Assessment Run 33 2011
CDX2

Material

The slide to be stained for CDX2 comprised:

1. Thyroid. 2. Pancreas. 3. Colon adenocarcinoma. 4. Appendix. 5. Lung adenocarcinoma. 6. Gastric adenocarcinoma. 7. Pancreas adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CDX2 staining as optimal included:

- A strong, distinct nuclear staining of virtually all the epithelial cells in the appendix.
- A moderate to strong, distinct nuclear staining of virtually all the neoplastic cells in the colon adenocarcinoma.
- An at least weak to moderate, distinct nuclear staining in the majority of neoplastic cells in the gastric adenocarcinoma.
- An at least weak to moderate, distinct nuclear staining in scattered neoplastic cells in the pancreas adenocarcinoma.
- An at least weak to moderate and distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas.
- As a maximum a weak cytoplasmic reaction in cells with strong nuclear staining. All other cells should be negative.

148 laboratories participated in this assessment. 51 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CDX2, run 33**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **DAK-CDX2**	27	Dako	5	5	8	9	37 %	78 %
rmAb clone **EPR2764Y**	15	Cell Marque Epitomics Medac Abcam Master Diagnóstica	7	4	2	2	73 %	75 %
mAb clone **CDX2-88**	26	BioGenex Biocare Linaris	0	5	8	13	19 %	-
mAb clone **AMT28**	13	Novocastra/Leica	0	2	4	7	15 %	-
Ready-To-Use Abs								
mAb clone **DAK-CDX2, IR080/IS080**	33	Dako	7	18	7	1	76 %	91 %
rmAb clone **EPR2764Y,760-4380**	26	Ventana/Cell Marque	14	5	4	3	73 %	86 %
mAb clone **CDX2-88, E087**	2	Linaris	0	0	0	2	-	-
mAb clone **CDX2-88, PM 226**	2	Biocare	0	2	0	0	-	-
mAb clone **AMT28, PA0535**	4	Novocastra/Leica	0	1	1	2	-	-
Total	148		33	42	34	39	-	
Proportion			22 %	29 %	23 %	26 %	51 %	-

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

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **DAK-CDX2**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target retrieval solution (TRS) pH 9 (3-in-1, Dako) (3/4)* or Tris-EDTA/EGTA pH 9 (2/5). The mAb was diluted in the range of 1:20–1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 out of 9 (78 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **EPR2764Y**: The protocols giving an optimal result were all based on HIER using either standard Cell Conditioning 1 (CC1) (3/7) Tris-EDTA/EGTA pH 9 (1/2), TRS pH 9 (Dako) (1/1), TRS pH 9 (3-in-1,Dako) (1/1) or Bond Epitope Retrieval Solution 2 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 12 (75 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **DAK-CDX2** (IR080/IS080, Dako): The protocols giving an optimal result were all based on HIER using TRS pH 9, TRS pH 9 (3-in-1) or Tris-EDTA/EGTA pH 9 and an incubation time of 20 to 75 min in the primary Ab and a 2- or 3-step polymer system, EnVision (Dako K8000/K8002/K5007/K4007) or BrightVision+ (Immologic) as the detection system. Using these protocol settings 21 out of 23 (91 %) laboratories produced a sufficient staining.

rmAb clone **EPR2764Y** (760-4380, Ventana/Cell Marque): The protocols giving an optimal result were based on HIER using mild or standard Cell Conditioning 1, an incubation time of 24 to 44 min of the primary Ab at 36°C and UltraView (Ventana, 760-500) as the detection system. One protocol was based on using mild CC1, an incubation time of 16 min of the primary Ab at 36°C and OptiView (Ventana, 760-700) as the detection system. Using these protocol settings 19 out of 22 (86 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:

- Too low concentration of the primary antibody.
- Less successful performance of the mAb clones CDX2-88 and AMT28. Only 10 of 47 (21 %) laboratories using the mAb clones CDX2-88 and AMT28 produced a sufficient staining (none of these achieved optimal marks!).
- Less successful performance of the mAb clone DAK-CDX2 on the Ventana BenchMark platform. Only 4 of 15 (27 %) laboratories using the mAb clone DAK-CDX2 (RTU or concentrate) produced sufficient staining (none of these achieved optimal marks!).

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. Virtually all laboratories were able to demonstrate CDX2 in high antigen expressing cells in the appendix and the colon adenocarcinoma (Figs. 2a and 2b), whereas the low expressing cells in the gastric adenocarcinoma, the pancreas adenocarcinoma and the epithelial cells of the intercalating ducts in the pancreas could only be demonstrated with an optimal protocol (Fig. 1a, Fig. 1b, Fig. 3a and Fig. 3b).

In this assessment optimal staining could only be obtained with the mAb clone DAK-CDX2 and the rmAb EPR2764Y and the protocols were all based on the use of an alkaline HIER buffer and a sensitive detection system. Surprisingly mAb DAK-CDX2 had a significantly lower pass rate on the Ventana Benchmark platform compared to the general pass rate for this clone. Low CDX2 expressing cells like the epithelial cells of the ducts in the pancreas and the tumour cells of the pancreas adenocarcinoma were difficult to detect on the Ventana Benchmark platform with mAb DAK-CDX2 (Figs 4a and 4b). Only 4 of 15 (27 %) laboratories using the mAb clone DAK-CDX2 (RTU or concentrate) on the Ventana Benchmark platform produced a sufficient staining. In comparison 31 of 45 (69 %) non-Ventana platform laboratories using the mAb clone DAK-CDX2 (RTU or concentrate) produced a sufficient staining. The reason for this discrepancy is currently not known.

None of the laboratories using the mAb clones CDX2-88 and AMT28 produced optimal staining. In fact only 10 of 47 (21 %) laboratories using the mAb clones CDX2-88 and AMT28 produced a sufficient staining.

In concordance with previous observations, pancreas is a recommendable positive control for CDX2, provided that a distinct nuclear reaction is seen in the majority of the duct epithelial cells. Virtually all laboratories obtaining this reaction pattern in the pancreas were assessed as sufficient.

This was the third assessment of CDX2. The proportion of sufficient results was again quite low: 51% were sufficient in the current run compared to 46 % in run 27, 2009 and 64 % in run 22 2008 – see table 2. The low

pass rate is probably due to a more challenging tissue material circulated and many laboratories participating for the first time as well as the fact that one third of the laboratories were using the old and less robust mAb clones CDX2-88 and AMT28.

Table 2. **Proportion of sufficient results for CDX2 in the three NordiQC runs performed**

	Run 22 2008	Run 27 2009	Run 33 2011
Participants, n=	56	93	148
Sufficient results	64 %	46 %	51 %

Conclusion

The mAbs clone DAK-CDX2 and the rmAb clone EPR2764Y can both be used to obtain an optimal demonstration of CDX2. For both Abs efficient HIER and a sensitive detection system is mandatory to obtain an optimal staining. The performances of the mAb clone DAK-CDX2 seems to be influenced by the stainer platform, giving a significantly lower pass rate on the Ventana Benchmark platform compared to the general pass rate for the clone. Pancreas is an appropriate control for CDX2: A weak to moderate, distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas must be seen.

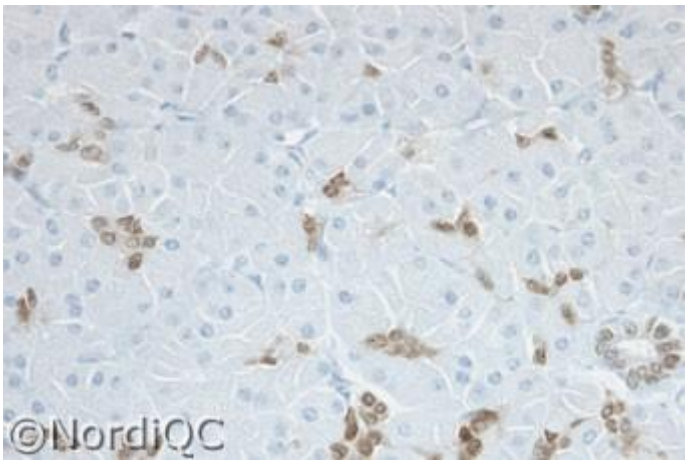

Fig. 1a
Normal pancreas (low expressor of CDX2) showing an optimal staining for CDX2 with the rmAb clone EPR2764Y as Ready-To-Use and performed at the Ventana BenchMark platform. A weak to moderate staining is seen in the majority of the ductal epithelial cells of the pancreas.

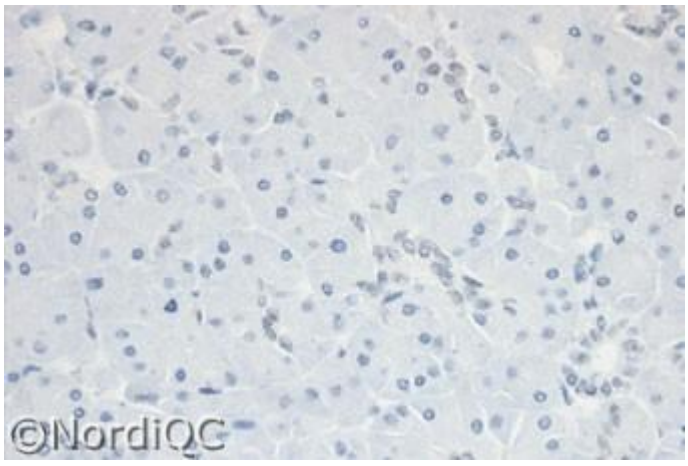

Fig. 1
Same pancreas as in Fig. 1a. Insufficient staining for CDX2 using the mAb clone CDX2-88 giving a too low sensitivity. Only a faint staining in very few ductal epithelial cells is seen. Also compare with Figs. 2b & 3b, same protocol.

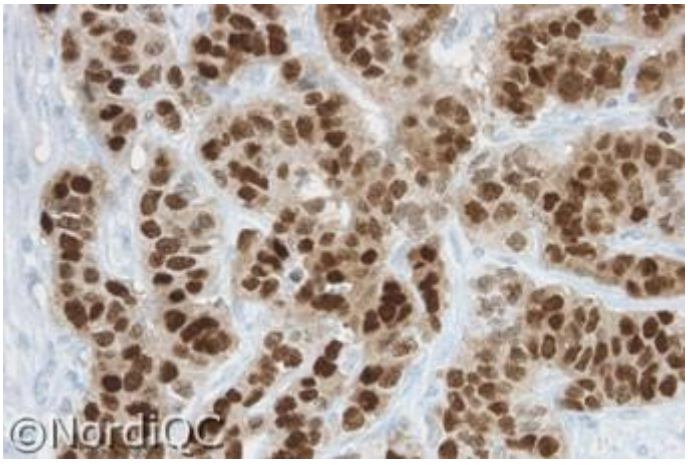

Fig. 2a
Colon carcinoma (high expressor of CDX2). Optimal staining for CDX2. Same protocol as in Fig. 1a. A strong nuclear staining is seen in virtually all tumour cells. The weak cytoplasmic staining accompanying the nurclear reaction is acceptable.

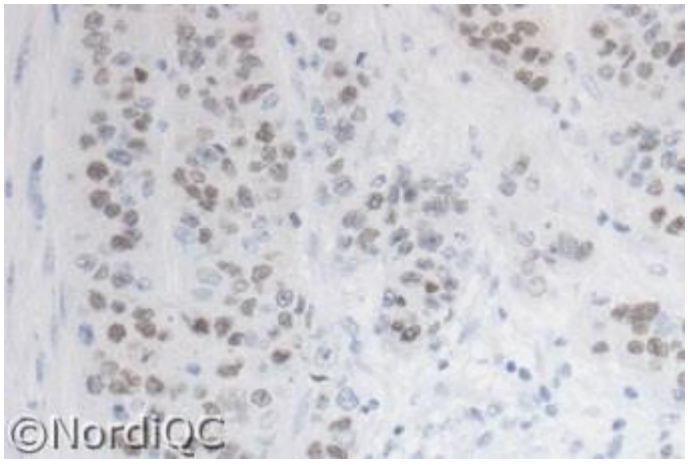

Fig. 2b
Same colon carcinoma as in Fig. 2a. Insufficient staining for CDX2. Same protocol as in Fig. 1b. The majority of the tumour cells are positive. Though the colon carcinoma is a high expressor of CDX2 the staining is rather weak and clearly not as strong compared to the optimal result in Fig. 2a.

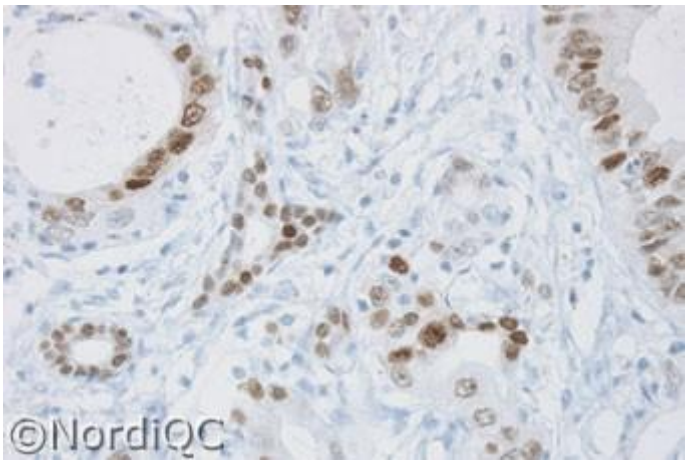

Fig. 3a
Pancreas adenocarcinoma (low expressor of CDX2). Optimal staining for CDX2. Same protocol as in Fig. 1a. A weak to moderate nuclear staining is seen in the majority of the tumour cells.

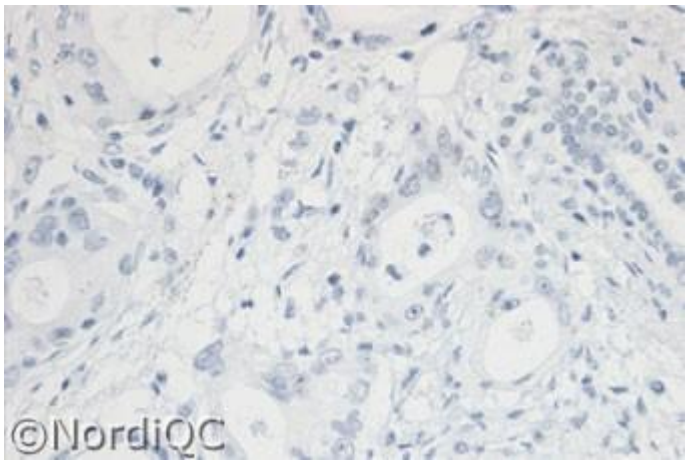

Fig. 3b
Same tumor as in Fig. 3a. Insufficient staining for CDX2. Same protocol as in Fig 1b. The tumour cells are negative.

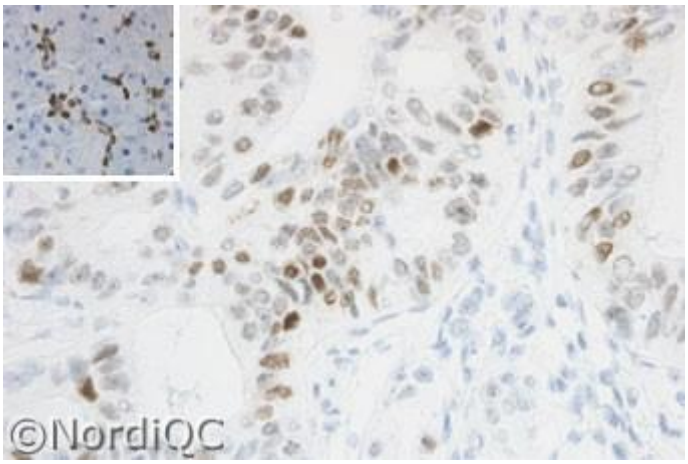

Fig. 4a
Pancreas adenocarcinoma and normal pancreas (insert) showing optimal staining for CDX2 with clone the mAb clone DAK-CDX2 in a Ready-To-Use format and performed at the Autostainer platform. A weak to moderate staining is seen in the majority of the ductal epithelial cells of the pancreas and in the majority of the tumour cells in the pancreas adenocarcinoma.

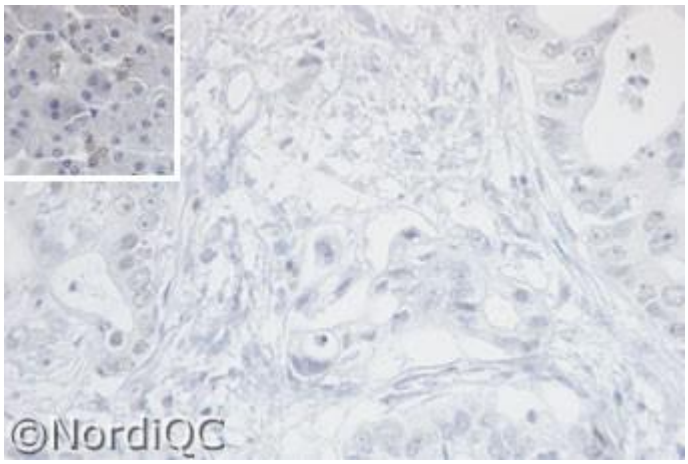

Fig. 4b
Pancreas adenocarcinoma and normal pancreas (insert) showing an insufficient staining for CDX2. Same Ready-To-Use product of the mAb clone DAK-CDX2 as in Fig. 4a was used, but performed at the Ventana Benchmark platform. Only a faint staining in very few ductal epithelial cells is seen and the tumour cells are negative. The mAb clone DAK-CDX2 was found to have an suboptimal performance on the Ventana Benchmark platform.

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