

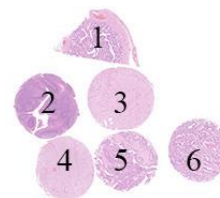
Purpose

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. Tonsil, 3. Kidney, 4. Renal clear cell carcinoma (RCC),
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 RCC.
- No nuclear staining reaction of B-cells. This was expected for antibodies (Ab) 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 to moderate 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 C12A32. 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 62	334
Number of laboratories returning slides	310 (93%)

At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data was not included in this report.

Results

310 laboratories participated in this assessment. 146 laboratories (46%) 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:

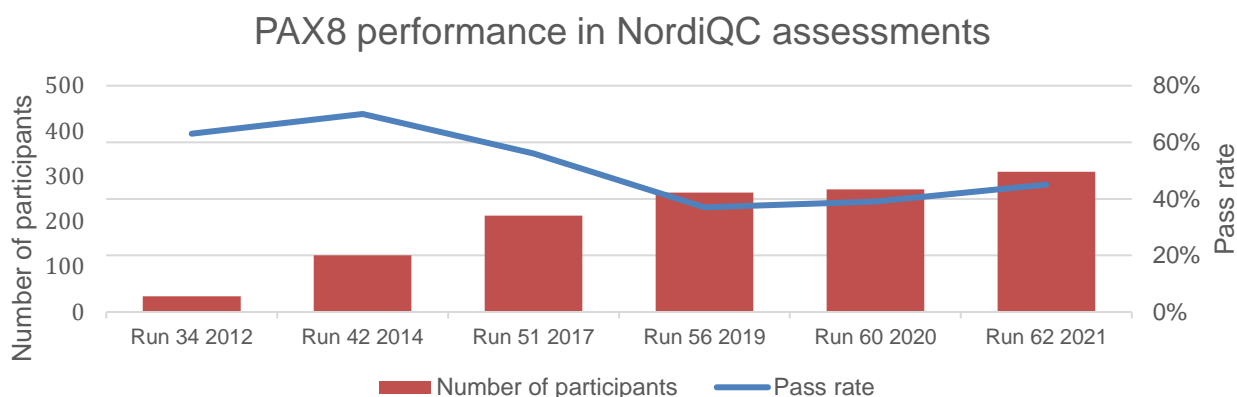
- Use of less successful antibodies primarily mAb clone MRQ-50 and rabbit monoclonal (rm)Ab clone EP331.
- Too short efficient Heat Induced Epitope Retrieval (HIER) time.
- Too low concentration of the primary Ab.

Performance history

This was the sixth NordiQC assessment of PAX8. The pass rate has increased since the previous run 60 in 2020. The proportion of participants using the mAb clone MRQ-50 was still relatively high (45% of all participants) and similar to run 60, this clone was found to be less successful especially when applied on the Ventana BenchMark and Dako Omnis platforms (see Table 5).

Furthermore, all results with a cross reaction to other PAX epitopes was downgraded due to interpretational challenges and in total only 19% of the results were scored optimal.

Graph 1. **Proportion of sufficient results for PAX8 in the six NordiQC runs performed**



Conclusion

Optimal staining results could be obtained with the rmAb clones **EP298, SP348, ZR-1, BC12, IHC048** and **RM436**. 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. The **EP331** also provided a low pass rate due to aberrant nuclear staining reaction in non-PAX8 expressing cells and poor signal-to-noise ratio.

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 62**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	OR ²
mAb clone BC12*	4	Biocare	-	3	1	2	50%	-
	1	Zytomed Systems						
	1	Diagnostic Biosystems						
mAb clone MRQ-50	34	Cell Marque	-	18	8	8	55%	-
mAb clone PAX8R1	2	Abcam	-	2	-	-	-	-
rmAb clone EP298*	7	Cell Marque						
	4	Epitomics ⁵	4	4	6	1	53%	27%
	3	BIO SB						
	1	Nordic Biosite						
rmAb clone EP331*	8	Cell Marque	-	5	7	1	39%	-
	4	Epitomics						
	1	Abcam						
rmAb clone SP348*	55	Abcam	47	10	1	2	95%	78%
	5	Gennova						
rmAb clone ZR-1*	5	Zeta Corporation						
	1	Abcam	3	1	3	-	57%	43%
	1	Bio SB						
pAb, 10336-1-AP	21	Proteintech	-	8	9	4	38%	-
pAb, 363A-15	1	Cell Marque	-	1	-	-	-	-
pAb, CP379	6	Biocare	-	1	4	1	-	-
Ab QR016*	2	Quartett	-	1	-	1	-	-
Unknown	2		-	-	2	-	-	-
Ready-To-Use antibodies							Suff. ¹	OR. ²
mAb clone, BC12* API438	4	Biocare Medical	2	2	-	-	-	-
rmAb clone EP298* Z2202	1	Bio SB	-	-	-	1		
rmAb ZR-1* GT210202	1	Gene Tech	-	1	-	-	-	-
rmAb clone, EP298* (VRPS)³ 8502-C010	2	Sakura Finetek	-	1	1	-	-	-
rmAb clone EP298* RMA-0817	2	Fuzhou Maixin	2	-	-	-	-	-
rmAb clone, EP331* 760-6077(VRPS)³	2	Ventana/Cell Marque	-	1	1	-	-	-
rmAb clone, EP331* 760-6077(LMPS)⁴	14	Ventana/Cell Marque	-	1	11	2	7%	-
rmAb clone EP331* 363M/AC0338	3	Cell Marque	-	1	2	-	-	-
mAb clone MRQ-50, 760-4618 (VRPS)³	1	Ventana/Roche	-	-	1	-	-	-
mAb clone MRQ-50, 760-4618 (LMPS)⁴	68	Ventana/Roche	-	7	44	17	10%	-
mAb clone MRQ-50, 363M-10/17/18	33	Cell Marque	-	7	17	9	21%	-
mAb clone MRQ-50, MAD-000550QD	3	Master Diagnostica	-	2	1	-	-	-
mAb clone MRQ-50, MAD-000550QD	1	Vitro SA	-	1	-	-	-	-
pAb clone 363A-XX	2	Cell Marque	-	-	2	-	-	-
rmAb clone RM436* 8257-C010	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone IHC048*	1	GenomeMe	1	-	-	-	-	-
mAb clone C12A32	1	Celnovte	-	1	-	-	-	-
mAb clone 4H7B3*	1	Diagnostic Biosystems	-	-	-	1	-	-
Total	310		60	79	121	50	-	
Proportion			19%	26%	39%	16%	45%	

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 62

The following protocol parameters were central to optimal staining:

Concentrated Antibodies

rmAb clone **EP298**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) High pH (3-in-1) (Dako/Agilent) (4/8). The rmAb was diluted in the range of 1:25-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 8 (75%) 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/Agilent), or Cell Conditioning 1 (CC1, Ventana/Roche) (3/8) and one using HIER in CC1 (Ventana/Roche) in combination with Protease 3 (Ventana/Roche) for 4 min. The rmAb was diluted 1:50-1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 50 of 52 (96%) laboratories produced a sufficient staining result.

rmAb clone **ZR-1**: Protocols with optimal results were based on HIER using TRS High pH (3-in-1) (Dako/Agilent) (2/2) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (1/1). The rmAb was diluted 1:25-50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 laboratories produced a sufficient staining result.

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

Concentrated antibodies	Dako/Agilent Autostainer		Dako/Agilent Omnis		Ventana/Roche BenchMark GX / XT / Ultra		Leica Biosystems 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	-	3/7 (43%)	-	0/6	-	-	-
rmAb SP348	0/3	-	17/21 (81%)	-	29/35 (83%)	0/1	-	-
rmAb ZR-1	2/2	-	-	-	0/2	-	1/1	-
rmAb EP331	0/2	-	0/6	-	0/4	-	0/1	-

* 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

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used Ready-To-Use (RTU) systems (≥5 assessed 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 MRQ-50 760-4618	0/1	0/1	7/67 (10%)	0/67
VMS Ultra/XT mAb EP331 760-6077	1/2	0/2	1/12 (8%)	0/12

* 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 83% of the insufficient results (141 of 171 laboratories). The remaining 17% of insufficient results were characterized by a poor signal-to-noise ratio and/or false positive staining reaction compromising interpretation.

As observed in run 60, the majority of the participating laboratories were able to demonstrate PAX8 in high-level antigen expressing cells, 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 RCC, 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 was also seen. This pattern was typically caused by use of a less successful primary antibody giving cross-reaction with e.g. PAX5. Cross-reactivity with PAX5 resulting in a distinct nuclear staining reaction of B-cells for antibodies raised against the N-terminal part of PAX8 was seen in 55% (169/310) of the returned slides (see Fig. 5a and 5b). This reaction applied for all polyclonal Abs and mAb clones MRQ-50, C12A32 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% (167 of 310) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for PAX8. The mAb clone MRQ-50, rmAb clones EP298, SP348, EP331 and the pAb 10336-1-AP were the most widely used antibodies (see Table 1). Out of these five Abs only the rmAbs clones EP298 and SP348 provided optimal staining results with a pass rate of 53% and 93% 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 two of the four main systems. The clone EP298 was during the last 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 93%, 76% optimal. The concentrate was used in a range of 1:50-1:1.000 and with HIER in an alkaline buffer as single pre-treatment. 2 laboratories used a proteolytic pre-treatment in combination with HIER for SP348, one assessed as optimal and one good.

In this assessment optimal results for the SP348 clone were only obtained on the Dako Omnis and Ventana Benchmark platforms but as only 3 slides submitted were stained on the Dako Autostainer and none from the Leica Bond no conclusion can be made whether these two platforms are less successful with this clone. In this context, it was observed that 2 out of 3 protocols based on clone SP348 and performed on Autostainer provided an optimal result.

Virtually all optimal results for clone SP348 were obtained by use of 3-step polymer/multimer based detections systems providing a high level of analytical sensitivity and expected performance in all tissues. In a few protocols the titre of SP348 was too high giving excessive background reaction and a final calibration of the titre must be established by use of appropriate positive and negative tissue control.

21 of the laboratories used concentrated antibodies on the Leica Bond instrument with a pass rate of 71% (15/21) one with an optimal result.

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 55%, no optimal. The clone frequently both provided a too low level of analytical sensitivity and cross reaction with PAX5 in B-cells. Similar to the observations generated in runs 56 and 60, the performance of mAb clone MRQ-50 was affected by the choice of platform. In this run the MRQ-50 clone only gave a pass rate of 9% on the Ventana BenchMark (9/97) and no sufficient mark on Dako Omnis (0/7) (see Table 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 (see Table 5) using HIER in an alkaline buffer for about 20 min. and the mAb clone MRQ-50 conc. diluted in the range of 1:50-200 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.

Table 5. Overview of the assessment marks for mAb clone MRQ-50 on the four main IHC instruments.

MRQ-50 score	Dako/Agilent Autostainer	Dako/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max
Optimal	-	-	-	-
Good	11	-	9	14
Borderline	3	5	59	3
Poor	-	3	31	-
Total	14	8	99	17
Sufficient %	79%	0%	9%	82%

The number of participants using the rmAb clone EP331 both as a RTU format and as a concentrate has increased immensely since the last run 60 with 4 participants to 32 in this run. In both the previous run 60 and in the current run none of the participants received an optimal result. In general, it seemed to be challenging to calibrate a protocol based on rmAb clone EP331 to obtain a high level of analytical sensitivity, appropriate specificity and optimal signal-to-noise ratio. If EP331 was used within protocols providing high level sensitivity identifying the critical structures as neoplastic cells in the RCC an aberrant nuclear staining was seen in non-PAX8 expressing stromal cells and simultaneously an aberrant cytoplasmic staining in e.g. tonsillar dendritic cells and the neoplastic cells in the colon adenocarcinoma. If the protocol was calibrated to reduce the aberrant background reaction, the critical structures typically were not demonstrated as expected. Sufficient results were only seen on the Dako Omnis and the Ventana Benchmark, whereas applying the EP331 clone on the Leica Bond or Dako Autostainer a false positive staining of the tonsil, colon adenocarcinoma and glycocalyx in the kidney was seen for both the concentrate and RTU (see Figs. 6a-7b).

In total 46% (143 of 310) of the laboratories used Abs in RTU formats. This was similar to the last run where 45% 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% (7 of 68) and 24% (7 of 33), 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 91 laboratories used the Ventana BenchMark with one of the two RTUs.

For the Ventana RTU format 760-4616 performed on BenchMark being used by 68 laboratories, only 1 laboratory followed the vendors recommended protocol settings without a sufficient result. The remaining 66 laboratories modified the protocol settings and 10% (7 of 67) 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 24-32 min. All used OptiView with amplification as detection system. Using these settings 25% (6/24) 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 showed a significantly reduced intensity. These settings also had a tendency to produce a poor signal-to-noise ratio and/or in some cases a false positive staining result.

In this assessment an increased number of participants used non-cross-reacting clones for PAX8 as SP348, contributing to the improved pass-rate from 39% to 45%. The concentrated formats had a pass-rate of 64% compared to the RTU formats with only 23% (see Table 1). The pass-rate is still at a very low level and the combination of limited access to non-cross reacting RTU systems for PAX8 and the platform depending performance of MRQ-50 complicates the optimization and validation process for the laboratories. However, there are still many available clones as SP348, ZR1, BC12 etc that has the potential to give optimal results on all platforms and at present seem to be the preferred choice.

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 to moderate 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).

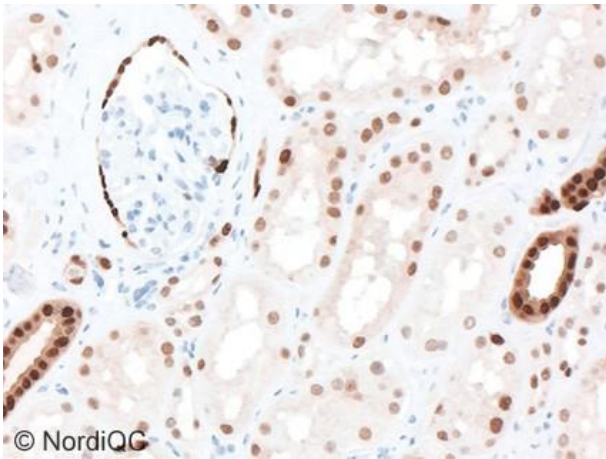


Fig. 1a
Optimal PAX8 staining of the kidney using the rmAb clone SP348 (Abcam) within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark. A weak to strong, distinct nuclear staining reaction is seen 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 weak cytoplasmic background staining is seen and accepted in the tubular cells (same protocol used in Figs. 1a - 5a) Compare with Fig. 1b.

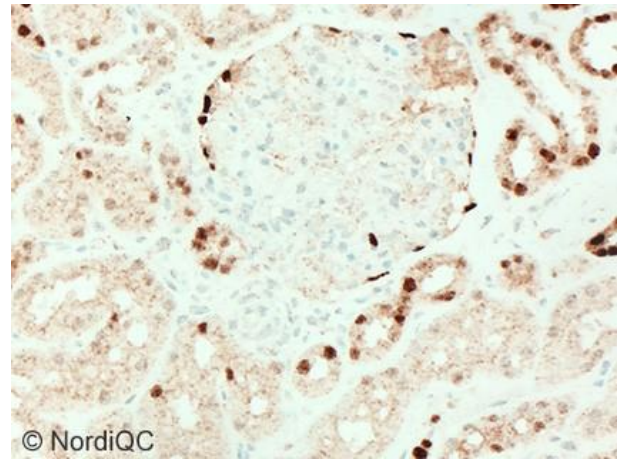


Fig. 1b
Insufficient PAX8 staining of the kidney using the mAb clone MRQ-50 (Ventana/Cell Marque) RTU within a laboratory developed assay, using an alkaline HIER pretreatment and a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate nuclear staining reaction 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-5b – same protocol.

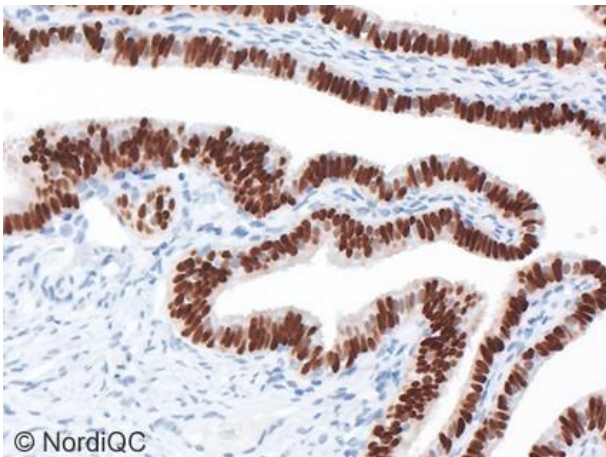


Fig. 2a
Optimal PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1a. A weak to moderate, distinct nuclear staining reaction of the majority of the ciliated epithelial cells and a strong nuclear staining reaction of the intercalated secretory epithelial cells is seen. A weak cytoplasmic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

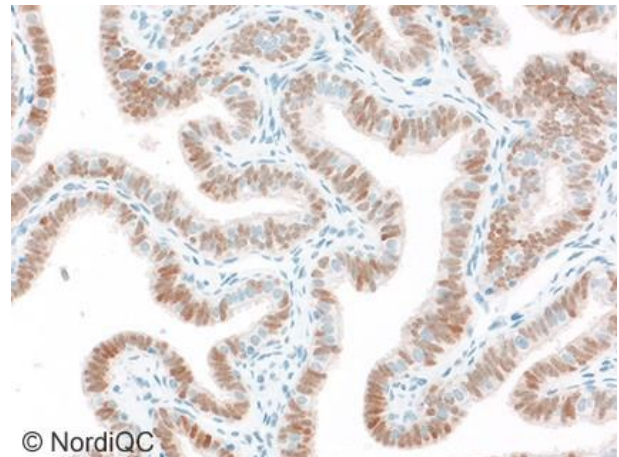


Fig. 2b
Insufficient PAX8 staining of the Fallopian tube using the same protocol as in Fig. 1b. A moderate nuclear staining of the intercalated secretory epithelial cells is seen whereas the number and intensity of ciliated epithelial cells is decreased. Compare with Fig. 2a.

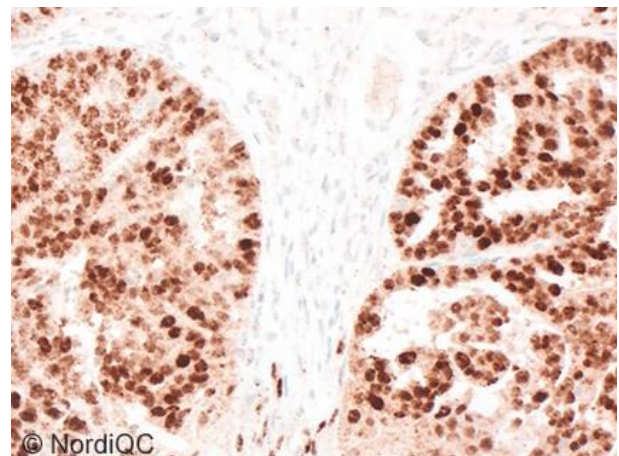
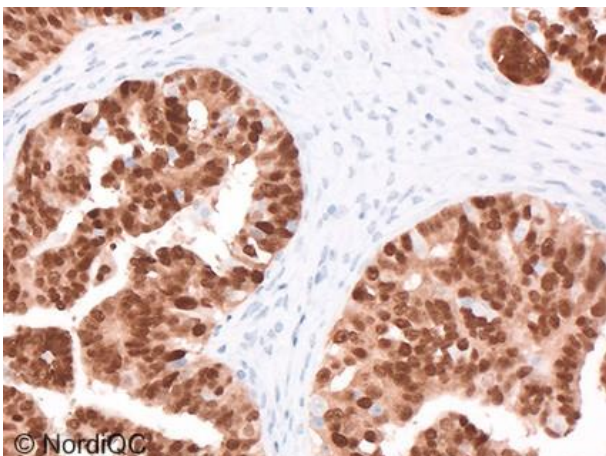


Fig. 3a
Optimal PAX8 staining of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1a and 2a. A very strong, nuclear staining reaction is seen in virtually all the neoplastic cells. Compare with Fig. 3b.

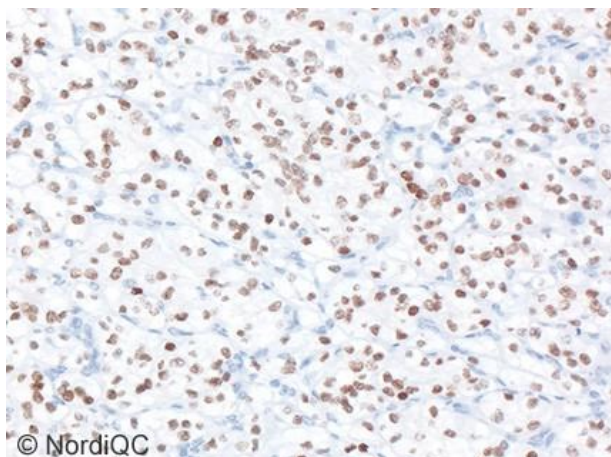


Fig. 4a
Optimal PAX8 staining of the RCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen. Compare with Fig. 4b.

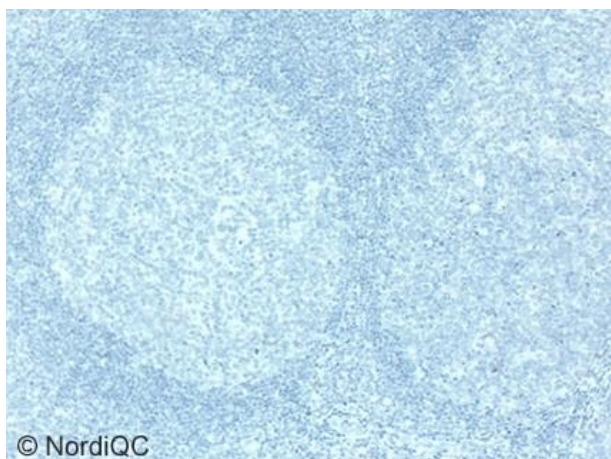


Fig. 5a
PAX8 staining without PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 do not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

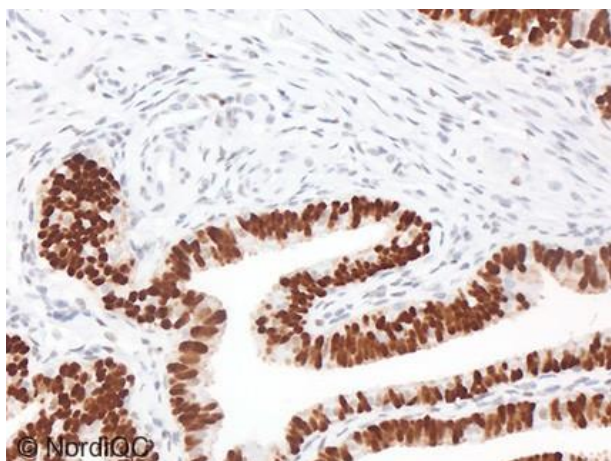


Fig. 6a
Staining result for PAX8 of the Fallopian using an insufficient protocol based on the rmAb clone EP331 as Nordic Immunohistochemical Quality Control, PAX8 run 62 2021

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.

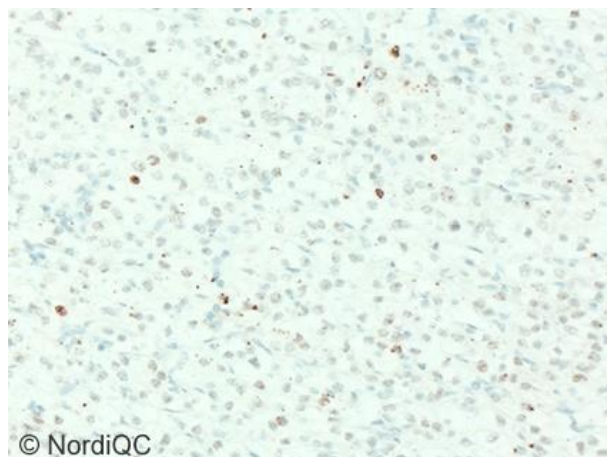


Fig. 4b
Insufficient PAX8 staining of the RCC using the same protocol as in Figs. 1b-3b. Only a weak nuclear staining is seen in dispersed neoplastic cells. Compare to Fig. 4a.

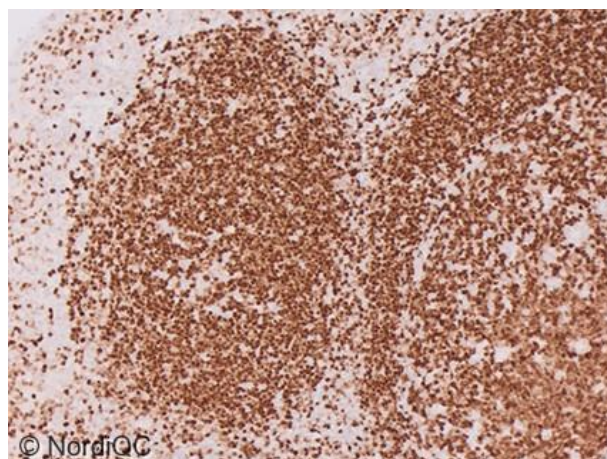


Fig. 5b
PAX8 staining with PAX5 cross reactivity. PAX8 staining in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a.

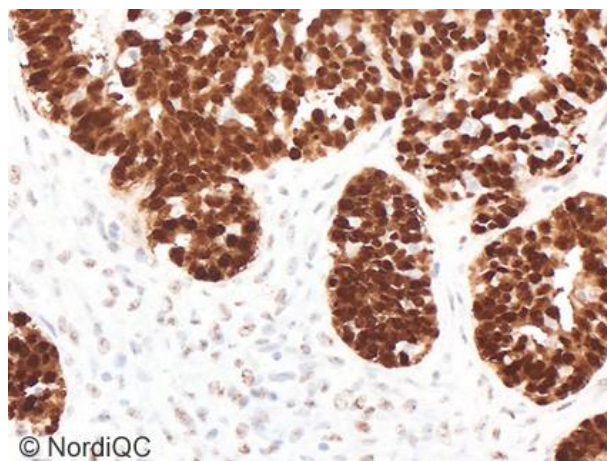


Fig. 6b
Insufficient PAX8 staining of the ovarian serous adenocarcinoma using the same protocol as in Fig. 6a. A

RTU (Abcam) within a laboratory developed assay, using HIER in an alkaline buffer as pretreatment, a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate, distinct nuclear staining reaction of the majority of the ciliated epithelial cells and a strong nuclear staining of the secretory epithelial cells is seen. The staining pattern in the epithelial cells is as expected and similar to Fig 2a., but in addition a weak but distinct aberrant staining of the nuclei of the stromal cells is seen. Also compare with Fig 6b – same protocol.

very strong, nuclear staining reaction is seen in virtually all the neoplastic cells, but also an aberrant moderate staining reaction is seen in the nuclei of virtually all the stromal cells.

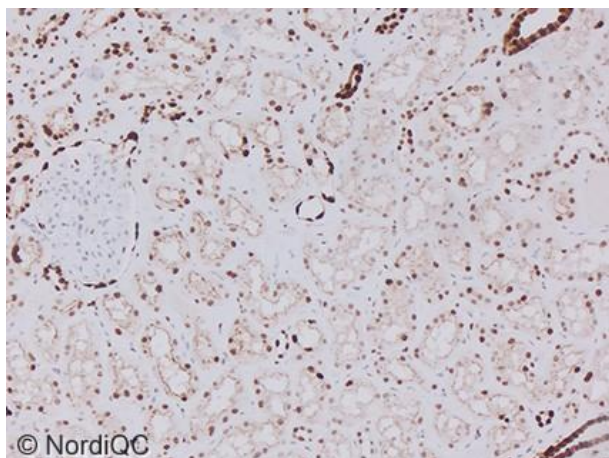


Fig 7a
 Insufficient staining of the kidney using the rmAb clone EP331 as RTU format (Abcam) on the Dako Autostainer platform by protocol settings giving a high level of analytical sensitivity (HIER in High ph and a 3-step polymer based detection system). Compared to the optimal result of kidney shown in Fig 1a, this protocol provides an excessive granulated cytoplasmic staining reaction and a strong staining of the glycocalyx in the proximal tubules. Also compare with the result shown in Fig. 7b - same protocol. Same result was typically also seen on the Leica Biosystems BOND platform using similar protocol settings.

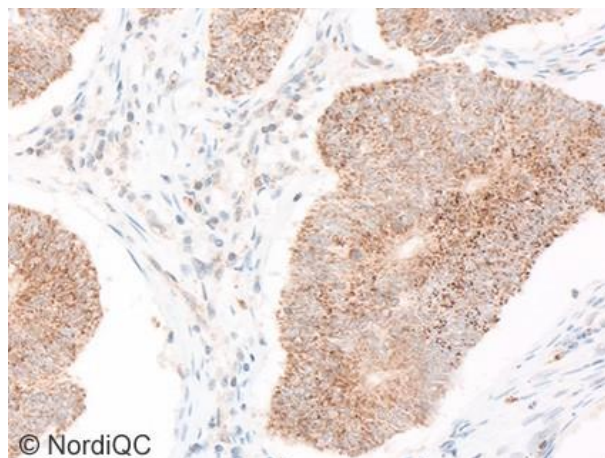


Fig 7b
 Insufficient staining of the colon adenocarcinoma using same protocol as Fig. 7a. An extensive and false positive cytoplasmic staining reaction is seen in the neoplastic cells compromising the interpretation and in addition an unspecific background staining is seen in the connective tissue.

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