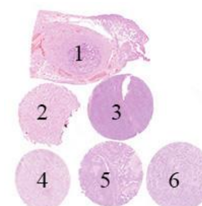
NordiQC

Assessment Run 60 2020

PAX8

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for PAX8 used to identify the origin of renal cell and ovarian carcinoma in the diagnostic work-up of cancer of unknown primary (CUP) origin. Relevant clinical tissues, both normal and neoplastic, were selected for a broad spectrum of antigen densities for PAX8 (see below).

Material
The slide to be stained for PAX8 comprised:



1. Fallopian tube, 2. Kidney, 3. Tonsil, 4. Renal clear cell carcinoma,
5. Ovarian serous adenocarcinoma. 6. Colon adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PAX8 staining as optimal included:

- A weak to moderate, distinct nuclear staining reaction of the majority of ciliated epithelial cells and a strong nuclear staining of intercalated secretory epithelial cells in the Fallopian tube.
- An at least weak to moderate, distinct nuclear staining reaction in the majority of epithelial cells of the proximal, distal/collecting renal tubules, loops of Henle and the parietal epithelial cells of Bowman's capsule in the kidney.
- A strong, nuclear staining reaction of virtually all neoplastic cells in the ovarian serous adenocarcinoma.
- A moderate to strong, nuclear staining reaction of the majority of neoplastic cells in the renal clear cell carcinoma.
- No nuclear staining reaction of B-cells. This was expected for antibodies raised against the C-terminal part of PAX8 - e.g. mAb clone BC12 and rmAbs clones EP298, SP348, EP331 and ZR-1.
- No staining reaction of neoplastic cells in the colon adenocarcinoma.

In cells with strong specific nuclear staining reaction, weak cytoplasmic staining was accepted.

In this assessment cross-reaction with other PAX epitopes e.g. to PAX5 in B-cells and/or PAX6 in neuroendocrine cells was downgraded, due to interpretational challenges especially in the diagnostic work-up of CUP. This applied for polyclonal Abs and mAb clones MRQ-50, PAX8R1 and DBM15.48. For these Abs the highest score consequently was "Good" providing an otherwise staining pattern as described above was obtained.

Participation

Number of laboratories registered for PAX8, run 60	317
Number of laboratories returning slides	278 (88%)

The number of laboratories returning slides has decreased in this run 60 compared to previous assessments, due to the Covid-19 pandemic and associated postal delays. All slides returned after the assessment were assessed, and received advice if the result being insufficient, but data is not included in this report.

During the assessment a limited number of participants have experienced issues with the circulated NordiQC slides, providing a partial or entire aberrant/false negative staining result in some cases. During assessment, this observation was taken into account and for PAX8, 6 slides were potentially affected. All six were excluded. 1 slide was returned from run 56 and was not assessed. If performance was characterized by uneven staining or a completely false negative result that could be related to the quality of the slide and not the protocol submitted, this was commented in the individual assessment feed-back.

Results
271 laboratories participated in this assessment. 105 laboratories (38%) achieved a sufficient mark (optimal or good). Abs used and assessment marks are summarized in Table 1 (see page 3)

The most frequent causes of insufficient staining were:
- Use of less successful antibodies primarily mAb clone MRQ-50.
- Too short efficient Heat Induced Epitope Retrieval (HIER) time.
- Too low concentration of the primary Ab.

Performance history
This was the fifth NordiQC assessment of PAX8. The pass rate was almost identical to the previous run 56 in 2019. The proportion of participants using the mAb clone MRQ-50 was relatively high (56% of all participants) and similar to run 56 this clone was found to be less successful especially when applied on the Ventana BenchMark and Dako Omnis platforms. Furthermore all results with a cross reaction to other PAX epitopes was downgraded due to interpretational challenges and in total only 10% of the results were scored optimal.

Table 2. **Proportion of sufficient results for PAX8 in the fifth NordiQC runs performed**

	Run 34 2012	Run 42 2014	Run 51 2017	Run 56 2019	Run 60 2020
Participants, n=	35	125	213	264	271
Sufficient results	63 %	70 %	56 %	37%	39%

Conclusion
Optimal staining results could be obtained with the rmAb clones **EP298**, **SP348, ZR-1** and **BP6157**. Irrespective of the clone applied, efficient HIER, use of a sensitive 3-step polymer/multimer based detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result.
The rmAbs clones **EP298**, **SP348** and **ZR-1** gave encouraging results and a high proportion of sufficient results on the main fully automated platforms and no cross-reaction with e.g. PAX5 was observed. In contrast, the mAb clone **MRQ-50** provided a poor performance especially on the Ventana BenchMark and Dako Omnis platforms and at the same time also labelled PAX5 in B-cells.
Kidney and Fallopian tube are both recommendable as positive tissue controls for PAX8. In kidney, an at least weak to moderate, distinct nuclear staining reaction in the majority of the epithelial cells of the proximal, distal/collecting renal tubules and parietal epithelial cells of Bowman’s capsule must be seen. In Fallopian tube, an at least weak to moderate, distinct nuclear staining reaction of the majority of ciliated epithelial cells and a strong nuclear staining of intercalated secretory epithelial cells must be seen. Tonsil can be used as negative tissue control for PAX8, as no staining should be seen in e.g. squamous epithelial cells and lymphocytes (positive nuclear staining in B-cells indicate cross reaction with PAX5).

Table 1. **Antibodies and assessment marks for PAX8, run 60**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC12***	3 2	Biocare Zytomed Systems	-	3	2	-	60%	-
mAb clone **DBM15.48**	1	Diagnostic Biosystems	-	-	-	1	-	-
mAb clone **MRQ-50**	38	Cell Marque	-	20	13	5	53%	-
mAb clone **PAX8R1**	2	Abcam	-	-	2	-	-	-
rmAb clone **EP298***	14 9 1 3 1	Cell Marque Epitomics[5] BIO SB Nordic Biosite Protein Tech	8	11	7	2	66%	28%
rmAb clone **EP331***	2	Cell Marque	-	1	1	-	-	-
rmAb clone **SP348***	25 1 1	Abcam Gennova Spring Biosciences	18	4	5	-	82%	64%
rmAb clone **ZR-1***	3 1	Zeta Corporation Abcam	1	-	3	-	-	-
rmAb clone **BP6157**	1	Bailing Biotechnology	1	-	-	-	-	-
pAb, **10336-1-AP**	22	Proteintech	-	11	7	4	50%	-
pAb, **363A-15**	3	Cell Marque	-	1	1	1	-	-
pAb, **CP379**	6	Biocare	-	1	2	3	17%	-
pAb, **RBK047**	1 1	Diagomics Zytomed Systems	-	-	2	-	-	-
pAb, **AIB-30190**	1	Nordic Biosite	-	1	-	-	-	-
Unknown	5		-	1	3	1	-	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **MRQ-50, MAD-000550QD**	2	Master Diagnostica	-	2	-	-	-	-
mAb clone, **BC12***	2	Biocare Medical	-	1	1	-	-	-
rmAb clone **SP348*, M6481**	1	Spring Biosciences	-	1	-	-	-	-
rmAb clone, **EP331***	1	Path n Situ	-	-	1	-	-	-
pAb, **363A-17**	2	Cell Marque	-	1	1	-	-	-
pAb, **RBG047**	1	Zytomed Systems	-	1	-	-	-	-
mAb clone **MRQ-50 363M-XX**	25	Cell Marque	-	6	14	5	24%	-
rmAb clone, **EP298* (VRPS)[3]**	1	Sakura Finetek	-	-	1	-	-	-
rmAb clone, **EP331* (VRPS)[3] 760-6077**	1	Ventana/Cell Marque	-	-	1	-	-	-
mAb clone **MRQ-50, 760-4618 (VRPS)[3]**	4	Ventana Roche	-	-	2	2	-	-
mAb clone **MRQ-50, 760-4618 (LMPS)[4]**	80	Ventana Roche	-	8	62	10	10%	-
Unknown	2		1	1	0	0	-	-
Total	271		29	76	132	34	-	
Proportion			11%	28%	49%	12%	39%	

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Ab terminated by vendor.
*Clones that do not show cross reactivity with PAX5.

Detailed analysis of PAX8, Run 60
The following protocol parameters were central to optimal staining:

Concentrated Antibodies
rmAb clone **EP298**: Protocols with optimal results were all based on HIER using either Target Retrieval Solution (TRS) High pH (6/13) (Dako), TRS High pH (3-in-1) (Dako) (1/1) or Cell Conditioning 1 (CC1, Ventana) (1/13). The rmAb was diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 17 of 26 (65%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **SP348**: Protocols with optimal results were based on HIER using TRS High pH (12/16) (Dako), TRS High pH (3-in-1) (Dako) (2/3) or CC1 (Ventana) (3/8) and one HIER in CC1 (Ventana) in combination with Protease 3 (Ventana) for 4 min. The rmAb was diluted 1:100-1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 19 of 23 (83%) laboratories produced a sufficient staining result.

rmAb clone **ZR-1**: One lab obtained an optimal result using the rmAb ZR-1 clone. The protocol was based on HIER using CC1 (Ventana). The rmAb was diluted 1:50 and incubated for 24 min. and visualized with a 3-step multimer based detection system (OptiView, Ventana).

rmAb clone **BP6157:** One lab obtained an optimal result using the rmAb BP6157 clone. The protocol was based on HIER using TRS high (3-in-1) (Dako). The rmAb was diluted 1:800 and incubated for 30 min. and visualized with a 3-step polymer based detection system (EnVision Flex, Dako).

Table 3. **Proportion of optimal results for PAX8 for the used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako Autostainer**		**Dako Omnis**		**Ventana 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
rmAb **EP298**	1/1	-	6/13 (46%)	-	1/13 (8%)	-	0/1	-
rmAb **SP348**	2/3	-	12/15 75%	-	4/8 (50%)	-	-	-
rmAB **ZR-1**	-	-	-	-	1/3	-	-	-
rmAb **BP6157**	1/1	-	-	-	-	-	-	-

* 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
126 laboratories used an RTU format for PAX8 in this assessment, and none of the RTU formats and corresponding systems scored an optimal result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥5 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly 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 4. **Proportion of sufficient and optimal results for PAX8 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb PAX8 **760-4618**	(0/4)	(0/4)	10% (8/80)	0% (0/80)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment and in concordance with the previous NordiQC PAX8 assessments, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 85% of the insufficient results (141 of 166 laboratories). The remaining 15% of insufficient results were characterized by a poor signal-to-noise ratio and false positive staining reaction compromising interpretation.
As observed in run 56, the majority of the participating laboratories were able to demonstrate PAX8 in high-level antigen expressing cell, such as secretory epithelia cells of the Fallopian tube and neoplastic cells of the ovarian serous adenocarcinoma, whereas demonstration of PAX8 in low-level antigen expressing cells as the neoplastic cells of the renal clear cell carcinoma, epithelial cells of collecting ducts, parietal cells lining the Bowman capsules of the kidney and in particular ciliated epithelial cells of the Fallopian tube and epithelial cells of the proximal tubules in the kidney were more challenging and only seen with appropriate protocol settings (see Fig. 1a to Fig. 5b). Cases of insufficient staining due to false positive cytoplasmic and/or aberrant nuclear reaction of cells not expressing PAX8 were also seen. This pattern was typically caused by use of a less successful primary antibody giving cross-reaction with e.g. PAX5.
Cross-reactivity to PAX5 resulting in a distinct nuclear staining reaction of B-cells for antibodies raised against the N-terminal part of PAX8 was seen in 70% (194/278) of the returned slides (see Fig. 5a and 5b). This reaction applied for all polyclonal Abs and clones MRQ-50, DBM15.48 and PAX8R1. Within the last couple of years well-performing rmAbs without cross reactivity has been introduced to the market (see Table 1). Based on this, cross-reactivity with PAX5 was downgraded due to the risk of misinterpretation in the diagnostic work-up of CUP. The diagnostic challenges and different reaction profiles related to the choice of PAX8 Ab has e.g. been described by Kamaljeet Singh et al.; *AIMM 2020, Aug;28(7):558-561; Comparison of PAX8 Expression in Breast Carcinoma Using MRQ-50 and BC12 Monoclonal Antibodies* and Tacha D et al., *AIMM 2013, Jan;21(1):59-63; PAX8 mouse monoclonal antibody [BC12] recognizes a restricted epitope and is highly sensitive in renal cell and ovarian cancers but does not cross-react with b cells and tumors of pancreatic origin*.

54% (147 of 271) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for PAX8. The mAb clone MRQ-50, rmAb clones EP298, SP348 and the pAb 10336-1-AP were the most widely used antibodies (see Table 1). Out of these four Abs only the rmAbs clones EP298 and SP348 provided optimal staining results with a pass rate of 66% and 82% respectively (see Table 1).

Data focusing on the four main IHC systems (see Table 3) showed that the clones EP298 and SP348 could be used to obtain an optimal result on three of the four main systems. The clone EP298 was during the planning and execution of this assessment however announced to be terminated from production from Epitomics due to instabilities (communication from distributor to NordiQC) and might no longer be purchased

The SP348 clone was the most successful concentrate with a pass-rate of 82%, 64% optimal. The concentrate was used in a range of 1:100-1:1.000 and typically with HIER in an alkaline buffer as single pre-treatment. No slide stained on the Leica Bond was submitted with this clone.
4 laboratories used a proteolytic pre-treatment either as a single retrieval method or in combination with HIER for SP348. All 4 laboratories applied the Ab on the Ventana BenchMark platform. The results based on HIER in combination with proteolysis were mixed as 1 was optimal, 1 good and 1 borderline. All 3 laboratories applied similar protocols and the only factor separating the optimal protocol from the others were the use of Renoir red as antibody diluent instead of Ventanas own diluent.
The use of proteolytic pretreatment in combination with HIER on the Ventana BenchMark platform was not mandatory to obtain a sufficient result for the SP348 clone. It was seen that 3 laboratories using this clone on the Ventana BenchMark received optimal results using a higher concentration of the antibody and a prolonged HIER. In addition, all 3 used Ventanas own diluent and not Renoir Red as diluent. The Renoir Red diluent induces a higher affinity for certain Abs and was found more successful for the combined pre-treatment. In this context it has also been emphasized that the morphology often is compromised using

enzymatic pretreatment. The protocol using proteolysis as single retrieval method gave a result evaluated as insufficient.

19 of the laboratories used concentrated antibodies on the Leica Bond instrument with a pass rate of 63% (12/19) none with optimal results.

The most widely used concentrate mAb clone MRQ-50 was observed to be inferior to e.g. rmAb clone SP348 and gave a relatively low pass rate of 53%, no optimal. The clone both provide a too low level of analytical sensitivity and cross reaction with PAX5 in B-cells. Similar to the observations generated in runs 51 and 56, the performance on mAb clone MRQ-50 in addition was affected by the choice of IHC platform used. In this run the concentrated format could not be used to provide a sufficient result on the Ventana BenchMark (0/11) and also provided a low pass rate on Dako Omnis with only one sufficient result (1/5). This inferior performance and reduced analytical sensitivity can potentially be related to the washing conditions and/or influence of elevated temperature settings (32°C on the Omnis and 36°C on the BenchMark) compared to systems using room temperature for incubation and washings. Whether this is the case with the mAb clone MRQ-50 is so far uncertain. Both the Autostainer and the Bond platform could be used to provide sufficient results using HIER in an alkaline buffer for about 20 min. and the mAb clone MRQ-50 conc. was diluted in the range of 1:100-200 with an incubation time of 15-30 min. depending on the total sensitivity of the protocol employed. For both platforms 3-layer detection systems were most successful.

With the pAb 10336-1-AP, sufficient staining results could be obtained on the Dako Autostainer (1 of 3), Dako Omnis (3 of 6) and Ventana Benchmark (7 of 12) platforms.

45% (122 of 271) of the laboratories used Abs in Ready-To-Use (RTU) formats. This was similar to the last run were 46% used RTU formats. The most widely used RTU systems for PAX8 were based on the mAb clone MRQ-50, prod. no **760-4616** from Ventana/Cell Marque and prod. no **363M-18** from Cell Marque. Both RTU products had an alarmingly low pass rate of 10% (8 of 81) and 22% (6 of 28), respectively. However, these data are in line with the observation for the MRQ-50 based LD assays and supports the observation that the mAb clone MRQ-50 is very difficult to optimize on the Ventana BenchMark platform. In total 102 laboratories used the Ventana BenchMark with one of the two RTUs (see Tabel 4).
For the Ventana RTU format 790-4616, only 4 laboratories followed the vendors recommended protocol settings, and none of these laboratories achieved a sufficient staining result. The remaining 80 laboratories modified the protocol settings and 10% (8 of 80) achieved a sufficient staining result. The laboratories using the Ventana platform and producing a sufficient result with a laboratory modified protocol used HIER (CC1, Ventana) for 52-64 min and a primary Ab incubation for 24-36 min. All used OptiView with amplification as detection system. Using these settings 21% (6/28) of the result were sufficient (good). The protocol still had difficulties demonstrating PAX8 in the proximal tubular cells in the kidney (see Fig. 1b) and the neoplastic cells in the ovarian serous adenocarcinoma were significantly reduced in intensity. These settings also had a tendency to produce a poor signal-to-noise ratio or in some cases a false positive staining result. Carefully calibrating these settings were of the outmost importance to produce a sufficient result.

It was observed, that a superior performance of the two above mentioned RTU formats based on mAb clone MRQ-50 was seen, when they were used on non-Ventana systems giving a pass rate of 71% (10 of 14) – all 10 assessed as good.

In this assessment some new RTU formats were introduced as the BC-12, SP348 and EP331 now are available as RTU products (see Table 1). Only a few laboratories submitted protocols using these clones as RTU giving only limited data to analyze, but so far none of these protocols received an optimal result. Analyzing the data for the RTU products in general on the different platforms did not reveal which modifications to the RTU protocols that would provide optimal or reproducible sufficient staining results. In general, the recommendation would be to change to one of the rmAb concentrates listed in Table 1 that does not show a cross reactivity to PAX5 and to perform a careful optimization and validation process.

Controls
Kidney and Fallopian tube are both recommended as positive tissue controls for PAX8. In kidney, optimally calibrated protocols must provide an at least weak to moderate, distinct nuclear staining reaction in the majority of epithelial cells of the proximal and distal renal tubules, loops of Henle, collecting ducts, and the parietal epithelial cells of Bowman's capsule. A weak cytoplasmic staining reaction in the same cells can be expected. In Fallopian tube, the protocol must be calibrated to provide an at least weak to moderate, distinct nuclear staining in the majority of the ciliated epithelial cells and a strong nuclear staining of the intercalated secretory epithelial cells. A weak cytoplasmic staining in the intercalated secretory epithelial

cells can be expected and must be accepted. Internal observations show that inadequate fixation (too short time / delayed) in formalin can reduce epitope availability in low-level PAX8 expressing structures. Tonsil can be used as negative tissue control for PAX8, as no staining should be seen in e.g. squamous epithelial cells and lymphocytes (positive nuclear staining in B-cells indicate cross reaction with PAX5).

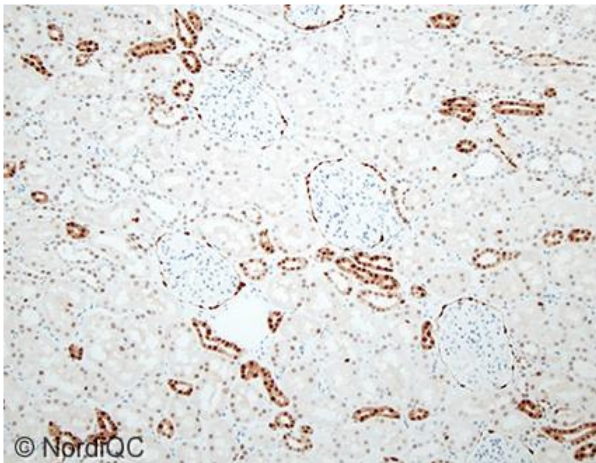


Fig. 1a
Optimal PAX8 staining of the kidney using the rmAb clone SP348 (Abcam) within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step polymer system (Dako/Agilent) and performed on the Dako Omnis stainer. A moderate to strong, distinct nuclear staining of the distal/collecting tubular cells in the kidney is seen. A weak to moderate nuclear staining is seen in the majority of proximal tubular cells. A weak cytoplasmic background staining is seen and accepted in the tubular cells (same protocol used in Figs. 1a - 6a) Compare with Fig. 1b.

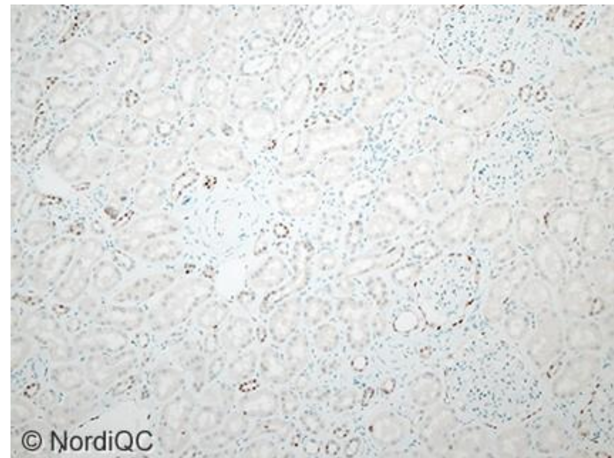


Fig. 1b
Insufficient PAX8 staining of the kidney using the mAb clone MRQ-50 (Ventana/Cell Marque) RTU within a laboratory developed assay, using an alkaline HIER pretreatment and a 3-step polymer system with OptiView on the Ventana BenchMark system. A weak to moderate nuclear staining of the distal/collecting tubular cells is seen. The proximal tubular cells are virtually negative. Compare with Fig. 1a. This was the typical pattern for the MRQ-50 clone when applied on the Ventana Benchmark and Dako Omnis platform. Also compare with Figs. 2b-6b – same protocol.

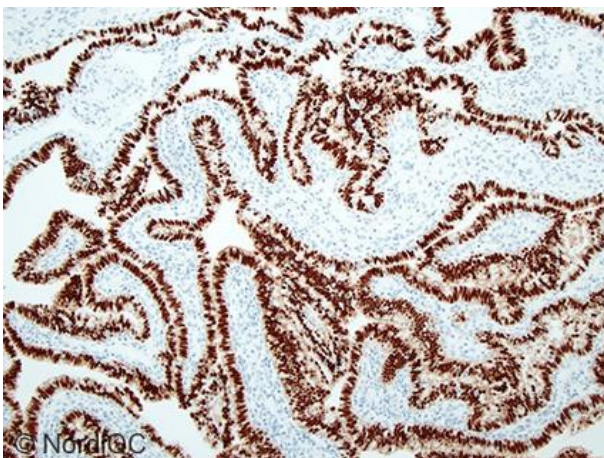


Fig. 2a
Optimal PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1a. A weak to moderate, distinct nuclear staining of the majority of the ciliated epithelial cells and a strong nuclear staining of the intercalated secretory epithelial cells is seen. A weak cytoplasmic background staining is seen and accepted in the epithelial cells. Compare with Fig. 2b.

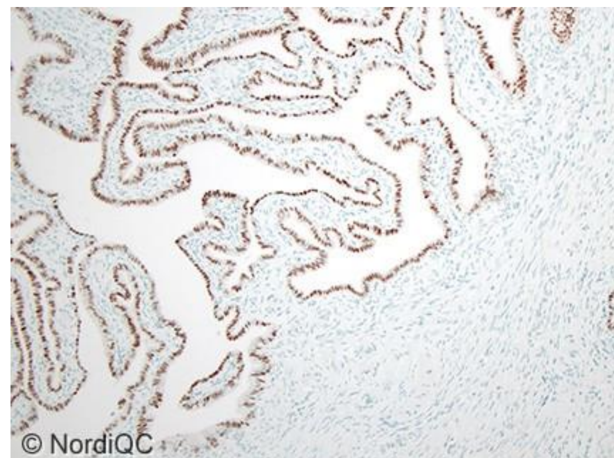


Fig. 2b
Insufficient PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1b. A moderate nuclear staining of the intercalated secretory epithelial cells is seen whereas the number of ciliated epithelial cells being decreased. Compare with Fig. 2a.

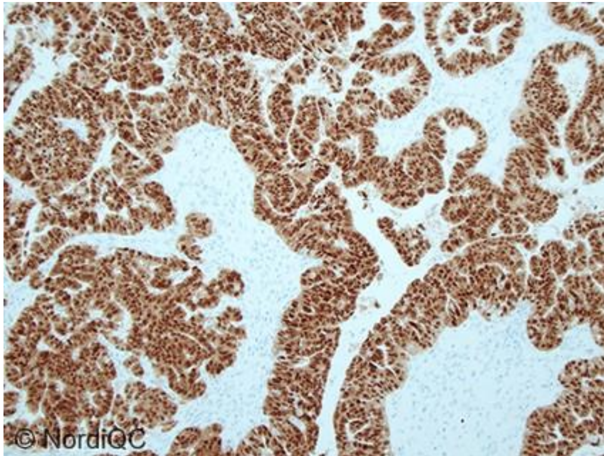


Fig. 3a
Optimal PAX8 staining of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1a and 2a. A very strong, nuclear staining is seen in virtually all the neoplastic cells. Compare with Fig. 3b.

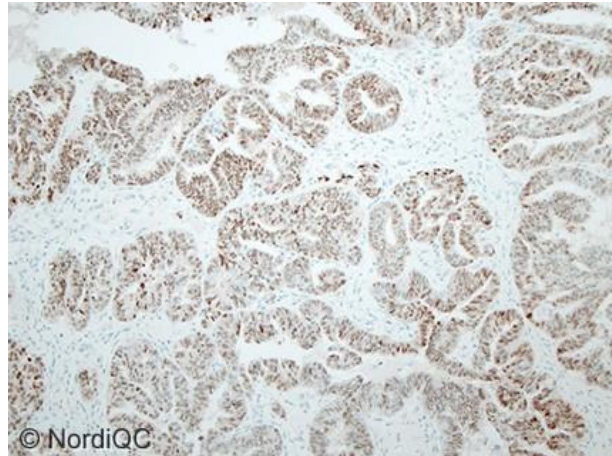


Fig. 3b
PAX8 staining of the ovarian serous adenocarcinoma using the same insufficient protocol as in Figs. 1b and 2b. The majority of the neoplastic cells display only a weak to moderate nuclear staining reaction. Compare with Fig. 3a.

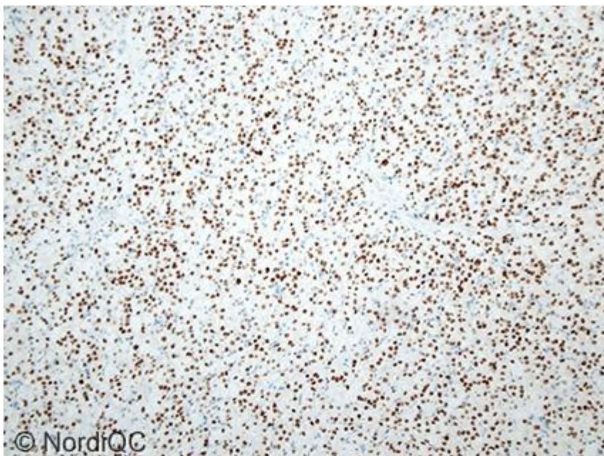


Fig. 4a
Optimal PAX8 staining in the renal clear cell carcinoma using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig 4b.

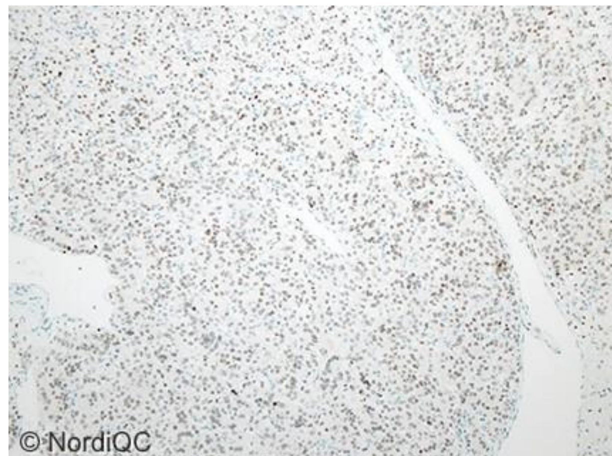


Fig. 4b
Insufficient PAX8 staining in the renal clear cell carcinoma using the same protocol as in Figs. 1b-3b. Only a weak nuclear staining is seenin the majority of the neoplastic cells. Compared to Fig. 4a.

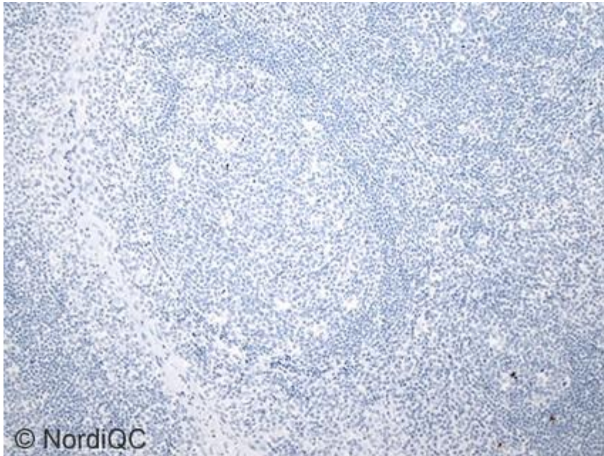


Fig. 5a
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

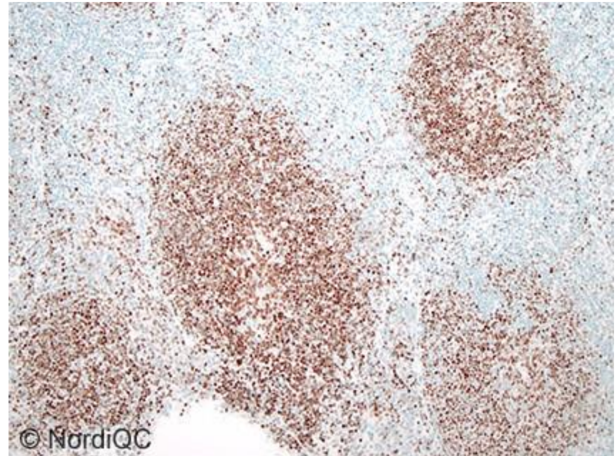


Fig. 5b
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining in virtually all B-cells. Compare with Fig. 5a.

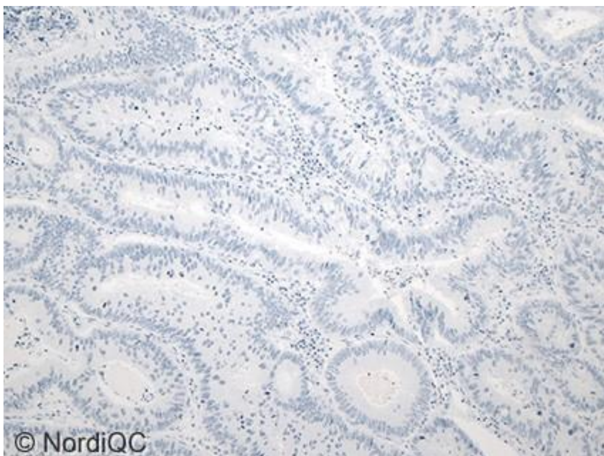


Fig. 6a
PAX8 staining in colon adenocarcinoma using the same protocol as in Figs. 1a-5a. No staining reaction is seen either in the normal tissue or the neoplastic cells. Compare with Fig. 6b.

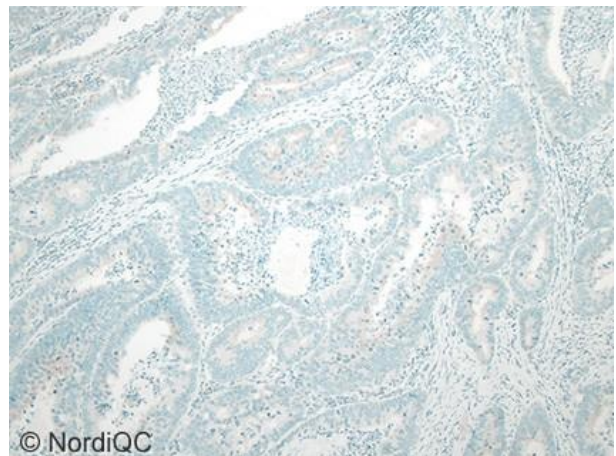


Fig. 6b
Insufficient PAX8 staining in the colon adenocarcinoma using the same protocol as in Figs. 1b-5b. A cytoplasmic staining reaction is seen in the neoplastic cells. Compare with Fig. 6a.

TJ/LE/SN 07.12.2020