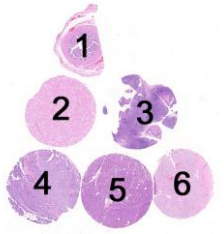
Assessment Run 34 2012

Paired box gene-8 protein (PAX8)

Material
The slide to be stained for PAX8 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 PAX8 staining as optimal included:

- An at least weak to moderate, distinct nuclear staining of the majority of the ciliated epithelial cells and a strong nuclear staining of the intercalated secretory epithelial cells in the fallopian tube.
- An at least weak to moderate, distinct nuclear staining of the epithelial cells lining the Bowman capsule and of the distal/collecting tubular cells in the kidney. A faint cytoplasmic staining was accepted.
- A strong, nuclear staining of virtually all neoplastic cells of the serous ovarian carcinoma.
- A moderate to strong, nuclear staining of the majority of the neoplastic cells in the renal clear cell carcinoma.

For all Abs used except the mAb clone BC12, Biocare, a 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 and a weak to moderate, distinct nuclear staining of the neuro-endocrine cells in the pancreas was expected and accepted.

35 laboratories participated in this assessment. Out of the 35 laboratories, 63 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for PAX8, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MRQ-50**	5	Cell Marque	3	1	0	1	80%	100%
mAb clone **BC12**	1	Biocare	1	0	0	0	-	-
mAb clone **PAX8R1**	1	Abcam	0	0	1	0	-	-
pAb **10336-1-AP**	7 1	ProteinTech group Bio-Connect	2	4	2	0	75 %	100%
pAb **A363**	5 1	Cell Marque Medac	0	5	1	0	83%	-
pAb **CP 379**	2	Biocare	2	0	0	0	-	-
pAb **MAD-000505QD**	1	Master Diagnostica	0	0	1	0	-	-
pAb **RBK047**	1	Zytomed	0	1	0	0	-	-
Ready-To-Use Abs:								
mAb clone **MRQ-50 760-4618**	2	Ventana	0	1	1	0	-	-
mAb clone **BC12 API 438**	1	Biocare	1	0	0	0	-	-
pAb **A363-17/18**	5	Cell Marque	0	1	3	1	-	-
pAb **PP 379**	1	Biocare	0	0	1	0	-	-
pAb **ZA0470**	1	Zhongshan	0	0	0	1	-	-
Total	35		9	13	10	3	-	
Proportion			26 %	37 %	29 %	8%	63 %	

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 **MRQ-50**: The protocols giving an optimal result were based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 6.1 (TRS Low pH, Dako) (1/1)* or TRS pH 9 (3-in-1) (TRS High pH, Dako) (2/3)* as the retrieval buffer. The mAb was typically diluted in the range of 1:100-1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **BC12**: The protocol giving an optimal result were based on HIER using TRS High pH (Dako) (1/1) as the retrieval buffer. The mAb was diluted 1:150 and EnVision FLEX+ (K8002) used as the detection system.

pAb **10336-1-AP**: The protocols giving an optimal result were based on HIER using either TRS pH 6.1 (TRS Low pH, Dako) (1/1) or Cell Conditioning 2 (CC2; BenchMark, Ventana) (1/1) as the retrieval buffer. The pAb was typically diluted in the range of 1:20-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining (optimal or good).

pAb clone **CP379**: The protocols giving an optimal result were based on HIER using either Tris-EDTA/EGTA pH 9 (1/1) or Cell Conditioning 1 (CC1; BenchMark, Ventana) (1/1) as the retrieval buffer. The pAb was diluted 1:25 and EnVision FLEX+ (K8002, Dako) or OptiView (760-700, Ventana) with OptiView amplification (760-099, Ventana) as the detection system.

Ready-To-Use Abs
mAb clone **BC12** (product.no. **API 438**, Biocare): The protocol giving an optimal result was based on HIER using Reveal Decloaker (Biocare) in a Pressure Cooker, an incubation time of 30 min in the primary Ab and MACH 4 (Universal HRP-polymer; M4U534, Biocare) as the detection system. The staining was performed on the intelliPATH FLX platform from Biocare.

The most frequent causes of insufficient staining reaction were:

- Less successful performance of the Ready-To-Use Abs
- Use of low sensitive detection systems

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative reaction of the cells expected to be demonstrated. The majority of the participating laboratories were able to demonstrate PAX8 in the secretory epithelia cells of the fallopian tube and in the neoplastic cells of the serous ovarian carcinoma, whereas the demonstration of PAX8 in the neoplastic cells of the renal clear cell carcinoma, the epithelial cells of collecting ducts and the parietal cells lining the Bowman capsules of the kidney and in particular the ciliated epithelial cells of the fallopian tube was more challenging and only seen with appropriate protocol settings.
All participants used HIER, and a sufficient result could be obtained with both high and low pH buffers. Using HIER in a buffer with high pH (pH 8-9) 13 out 25 (52%) protocols were assessed as sufficient out of which 5 (20%) were assessed as optimal, whereas 9 out 10 (90%) protocols based on HIER in a low pH buffer were assessed as sufficient out of which 4 (40%) were optimal.
In spite of the low number of optimal results, an optimal staining could be produced with all antibodies applied in this assessment except for the mAb clone PAX8R1.
For the overall use of PAX8 antibodies (mAbs and pAbs) the proportion of sufficient results was highly influenced by the choice of the detection system used. If a 2-step polymer/multimer (e.g. EnVision Flex/UltraView) based system was applied, 6 out of 13 obtained a sufficient staining result (46%) out of which none (0%) produced an optimal staining. If a more sensitive 3-step polymer/multimer (e.g. EnVision Flex+/Ultraview + amplification) based system was applied, 16 out of 22 obtained a sufficient staining result (73%) of which 9 (41%) were assessed as optimal.
A significant difference in the overall performance of concentrated versus Ready-To-Use (RTU) formats was observed. With a concentrated format, 19 out of 25 (76 %) protocols were assessed as sufficient, out of which 8 (32%) were optimal With an RTU format 3 out of 10 (30%) protocols were assessed as sufficient out of which 1 (10%) was optimal.

The general conception that B-lymphocytes express PAX8 has been disputed by Moretti et al. (1). Antibodies raised against the N-terminal region of PAX8 may cross-react with PAX5 due to high sequence homology in that region. In contrast to all the other antibodies used in this run, the mAb BC12 didn't react with B-lymphocytes. As this preliminary result needs further investigations, both reaction patterns were

accepted in this assessment (i.e., positive and negative nuclear staining of the B-cells). We observed the same reaction pattern in the pancreatic neuroendocrine cells, which were negative with mAb clone BC12 while positive with all other Abs.

The most successful and robust assays for PAX8 in this assessment was obtained with the mAb clone MRQ-50 from Cell Marque followed by the mAb clone BC12, pAb CP 379 (Biocare) and pAb 10336-1-AP (ProteinTech group). Although the data are limited due to the low number of participants in this assessment, optimal results could only be obtained with a high sensitive detection system e.g. Flex+ (K8002,Dako), Optiview + Amplification (760-700 + 760-099,Ventana) or MACH-4 (M4U534,Biocare) for these Abs. It was not possible to evaluate if the performance of the applied Abs was depending on the IHC platform used.

The Fallopian tube was found to be an applicable critical stain quality indicator for PAX-8: An at least weak to moderate, distinct nuclear staining must be seen in the majority of the ciliated epithelial cells, and a strong nuclear staining in the secretory cells (a weak cytoplasmic staining is accepted).
Alternatively kidney can be used as a positive control: The majority of the epithelial cells of the collecting ducts must show an at least weak to moderate distinct staining reaction.

Conclusion
The mAb clones **MRQ-50** and **BC12**, and the pAbs **10336-1-AP** and **CP 379** are all recommendable Abs for PAX8. These Abs in combination with efficient HIER andthe application of a sensitive and specific IHC system is mandatory for optimal performance. In this assessment the Ready-To-Use Abs / systems gave an inferior performance compared to the use of the Abs as a concentrate.
Normal Fallopian tube is an appropriate control provided that the majority of the ciliated epithelial cells show an at least weak to moderate, distinct nuclear staining reaction.

1: Moretti L, Medeiros LJ, Kunkalla K, Williams MD, Singh RR, Vega F. N-terminal PAX8 polyclonal antibody shows cross-reactivity with N-terminal region of PAX5 and is responsible for reports of PAX8 positivity in malignant lymphomas. Mod Pathol. 2012 Feb;25(2):231-6. doi: 10.1038/modpathol.2011.162. Epub 2011 Oct 28. PubMed PMID: 22037256.

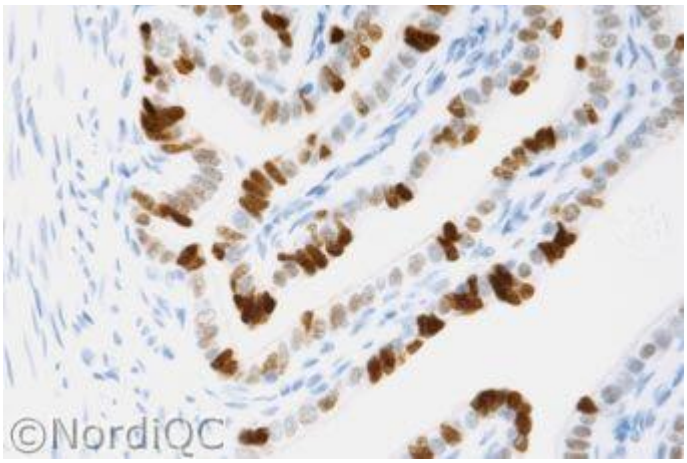


Fig. 1a
Optimal staining for PAX8 of the fallopian tube using the mAb clone MRQ-50 as a concentrate, HIER in TRS pH 6.1 and a 3-step polymer based detection system. Virtually all the ciliated epithelial cells show a distinct, weak to moderate nuclear staining reaction, while the secretory epithelial cells are strongly labelled.

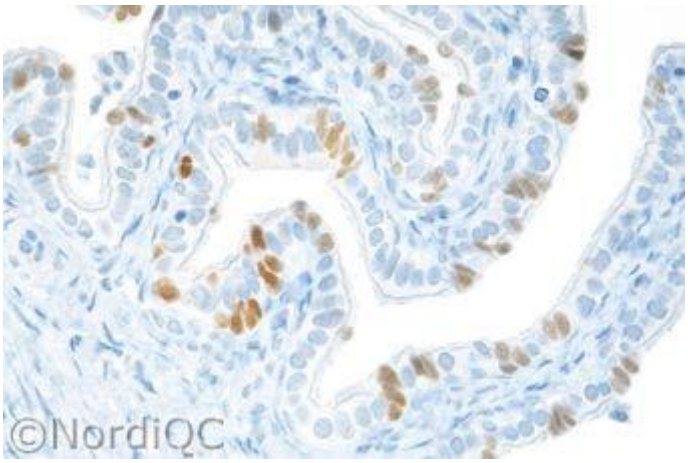


Fig. 1b
Insufficient staining for PAX8 of the fallopian tube using the mAb clone MRQ-50 as a concentrate with a protocol giving a too low sensitivity (a too low conc. of the primary Ab and a 2-step multimer based detection system) - same field as in Fig. 1a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 1a.
Also compare with Fig. 2b, same protocol.

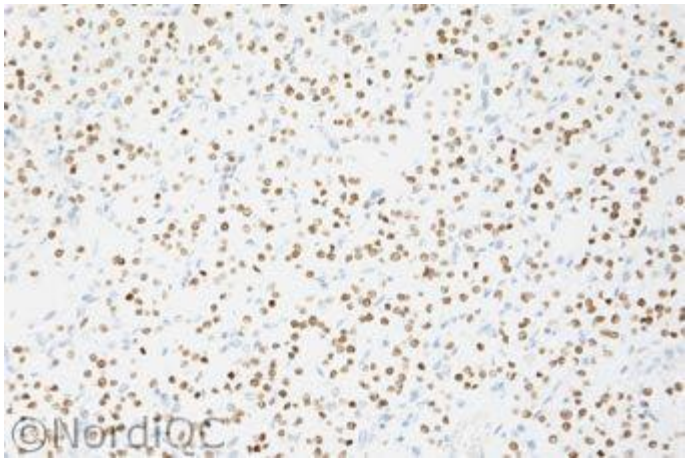


Fig. 2a
Optimal staining for PAX8 of the renal clear cell carcinoma using same protocol as in Fig. 1a.
The majority of the neplastic cells show a moderate to strong nuclear staining reaction.

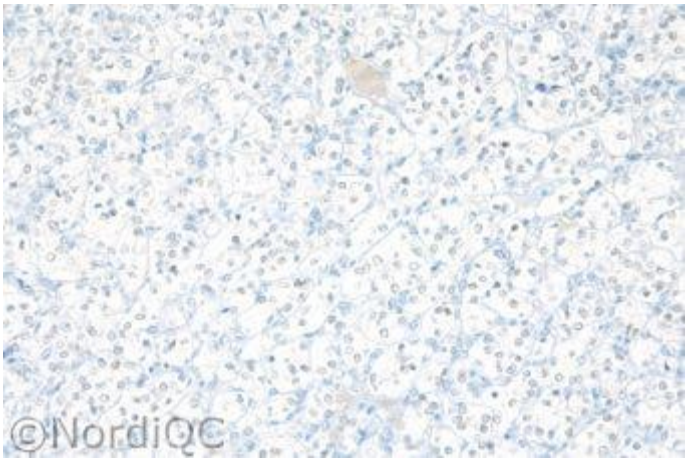


Fig. 2b
Insufficient staining for PAX8 of the renal clear cell carcinoma using same protocol as in Fig. 1b - same field as in Fig. 2a.
Only scattered neoplastic cells show a weak and equivocal staining reaction.

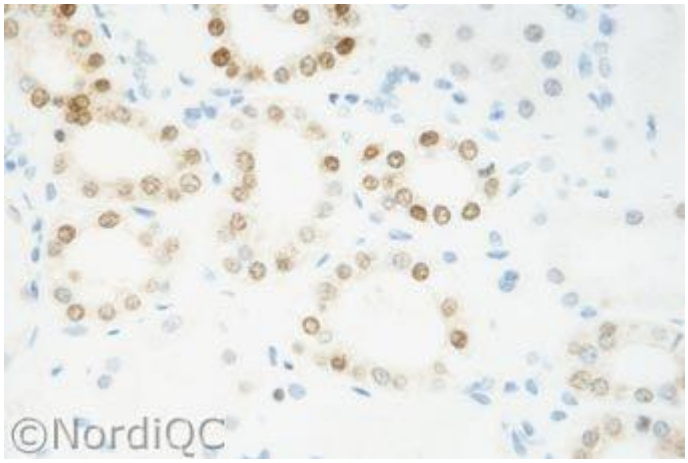


Fig. 3a
Optimal staining for PAX8 of the kidney using same protocol as in Figs. 1a and 2a. The epithelial cells of the collecting tubuli show a distinct, weak to moderate nuclear staining reaction.
Only a faint cytoplasmic staining is observed.

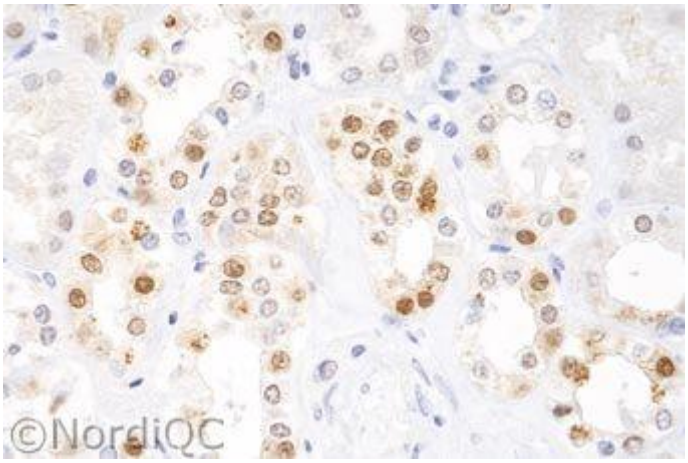


Fig. 3b
Optimal staining for PAX8 of the kidney using the mAb clone BC12 - same field as in Fig. 3a.
The proportion of positive cells and the intensity of the staining reaction are similar to the result obtained in Fig. 3a.
However also compare with Fig. 4b, same protocol.

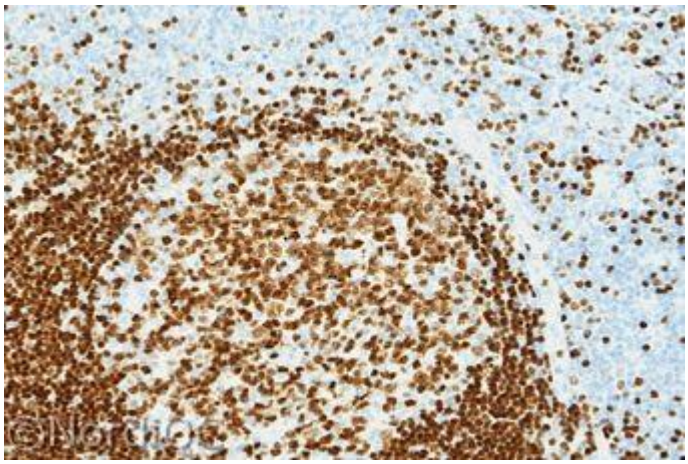


Fig. 4a
Staining for PAX8 of the tonsil using same protocol as in Figs. 1a - 3a. Virtually all the B-cells show a strong nuclear staining reaction.
Also compare with Figs. 4b.

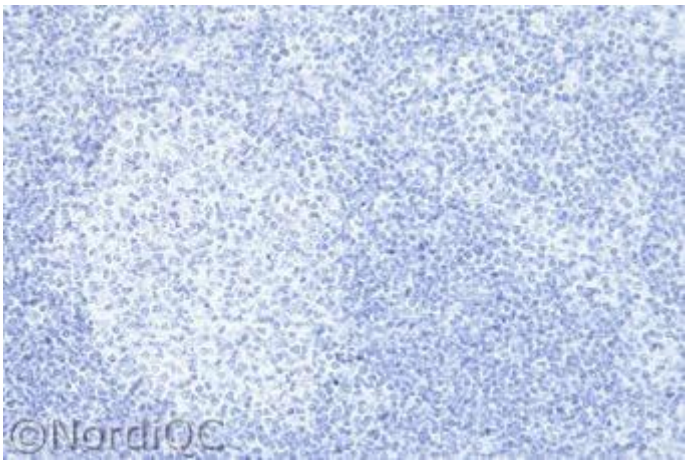


Fig. 4b
Staining for PAX8 of the tonsil using same protocol as in Fig. 3b. No staining of the B-cells is seen.
Also compare with Figs. 4a.

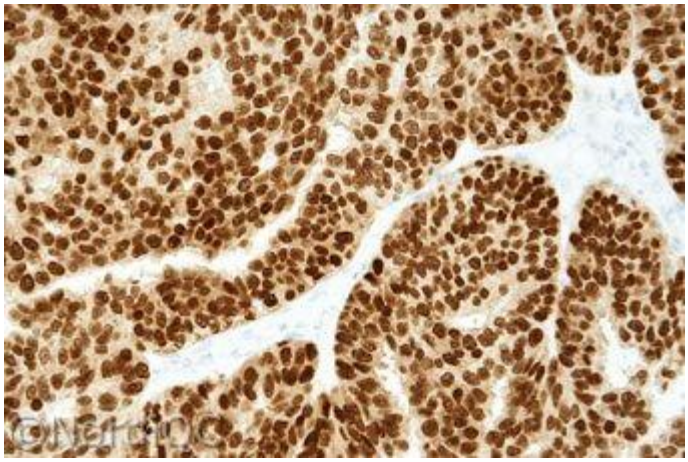


Fig. 5a
Optimally calibrated PAX8 (pAb 10336-1-AP) staining of the serous ovarian carcinoma using UltraView with amplification (a 3-step multimer based detection system). Virtually all the neoplastic cells show a strong, distinct nuclear staining.
Also compare with Figs. 5b.

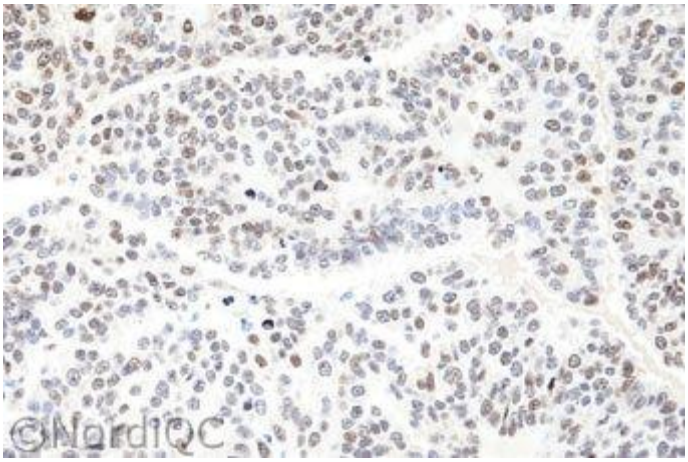


Fig. 5
Insufficient PAX8 staining of the serous ovarian carcinoma using same protocol settings as in Fig. 5a except for using UltraView without amplification (a 2-step multimer based detection system). The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result obtained in Fig. 5a.

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