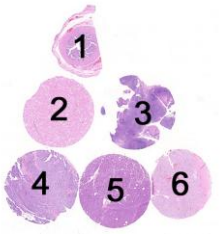
Assessment Run 34 2012

Paired box gene-2 protein (PAX2)

Material
The slide to be stained for PAX2 comprised:

1. Fallopian tube, 2. Kidney, 3. Tonsil, 4. Serous ovarian carcinoma, 5. Pancreas, 6. Renal clear cell carcinoma



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PAX2 staining as optimal included:

- An at least weak but distinct nuclear staining reaction of scattered ciliated epithelial cells and a strong nuclear staining reaction of the intercalated secretory epithelial cells in the Fallopian tube.
- An at least weak to moderate, distinct nuclear staining reaction of the epithelial cells lining the Bowman capsule and collecting ducts in the kidney. A faint cytoplasmic staining was accepted.
- A moderate to strong, nuclear staining of virtually all the mantle zone B-cells, the germinal centre B-cells and the interfollicular peripheral B-cells in the tonsils.
- A moderate to strong, nuclear staining of the majority of the neoplastic cells in the renal clear cell carcinoma.
- A negative staining of the neoplastic cells in the serous ovarian carcinoma.
- A negative staining reaction of all cells in the pancreas.

9 laboratories participated in this assessment. Out of the 9 laboratories, 1 participant (11%) achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PAX2, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **EP3251**	2	Epitomics Abcam	0	0	1	1	0%	-
pAb **71-6000**	4	Invitrogen/Zymed	0	1	2	1	25%	-
pAb **311A-14**	1	Cell Marque	0	0	1	0	-	-
Ready-To-Use Abs:								
pAb **311A-17**	1	Cell Marque	0	0	0	1	-	-
pAb **760-4393**	1	Ventana	0	0	1	0	-	-
Total	9		0	1	5	3	-	
Proportion			0 %	11 %	55 %	33%	11 %	

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

No laboratory were able to produce an optimal staining result and only 1 (11%) out of 9 protocols was assessed as sufficient.
This laboratory used the pAb 71-6000 from Invitrogen/Zymed (lot. no. 428076A), HIER in an alkaline buffer with Bond Epitope Retrieval Solution 2, Bond Refine (DS9800,Leica) as the detection system on the Bond-max platform.
Different staining results were obtained with the same pAb but different lot numbers 3 out of 3 (100%) protocols based on lots 792018A and 954637A were assessed as insufficient due to a false positive high background staining.
Although the number of participants was low, there was a generally tendency towards lack of specificity and sensitivity with all the Abs used in this run.

The most frequent causes of insufficient staining were:
- Poor signal-to-noise ratio
- False positive staining

- Too weak specific signal

In this assessment the prevalent feature of an insufficient staining was a poor signal-to-noise ratio, a false positive staining and/or a too weak or completely false negative reaction of the cells expected to be demonstrated. The majority of the participating laboratories were **not** able to demonstrate PAX2 in low antigen expressing structures such as the ciliated epithelial cells of the salpinx and in particular the epithelial cells of the collecting ducts and the Bowmann capsules without giving a high background staining.

The same multitissue block was used in the PAX2 and PAX8 assessments. Although the number of participants for both PAX2 and PAX8 was limited, the proportion of sufficient results was significantly higher for PAX8 than PAX2. This most likely was due to better PAX8 Abs as the applied protocols were based on the same settings in the two assessments.

Conclusion
Only 1 out of 9 participants produced a sufficient result using the pAb 71-6000 / lotnr. 428076A.
No Abs or protocols provided an optimal staining result for PAX2.
Due to this inappropriate performance of the PAX2 Abs, the laboratories should consider substituting PAX2 with PAX8.

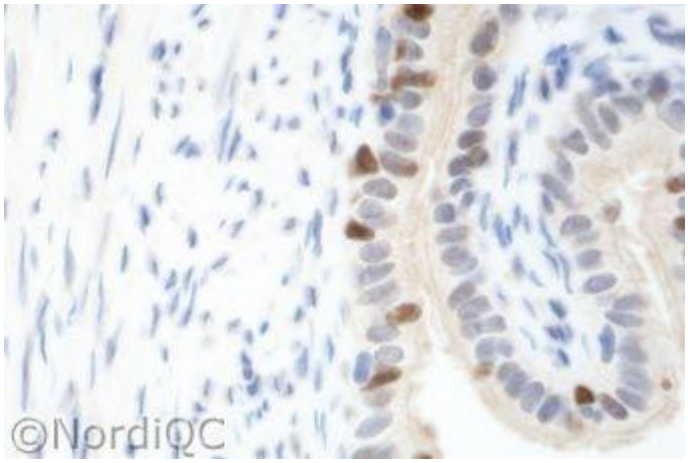


Fig. 1a
Sufficient staining, assessed as good, for PAX2 of the fallopian tube using the pAb 71-6000 / lot. no. 428076A, Invitrogen, HIER in BERS 2 and Bond Refine (DS9800) as the detection system. A weak distinct nuclear staining reaction is seen in dispersed ciliated epithelial cells, while the secretory epithelial cells are strongly labelled with minimal background staining.

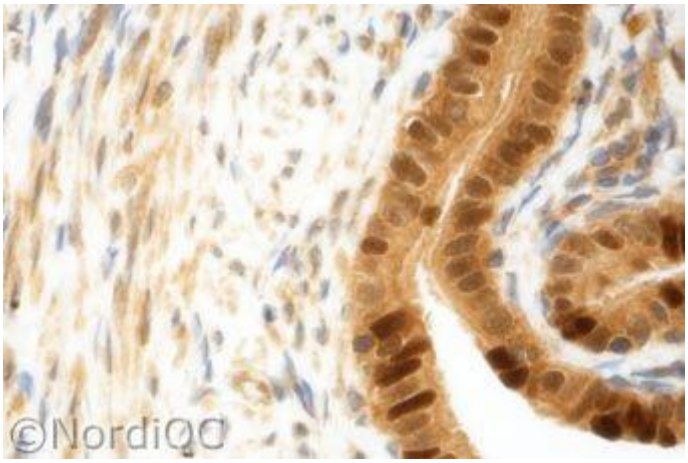


Fig. 1b
Insufficient staining for PAX2 of the salpinx using the pAb 71-6000 / lot. no. 954637A, Invitrogen and the same protocol settings as in Fig. 1a - same field as in Fig. 1a. The interpretation of the specific signal is hampered due to a heavy background and cytoplasmic staining reaction.

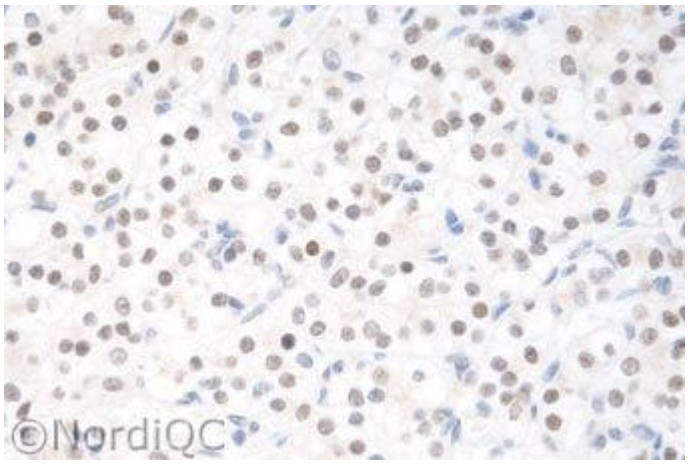


Fig. 2a
Sufficient staining for PAX2 of the renal clear cell carcinoma using same protocol as in Fig. 1a.
The majority of the neplastic cells show a moderate to strong nuclear staining reaction.

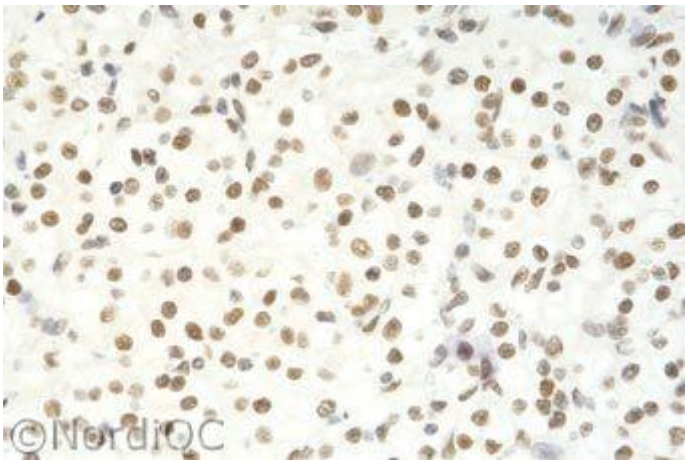


Fig. 2b
Staining for PAX2 of the renal clear cell carcinoma using an insufficient protocol based on the mAb clone EP3251 - same field as in Fig. 2a.
The majority of the neplastic cells show a strong nuclear staining reaction. However, also compare the staining reaction

with Figs. 3a and 3b.

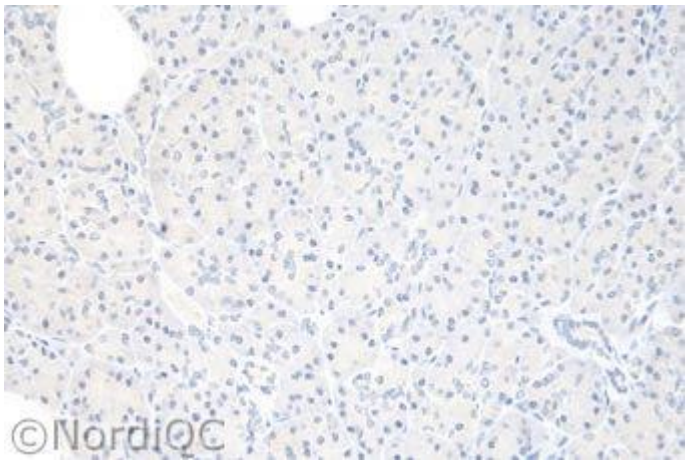


Fig. 3a
Sufficient staining for PAX2 of the pancreas using same protocol as in Figs. 1a & 2a.
No nuclear staining reaction is seen in the normal pancreatic cells.

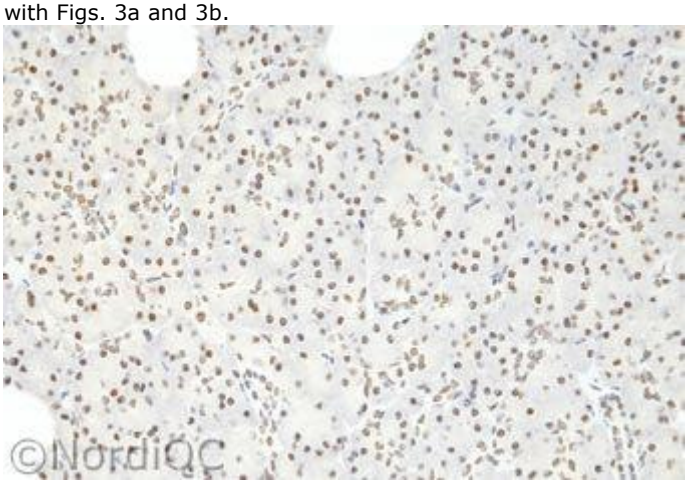


Fig. 3b
Insufficient staining for PAX2 of the pancreas using the same protocol as in Fig. 2b. Virtually all the normal pancreatic cells show a strong false positive nuclear staining reaction.

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