Assessment Run 22 2008
CDX2

The slide to be stained for CDX2 comprised:
1. Colon, 2. Breast ductal carcinoma, 3. Pancreas blood type A, 4. Pancreas blood type 0. 5-7. Colon adenocarcinomas.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an CDX2 staining as optimal included:

- A strong and distinct nuclear staining of virtually all enterocytes in the normal colon epithelium.
- A strong and distinct nuclear staining of virtually all the neoplastic cells in the three colon adenocarcinomas.
- A weak to moderat, distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas.

A weak cytoplasmic reaction in the enterocytes and the neoplastic cells of the colon adenocarcinomas was accepted. All other cells should be negative.

56 laboratories participated in the assessment. At the assessment 17 achieved optimal (30 %), 19 good (34 %), 14 borderline (25 %) and 6 (11 %) poor marks.

The following antibody clones were used:
mAb clone **CDX2-88** (BioGenex, n=31; BioCare, n=1; Linaris, n=1)
mAb clone **AMT28** (Novocastra, n=23)

In this assessment optimal staining could be obtained with both clone **CDX2-88** (14/33)* and clone **AMT28** (3/23). Sufficient stains (optimal or good) were seen in 25 cases (76%) with clone CDX2-88 and in 11 cases (44%) with clone AMT28.
* (number of optimal results/number of laboratories using this Ab).

CDX2-88: The protocols giving an optimal result were all but one based on heat induced epitope retrieval (HIER) in an alkaline buffer such as Tris-EDTA/EGTA pH 9.0 (11/15)**, Bond Epitope Retrieval Solution 2 (2/2) or EDTA pH 8 (1/1). Citrate buffer in combination with a pressure cooker as HIER device could also be used (1/4). The mAb was typically diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed.
** (number of optimal results/number of laboratories using this buffer).

AMT28: The protocols giving an optimal result were all based on HIER in Tris-EDTA/EGTA based buffer (2/14) or HIER in citrate buffer pH 6.0 (1/3). The mAb was typically diluted in the range of 1:25 – 1:50 depending on the total sensitivity of the protocol employed.

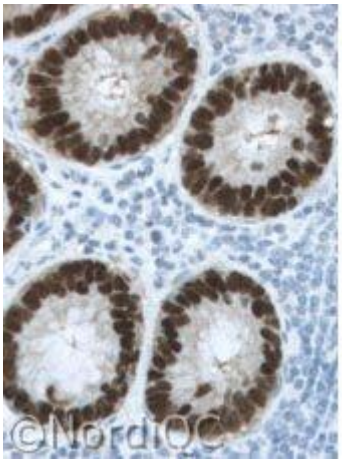
The most frequent causes of insufficient staining were:
- Insufficient HIER – too short HIER time and/or use of citrate pH 6.0
- Too low concentration of the primary Ab.

The prevalent feature of an insufficient staining with both clones was a negative staining of the duct epithelial cells in the pancreas (Fig. 4a and Fig. 4b) and a too weak or negative staining of tumour cells in one or more of the three colon adenocarcinomas (Fig. 3a and Fig. 3b). The assessment identified pancreas as a robust and easy interpretable stain quality indicator. It was observed that a weak to moderate but distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas (Fig. 4a) is a far better quality indicator than the very strong reaction of the normal enterocytes of colon (Fig. 1a). In several of the insufficient results the normal colon enterocytes were stained, while one of the three colon adenocarcinomas was false negative, which stresses that colon cannot be recommended as a positive control for CDX2.

In this assessment pancreas from two patients were included. One patient was blood type A and the other blood type 0. In a number of laboratories using clone CDX2-88 a "Mouse Ascites Golgi" (MAG) reaction was seen (15

out of 33). The reaction was only seen in the tissue from the patient with blood type A. The intensity of this unspecific staining varied from weak to strong (Figs. 4b and 5). In general the strongest MAG reaction was seen using the Ready To Use (RTU) format of clone CDX2-88. All the clone CDX2-88 antibodies were supplied as an ascites format. Clone AMT28 were all supernatant antibodies and no MAG reaction was seen with that clone.
In the assessment MAG reaction was noted, but had no impact on the marks given to the laboratories. However, it should be stressed that the contaminant antibodies in some of the CDX2-88 ascites mAbs may cause difficulties in interpreting stains in tumours from blood type A patients. As the unspecific MAG reaction is ascites related and not seen with mAbs produced as supernatants, the manufactures should be encouraged to avoid the ascites production of mAbs.

Conclusion
In this first CDX2 assessment the mAb clones **CDX2-88** and **AMT28** are both useful for the demonstration of the CDX2 protein. The clone CDX2-88 seems to be more sensitive and robust than clone AMT28. However it is a drawback that the clone CDX2-88 is an ascites format giving MAG reaction (Fig. 5). Both clones require efficient HIER and the concentration of the clones should be carefully calibrated. Pancreas is an appropriate control: A weak to moderate but distinct nuclear reaction in the majority of the duct epithelial cells in the pancreas should be seen.



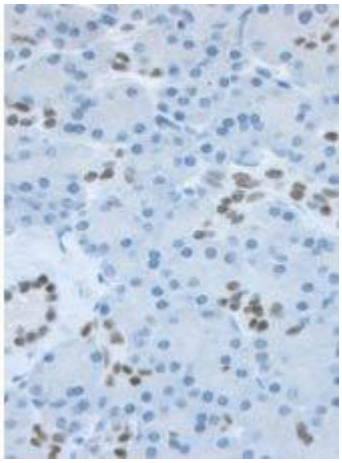
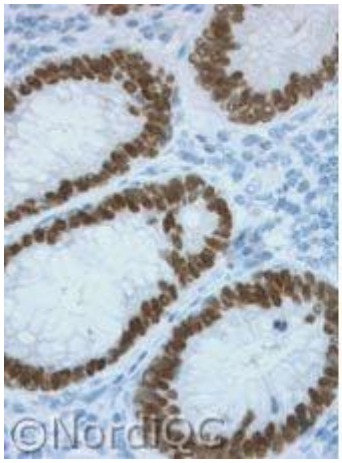


Fig. 1a
Optimal staining for CDX2 using the mAb clone CDX2-88.
Left, colon: A strong nuclear staining is seen in all the enterocytes with a minimal cytoplasmic reaction.
Right, pancreas: A weak to moderate staining is seen in the majority of the ductal epithelial cells.



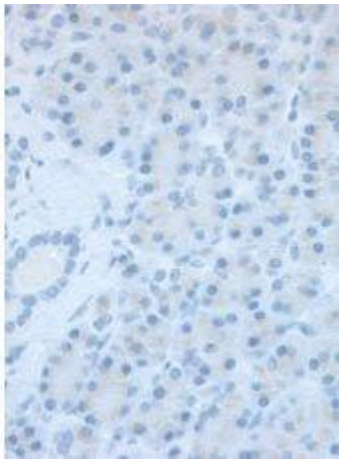
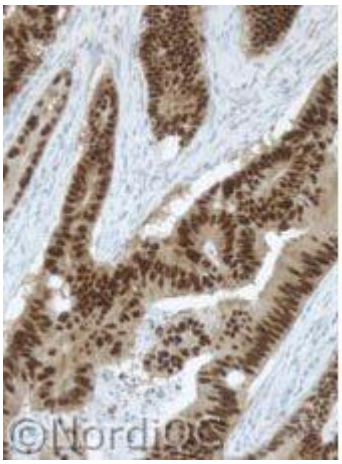


Fig. 1b
Staining for CDX2 using the mAb clone CDX2-88 with an insufficient protocol.
Left, colon: A moderate to strong nuclear staining is seen in all the enterocytes.
Right, pancreas: No nuclear staining is seen in the ductal epithelial cells. Also compare with Fig 2b – same protocol.



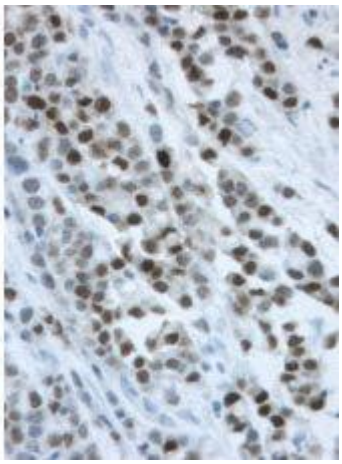
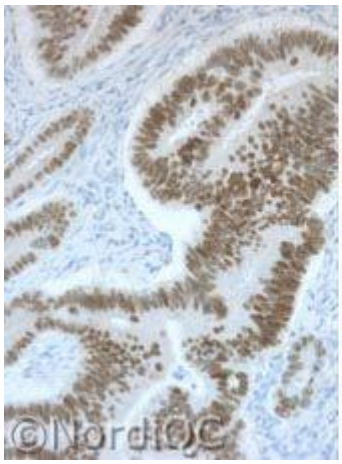


Fig. 2a
Optimal staining for CDX2 using same protocol as in Fig. 1a.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show an intense staining while the cytoplasmic compartment is weakly stained.
Right: Colon adenocarcinoma with low expression of CDX2: The majority of the neoplastic cells show a moderate to strong nuclear reaction.



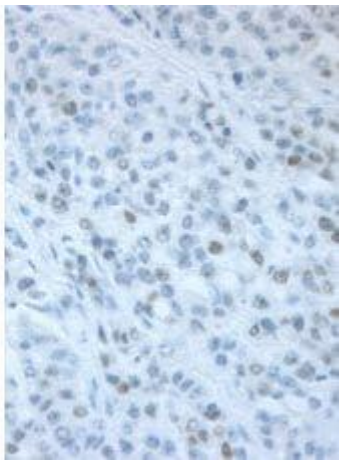


Fig. 2b
Insufficient staining for CDX-2 using same protocol as in Fig. 1b.
Left: Colon adenocarcinoma with high expression of CDX2: The nuclei of the neoplastic cells show a moderate staining, while the cytoplasmic compartment is almost negative.
Right: Colon adenocarcinoma with low expression of CDX2: Only scattered neoplastic cells show a weak nuclear reaction.

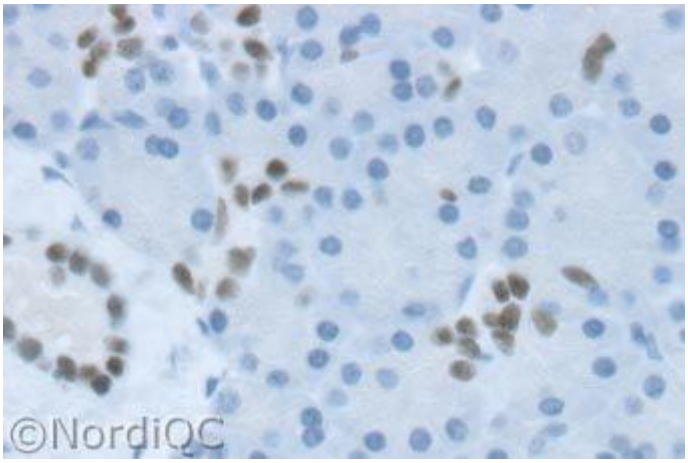


Fig. 3a
Optimal staining for CDX2 using the mAb clone CDX2-88 properly calibrated on the pancreas from the blood type 0 patient. The ductal epithelial cells show a moderate staining and the acinar cells are negative.

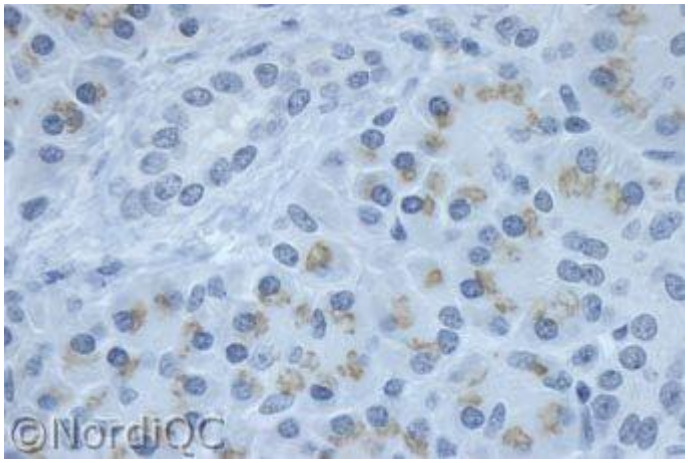


Fig. 3b
Insufficient staining for CDX2 on the pancreas from the blood type A patient. The RTU mAb clone CDX2-88 was used. No nuclear staining is seen in the ductal epithelial cells while a strong MAG reaction is seen in the majority of the acinic cells. Compare with Fig 3a.

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