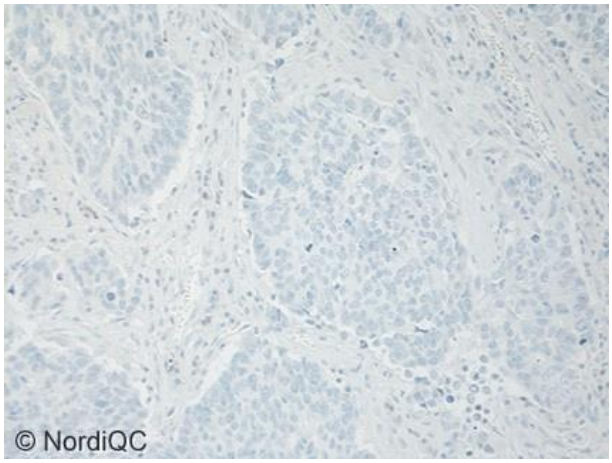


Fig. 4b (x200)
CDX2 staining of the lung adenocarcinoma using the same protocol as in Figs. 1b-3b. The neoplastic cells display the expected staining pattern. However, overall the protocol provided a too low level of analytical sensitivity, risking misdiagnosis of colon adenocarcinomas - compare with Figs. 5a – 6b.

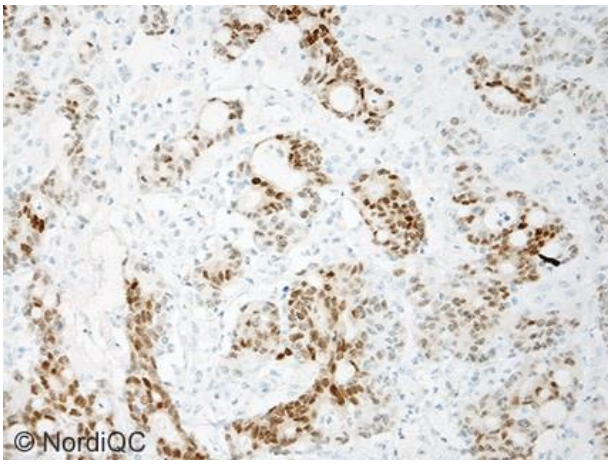


Fig. 5a (x200)
Optimal CDX2 staining of the colon adenocarcinoma, tissue core 5, using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a moderate to strong, and distinct nuclear staining reaction.

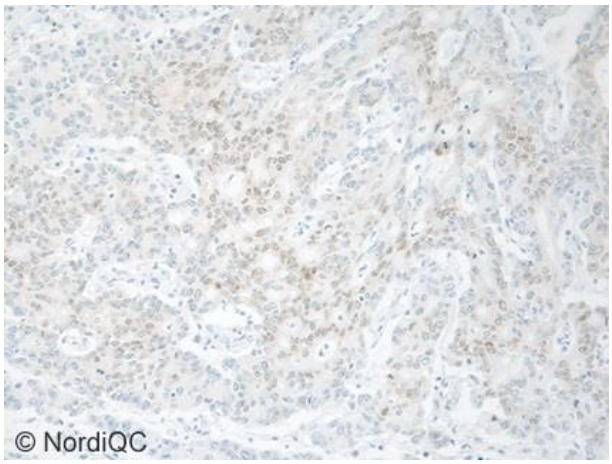


Fig. 5b (x200)
Insufficient CDX2 staining of the colon adenocarcinoma, tissue core 5, using same protocol as in Figs. 1b - 4b. The majority of the neoplastic cells are false negative or only display a weak nuclear staining reaction – compare with Fig. 5a.

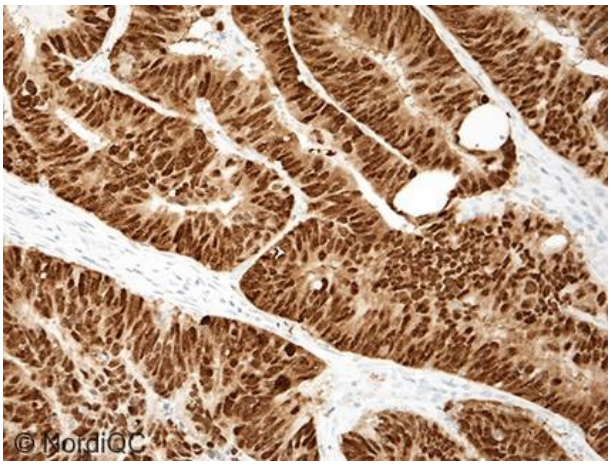


Fig. 6a (x400)
Optimal CDX2 staining of the colon adenocarcinoma, tissue core 6, using same protocol as in Fig. 1a - 5a. All the neoplastic cells show a strong and distinct nuclear staining reaction. A coexisting cytoplasmic staining reaction is seen and expected due to the high CDX2 expression level.

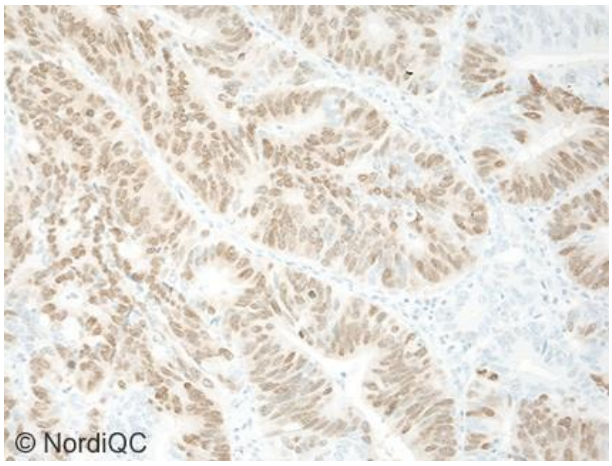


Fig. 6b (x200)
Insufficient CDX2 staining of the colon adenocarcinoma, tissue core 6, using same protocol as in Figs. 1b - 5b. Virtually all the neoplastic cells are demonstrated but display a significantly reduced and too weak nuclear staining reaction – compare with Fig. 6a.

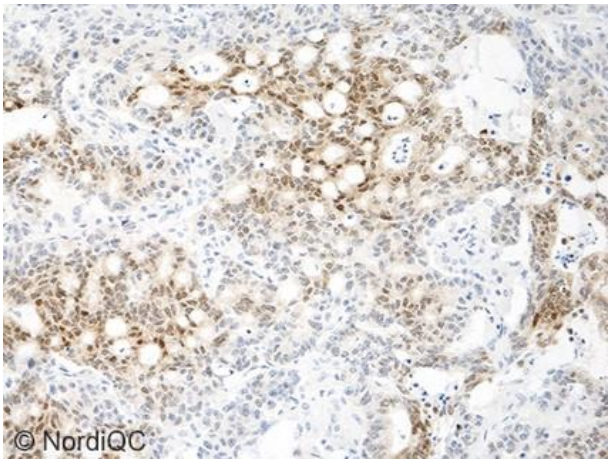


Fig. 7a (x200)
Optimal CDX2 staining of the colon adenocarcinoma, tissue core 5, using the mAb clone DAK-CDX2 as RTU format (Dako, GA080) on the Omnis, following recommendations given by the vendor including use of the EnVision Flex+ (with mouse linker) as the detection system (see description above). The neoplastic cells displayed the expected pattern as seen in Fig. 5a.

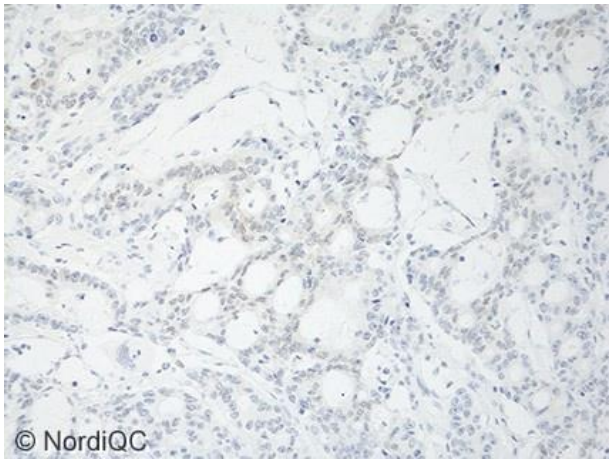


Fig. 7b (x400)
Insufficient CDX2 staining of the colon adenocarcinoma, tissue core 5, using the mAb clone DAK-CDX2 as RTU format (Dako, GA080) on the Omnis, modifying the recommended protocol settings by reducing incubation time in primary Ab from 25 min. to 20 min. and substituting EnVision Flex+ with EnVision Flex (without mouse linker) as the detection system. The vast majority of the neoplastic cells are false negative or only display faint staining reactions - compare with optimal staining in Fig. 7a.

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