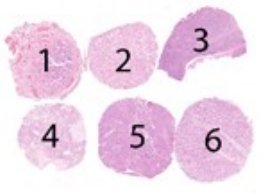
Assessment run 77 2026

Paired box gene-8 protein (PAX8)

Purpose
Evaluation of the technical performance, analytical sensitivity and specificity of PAX8 IHC tests among the NordiQC participants, typically used in the diagnostic work-up identifying the origin of renal cell and ovarian carcinomas in cancers of unknown primary origin (CUP). Clinically relevant normal and neoplastic tissues were selected to include a broad spectrum of PAX8 antigen expression levels (see below).

Material
The slide to be stained for PAX8 comprised:

1. Fallopian tube, 2. Kidney, 3. Tonsil, 4. Clear cell renal cell carcinoma (ccRCC), 5. Ovarian serous adenocarcinoma, 6. Colon adenocarcinoma.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing 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.
- 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 ccRCC.
- 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 SP348, EP331, IHC048, GR64, RM436, QR016 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. 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, 4H7B3 and IHC008. For these Abs the highest score consequently was "Good" provided that the staining pattern was otherwise as described above.

KEY POINTS FOR PAX8 IMMUNOASSAYS
- The rmAb clone SP348 was used by 48% of all participants with a pass rate of 90%.
- The mAb clone MRQ-50 and rmAb clone EP331 were in total used by 34% of all participants with a pass rate of 68%, only one optimal.
- The newly introduced RTU product **790-7263** based on the rmAb clone **SP348** from Ventana/Roche provided a 100% pass rate.
- For controls, the use of kidney or fallopian tube in combination with tonsil seems suitable to monitor the performance of the PAX8 assay.

Participation

Number of laboratories registered for PAX8, run 77	459
Number of laboratories returning slides	416 (91%)

Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results
416 laboratories participated in this assessment. One laboratory submitted a protocol stained on the wrong TMA. This was not included in the analysis. Of the remaining 415 laboratories, 266 (64%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarise the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Use of less successful antibodies primarily mAb clone MRQ-50 and rmAb clone EP331.
- Insufficient effective Heat Induced Epitope Retrieval (HIER) time.
- Too low concentration of the primary Ab.
- Limited access to robust and specific RTU systems for all main IHC platforms

Controls
Kidney and Fallopian tube are both recommended 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, 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 indicates cross reaction with PAX5).

Conclusion
Optimal staining results could be obtained with the mAb clones **BC12, BY087** and rmAb clones **SP348, MXR013, GR002, IHC048, BP6157** and **QR016**. 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 rmAb clone **SP348** 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. 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** clone also provided a low pass rate due to aberrant nuclear staining reaction in non-PAX8-expressing cells and poor signal-to-noise ratio. The newly introduced RTU product **790-7263** based on the rmAb clone **SP348** from Ventana/Roche provided a 100% pass rate. Laboratory developed (LD) tests based on concentrated Abs provided a significantly higher proportion of sufficient results (78%) compared to Ready-To-Use (RTU) Abs (53%), mainly related to the most successful Ab rmAb clone SP348 being newly introduced as RTU system and therefore used by relatively few laboratories.

Table 1a. **Overall results for PAX8, run 77**

	n	Optimal	Good	Borderline	Poor	Sufficient results[1]	Optimal results
Concentrated antibodies	184	88	55	35	6	78%	48%
Ready-To-Use antibodies	231	60	63	100	8	53%	26%
Total	415	148	118	135	14		
Proportion		36%	28%	33%	3%	64%	

1) Optimal and good

Table 1b. **Concentrated antibodies and assessment marks for PAX8, run 77**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC12***	7	Biocare	-	-	6	1	0%	0%
	1	Zytomed Systems			1			
mAb clone **MRQ-50**	10	Cell Marque	-	6	4	-	60%	0%
mAb clone **PAX8R1**	1	Abcam	-	-	1	-	-	-
mAb clone **ZM28**	1	Zeta Corporation	-	-	1	-	-	-
mAb clone **4H7B3**	1	BD scientific	-	-	1	-	-	-
rmAb clone **SP348***	102	Abcam	62	29	9	2	89%	60%
	36	Cell Marque	21	10	3	2		
	1	Sanbio	1	-	-	-		
	1	Unknown	-	1	-	-		
rmAb clone **QR016***	11	Quartett	3	7	1	-	91%	27%
rmAb clone **EP331***	1	Cell Marque	-	1	-	-	-	-
rmAb clone **ZR-1***	1	Zeta Corporation	-	-	1	-	-	-
	1	Bio SB	-	-	-	1		
	1	Abcam	-	1	-	-		
rmAb clone **IHC048***	1	GenomeMe	1	-	-	-	-	-
rmAb clone **GR64***	1	Genebio Solution	-	-	1	-	-	-
rmAb clone **RM436***	1	BioSB	-	-	1	-	-	-
pAb, **10336-1-AP**	3	ProteinTech	-	-	3	-	-	-
pAb, **CP379 AK**	1	Biocare Medical	-	-	1	-	-	-
pAb, **RBK047**	1	Zytomed Medical	-	-	1	-	-	-
Total	184		88	55	35	6		
Proportion			48%	30%	19%	3%	78%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
*Clones that do not show cross reactivity with PAX5.

Table 1c. **Ready-To-Use antibodies and assessment marks for PAX8, run 77**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **MRQ-50, 760-4618 (VRPS)[3]**	16	Ventana/Cell Marque	-	-	12	4	0%	0%
mAb clone **MRQ-50, 760-4618 (LMPS)[4]**	46	Ventana/Cell Marque	-	1	43	2	2%	0%
rmAb clone, **EP331* 760-6077 (VRPS)[3]**	6	Ventana/Cell Marque	-	4	2	-	67%	0%
rmAb clone, **EP331* 760-6077 (LMPS)[4]**	36	Ventana/Cell Marque	1	23	11	1	67%	3%
rmAb clone **SP348 790-7263 (VRPS)[3]**	22	Ventana/Roche	17	5	-	-	100%	77%
rmAb clone **SP348 790-7263 (LMPS)[4]**	6	Ventana/Roche	4	2	-	-	100%	67%
rmAb clone **RM436* 8257-C010**	2	Sakura Finetek	-	2	-	-	-	-
mAb clone **MRQ-50, 363M-10/17/18**	18	Cell Marque	-	3	15	-	17%	0%
mAb clone **MRQ-50, MAD-000550QD**	4	Master Diagnostica/ Vitro S.A	-	3	1	-	-	-
mAb clone, **BC12* API438**	5	Biocare Medical	2	2	1	-	80%	40%
mAb clone **BC12* MSG107**	3	Zytomed systems	1	1	1	-	-	-
mAb clone **4H7B3 PDM180**	4	Diagnostic BioSystems	-	-	4	-	-	-
mAb clone **C12A32 CPM-0252**	1	Celnovte	-	1	-	-	-	-
mAb clone **BY087* BFM-0403**	1	Bioin Biotechnology	1	-	-	-	-	-
mAb clone **IHC008-7 IHC008-7**	3	GenemeMe	-	-	3	-	-	-

rmAb clone **SP348* 363R-30/37/38**	30	Cell Marque	21	5	3	1	87%	70%
rmAb clone **SP348* M6481**	2	Spring Biosciences	-	2	-	-	-	-
rmAb clone **EP331* 363M/AC0338**	1	Cell Marque	-	1	-	-	-	-
rmAb clone **QR016* P-P008**	14	Quartett	8	5	1	-	93%	57%
rmAb clone **QR016* P-P008-150**	1	Anatopath	1	-	-	-	-	-
rmAb clone **ZR-1* Z2202**	1	Zeta corporation	-	-	1	-	-	-
rmAb clone **ZR-1* ab122944**	1	Abcam	-	-	1	-	-	-
rmAb clone **GR002* GT210202**	1	GeneTech	1	-	-	-	-	-
rmAb clone **IHC048* IHC048-7**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **282J3C4* PA244**	1	Abcarta	-	1	-	-	-	-
rmAb clone **BP6157* I11562E**	1	Biolynx Biotechnology Co.	1	-	-	-	-	-
rmAb clone **MXR013* RMA-1024**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **DY49963* 4921362**	1	ImmunoLogic	-	1	-	-	-	-
rmAb clone **JE80-01* BL5013**	1	Guangzhou Biotron	-	1	-	-	-	-
pAb clone **NOA-RBG047**	1	Zytomed Systems GmbH	-	-	1	-	-	-
Total	231		60	63	100	8		
Proportion			26%	27%	43%	4%	53%	

1) Proportion of sufficient results (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).
*Clones that do not show cross reactivity with PAX5.

Detailed analysis of PAX8, run 77
The following protocol parameters were central to obtaining optimal staining:

Concentrated antibodies
rmAb clone **SP348**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) High pH (42/51)* (Dako/Agilent), or Cell Conditioning 1 (CC1, Ventana/Roche) (37/68), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (3/14) or HIER in CC1 (Ventana/Roche) in combination with Protease 3 (Ventana/Roche) for 4 min. (1/3). The rmAb was diluted 1:10-1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 123 of 139 (88%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **QR016**: three protocols produced optimal results. 2 were based on HIER in CC1 (Ventana/Roche) (2/6) and the last one was based on BERS2 (Leica Biosystems) (1/3). The rmAb was diluted 1:100. Using these protocol settings 4 of 4 laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for PAX8 on the four main IHC systems by optimal titration settings as listed above**

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**			**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	P3+CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
rmAb clone **SP348*** 1:10-1:2000	2/2	-	80% (41/51)	-	54% (37/68)	1/3	0/1	21% (3/14)	-
rmAb clone **QR016*** 1:100	-	-	-	-	2/3	-	-	1/1	-

1) Autostainer Link 48.
2) BenchMark GX, Ultra, Ultra plus
3) Bond III, Prime

*Clones that do not show cross reactivity with PAX5.

Ready-To-Use antibodies and corresponding systems
mAb clone **EP331**, product no.**760-6077**, Ventana/Roche, BenchMark GX/Ultra/Ultra Plus:
Only one protocol produced an optimal result and was based on OptiView (760-700) as detection system,HIER using CC1 (efficient heating time 80 min.) and 36 min. incubation of the primary Ab.

rmAb clone **SP348**, product no.**790-7263**, Ventana/Roche, BenchMark Ultra/Ultra Plus:
Optimal protocols using UltraView (760-500) as detection system were typically based on HIER using CC1 (efficient heating time 64 min.) and 32 min. incubation of the primary Ab.
Optimal protocols using OptiView (760-700) as detection system were typically based on HIER using CC1 (efficient heating time 40-64 min.) and 32-36 min. incubation of the primary Ab.
Using these protocol settings, 26 of 26 (100%) laboratories produced a sufficient staining result.
The product was used by 1 laboratory on a non-intended platform. This data is not included.

Table 3 summarises the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems with changes to basic protocol settings. Only protocols performed on the intended IHC stainer are included.

Table 3. **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 BenchMark mAb MRQ-50 **760-4618 UltraView**	0% (0/14)	0% (0/14)	0% (0/11)	0% (0/11)
VMS BenchMark mAb MRQ-50 **760-4618 OptiView**	0% (0/2)	0% (0/2)	3% (1/35)	0% (0/35)
VMS BenchMark rmAb EP331 **760-6077 UltraView**	67% (4/6)	0% (0/6)	50% (5/10)	0% (0/10)
VMS BenchMark rmAb EP331 **760-6077 OptiView**			73% (19/26)	4% (1/26)
VMS BenchMark mAb SP348 **790-7263 UltraView**	100% (5/5)	60% (3/5)	(1/1)	(1/1)
VMS BenchMark mAb SP348 **790-7263 OptiView**	100% (17/17)	82% (14/17)	(4/4)	(3/4)

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

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 74% of the insufficient results (111 of 149 laboratories). The remaining 26% of insufficient results were characterized by a poor signal-to-noise ratio and/or excessive background or false positive staining reaction compromising interpretation.
As observed in previous runs, the majority of the participating laboratories were able to demonstrate PAX8 in high-level antigen expressing cells, such as secretory epithelial cells of the Fallopian tube and neoplastic cells of the ovarian serous adenocarcinoma and parietal cells lining the Bowman capsules of the kidney, whereas demonstration of PAX8 in low-level antigen expressing cells 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 Figs. 1a - 6b). Cases of insufficient staining due to extensive cytoplasmic staining reaction 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 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 34% (125/368) of the returned slides (see Figs. 5a and 5b). This reaction applied for all polyclonal Abs and mAb clones MRQ-50, 4H7B3, C12A32 and IHC008. Within the last couple of years well-performing rmAbs without cross reactivity have 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 have e.g. been described by Kamaljeet Singh et al[1].

44% (184 of 415) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for PAX8. The mAb clones MRQ-50, BC12, the rmAb clones SP348, QR016 and the pAb 10336-1-AP were the most widely used antibodies (see Table 1b). Out of these five Abs, only the rmAb clones SP348 and QR016 provided optimal staining results.

Data focusing on the four main IHC systems (see Table 3) showed that the **rmAb clone SP348** could be used to obtain optimal results on all of the four main systems.

The rmAb clone **SP348** was the most used concentrate (n=140) with a pass-rate of 89%, 60% optimal. Within the sufficient protocols, the concentrate was used in a range of 1:10-1:2.000 and with HIER in an alkaline buffer as single pre-treatment. 3 laboratories used a proteolytic pre-treatment in combination with HIER for SP348, 1 result was assessed as optimal and 2 good.
Data from this assessment underlines the need for platform-specific calibration of the applied titre of rmAb clone **SP348** for optimal performance. In general, optimal results required alkaline HIER and preferably a sensitive 3-step detection system, but the optimal titre differed between platforms. On the Dako/Agilent Autostainer, a dilution of 1:100 in combination with HIER in TRS High pH with EnVision FLEX+ was found optimal. On Dako Omnis, HIER in TRS High pH with FLEX+ protocols using a dilution of 1:50–1:400 gave sufficient staining in 95% of cases (38/40), whereas the application of DUAL linker detection system permitted higher dilution range of 1:1.500–1:2.000. On Ventana BenchMark, OptiView-based protocols with 48–64 min. HIER, 30–40 min. primary antibody incubation and dilution range of 1:75–1:200 achieved a 91% pass rate (30/33). Only one UltraView-based protocol was submitted, and this was insufficient.
14 laboratories used the rmAb clone SP348 on the Leica Bond with a pass rate of 57% (8/14) which is significantly lower than the level obtained on other IHC systems. Sufficient protocols were based on a dilution in the range of 1:25-100, HIER in BERS 2 pH 9 for 20-40 min. and an Ab incubation time for 15-30 min. These settings are similar to the above mentioned and more successful protocols providing optimal results on the other platforms. In total, only 3 protocols (21%) provided an optimal result on Bond platforms using otherwise "optimal protocol settings". The inferior performance for the rmAb clone SP348 may be related to the Bond Refine detection system which in the case of rabbit antibodies acts as only a 2-step detection system due to the post-blocking step or Linker being based on a rabbit anti-mouse antibody amplifying the signal of mouse primary antibodies with no effect on primary rabbit antibodies.

The rmAb clone **QR016** has increased in popularity compared to the last run where it is now the second most used concentrated product. A total of 11 laboratories used the product providing a pass rate of 91%, however only 27% optimal. The product was applied on all 3 fully automated main IHC platforms, but optimal results were only achieved on the Ventana BenchMark and Leica BOND platform. All 3 optimal protocols applied a titre of 1:100 of the primary Ab, HIER for 30 min (BERS2, Leica Biosystems) or 64 min (CC1, Ventana/Roche) and a relatively long incubation time (56-60 min) of the primary Ab. Results scored as good were mainly downgraded because fewer cells than expected were demonstrated or cells showed too weak staining reaction. In all cases, either a too high dilution of the primary Ab or too short incubation time was used compared to optimal protocols, however data is limited.

In previous runs, the mAb clone **MRQ-50** was the most popular Ab applied, however only 10 participants used this clone as a concentrated format in this run. This clone was observed to be inferior to e.g. rmAb clone SP348 and QR016 and gave a relatively low pass rate of 60%, with no optimal results. The clone frequently both provided a too low level of analytical sensitivity and cross-reaction with e.g. PAX5 in B-cells. Similar to the observations generated in previous runs, the performance of mAb clone MRQ-50 was affected by the choice of platform. In the cumulated data from runs 62, 64, 68 and 77, the MRQ-50 clone only gave a pass rate of 5% on the Ventana BenchMark platform (13/256) and no sufficient mark on Dako Omnis (0/18) (see Table 4).
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 Dako Omnis and 36°C on the Ventana BenchMark) compared to systems using room temperature for incubation and washings as e.g. the Dako Autostainer and Leica Bond platforms. Whether this is the case with the mAb clone MRQ-50 is so far uncertain, but as seen in table 4, the performance of clone MRQ-50 was superior on the Dako

Autostainer and the Leica Biosystems Bond platforms as both systems provided a significantly higher level of sufficient results. The sufficient results werebased on 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 4. Overview of the assessment marks for mAb clone MRQ-50 on the four main IHC instruments in runs 62, 64, 68 and 77 (cumulated data for both RTU and concentrate).

MRQ-50 score	Dako/Agilent Autostainer	Dako/Agilent Omnis	Ventana/Roche BenchMark GX / XT / Ultra	Leica Biosystems Bond III / Max/Prime
Optimal	-	-	-	-
Good	26	-	13	46
Borderline	7	15	177	10
Poor	-	3	66	-
Total	33	18	256	56
Sufficient %	79%	0%	5%	82%

The number of participants using the rmAb clone **EP331** both as a RTU format and as a concentrate has increased from 28 to 44 in this present run. Only one of the participants using this clone received an optimal mark. 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 rmAb clone EP331 was used within protocols providing high level of analytical sensitivity identifying the critical structures as neoplastic cells in the ccRCC an aberrant nuclear staining was seen in non-PAX8 expressing stromal cells and simultaneously giving 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 were typically not demonstrated as expected. Sufficient results were only seen on the Ventana BenchMark platforms and a single result on the Bond Prime platform (Leica Biosystems). Similar to the observations from the previous runs, the clone EP331 applied on the Leica Bond or Dako Autostainer induced a false positive staining of the tonsil, colon adenocarcinoma and glycocalyx in the kidney (Fig. 7b) and was seen for both the concentrate and RTU formats.

In total 56% (231 of 415) of the laboratories used Abs in RTU formats. Among the major IHC vendors, Ventana/Roche is currently the only provider of PAX8 RTU products for use on its proprietary IHC platforms, offering three different clones. The most widely used RTU system for PAX8 was based on the mAb clone **MRQ-50**, prod. No **760-4618** from Ventana/Cell Marque and used by 62 laboratories. The RTU product had an alarmingly low pass rate of 2%. However, these data are in line with both the observation for the corresponding concentrated format of clone MRQ-50 within LD assays but also data from previous assessments and support the observation that the mAb clone MRQ-50 is very difficult to optimize on the Ventana BenchMark platform.
The RTU system prod. No **760-6077** based on rmAb clone **EP331** was used by 42 laboratories, with a pass rate of 67% for both VRPS and LMPS settings. As mentioned previously the product seemed difficult to optimize and only one optimal result was obtained. For both **760-4618** (MRQ-50) and **760-6077** (EP331), replacement with the newly introduced **790-7263** product based on rmAb clone **SP348** should be considered, as this product demonstrated a superior performance (see Table 1c).

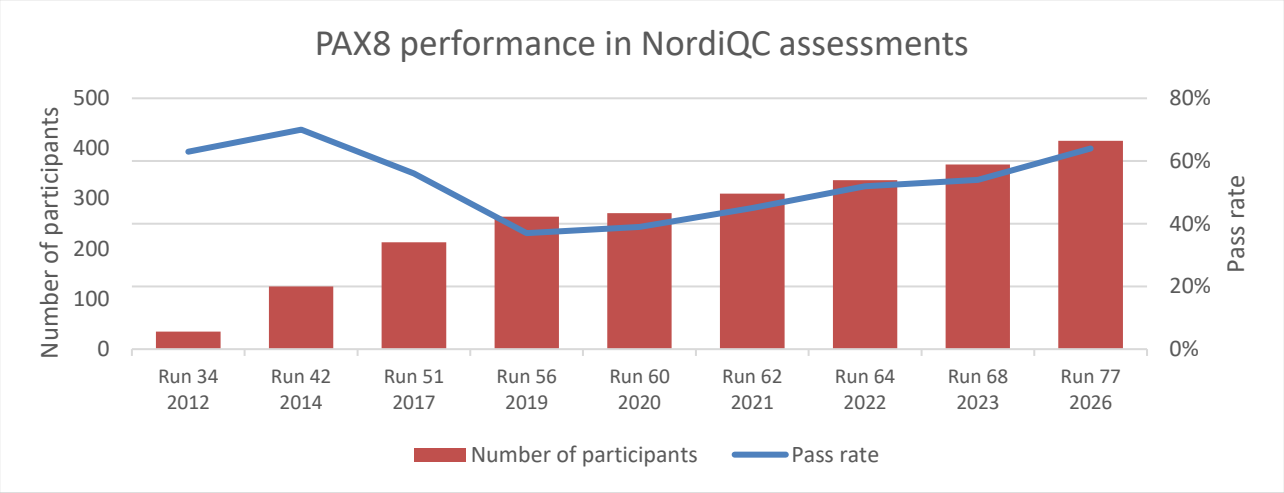
The newly introduced RTU product **790-7263** based on the rmAb clone **SP348** from Ventana/Roche, seems to be the preferred and best choice as the pass rate was 100% with 77% optimal results when following VRPS. Protocols giving sufficient results were available for both UltraView and OptiView however protocols using OptiView as detection system provided a higher proportion of optimal results of 82% compared to UltraView with only 60% optimal (see table 3). Only 28 laboratories used the product from Ventana/Roche as it is newly introduced.

A generic and platform-agnostic RTU format of the rmAb SP348 was also available from Cell Marque **363R-30/37/38** and used by 30 laboratories with a pass rate of 87%, 70% being optimal. The product was used on all of the 4 main IHC systems, but optimal results were only produced on the Dako/Agilent platforms and the Ventana BenchMark systems.

Performance history
This was the ninth NordiQC assessment of PAX8. The pass rate has increased slowly but consistently since run 56 in 2019. The proportion of participants using the mAb clone MRQ-50 and rmAb clone EP331 was still relatively high among the RTU users (59%) and similar to previous runs, these clones were found to be less successful (see Table 1c and 4). The pass rate is thus mainly related to the proportion of participants

using either inferior or superior Abs and in order to impact the pass rate further an extended use of robust Abs as clones SP348 and QR016 must be effectuated on the expanse of less successful Abs.

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



Summary

In this assessment, an increased number of participants used non-cross-reacting Abs for PAX8, as clone SP348, contributing to the improved pass-rate from 54% to 64%. The overall performance for protocols based on concentrated formats had a pass-rate of 78% compared to protocols based on RTU formats with a pass rate of 53% (see Table 1a). 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 dependent performance of MRQ-50 complicates the optimization and validation process for the laboratories. However, there are commercially available clones such as SP348, QR016 etc. that have the potential to give optimal results on all of the main IHC platforms and at present seem to be the preferred choices.

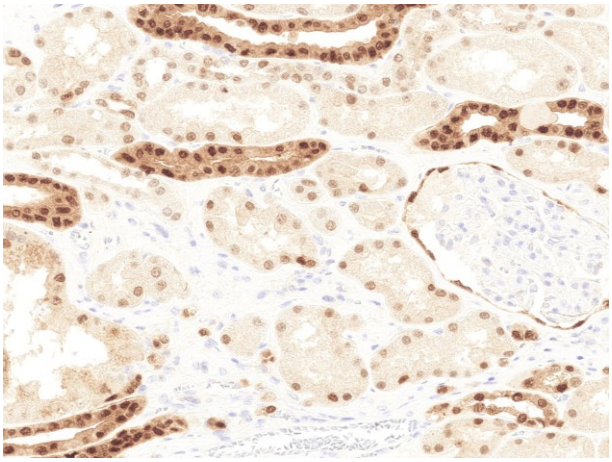


Fig. 1a x200
Optimal PAX8 staining reaction of the kidney using the rmAb clone SP348 (Cell Marque) as a concentrate within a laboratory developed assay optimally calibrated, using HIER in an alkaline buffer and a 3-step polymer-based detection system (EnVision FLEX+, Dako/Agilent) and performed on the Dako Omnis. A moderate 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 moderate to low cytoplasmic staining reaction is seen and accepted in the tubular cells with low level PAX8 expression. Same protocol settings used in Figs. 1a - 6a.

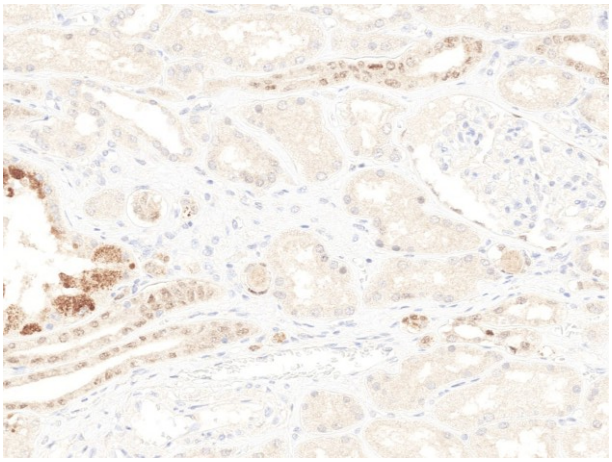


Fig. 1b x200
Insufficient PAX8 staining reaction of the kidney using the mAb clone MRQ-50 (Ventana/Cell Marque) as RTU within a laboratory developed assay, using HIER in an alkaline buffer, 40 min incubation of primary Ab, a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. A weak to moderate nuclear staining reaction of the distal/collecting tubular cells in the kidney is seen. The proximal tubular cells are virtually negative. Compare with Fig. 1a – same field. This was the typical pattern for the MRQ-50 clone when applied on the Ventana BenchMark and Dako Omnis platforms. Also compare with Figs. 2b-5b – same protocol settings.

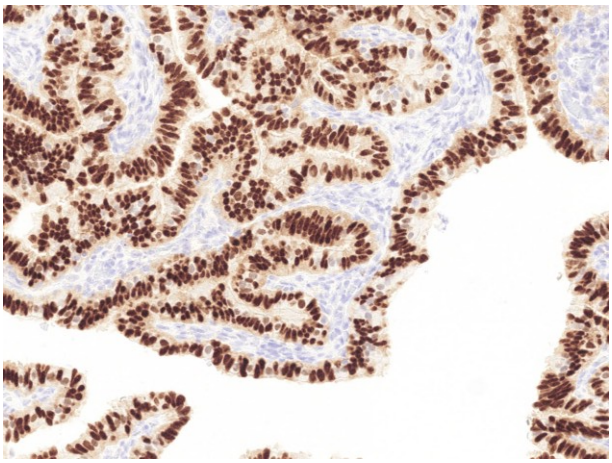


Fig. 2a x200
Optimal PAX8 staining reaction of the Fallopian tube using the same protocol as in Fig. 1a. A weak to moderate, distinct nuclear staining reaction in the majority of the ciliated epithelial cells and a strong nuclear staining reaction of the intercalated secretory epithelial cells are seen. A weak cytoplasmic staining reaction is seen and accepted in the epithelial cells, as no general background staining is seen. Compare with Fig. 2b.

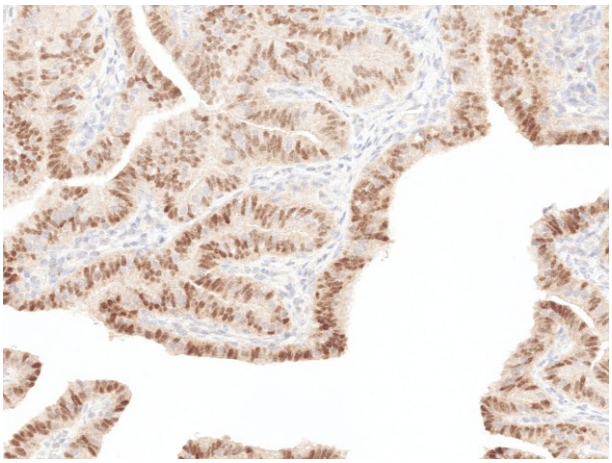


Fig. 2b x200
Insufficient PAX8 staining reaction of the Fallopian tube using the same protocol as in Fig. 1b. A moderate nuclear staining reaction of the intercalated secretory epithelial cells is seen whereas the number and intensity of ciliated epithelial cells are decreased. Compare with Fig. 2a – same field.

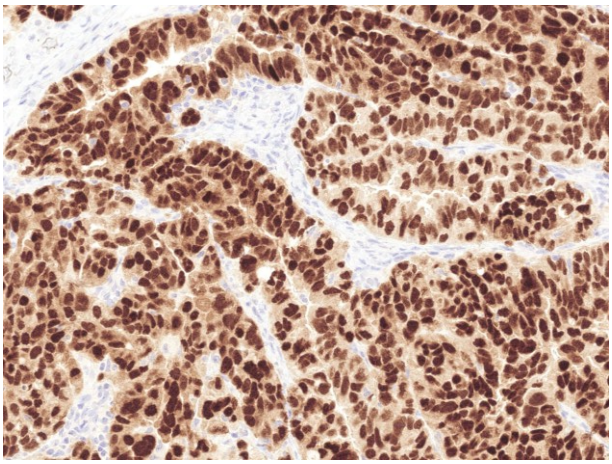


Fig. 3a x200
Optimal PAX8 staining reaction of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1a and 2a. A very strong nuclear staining reaction is seen in virtually all the neoplastic cells. Compare with Fig. 3b.

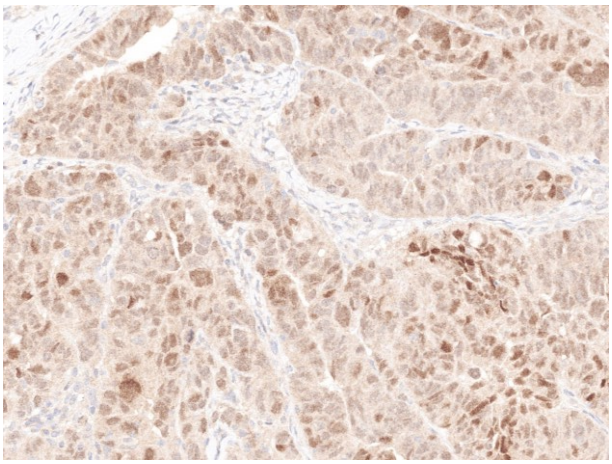


Fig. 3b x200
Insufficient PAX8 staining reaction of the ovarian serous adenocarcinoma using the same protocol as in Figs. 1b and 2b. The majority of the neoplastic cells are demonstrated but display only a weak to moderate nuclear staining reaction. In addition the diffuse cytoplasmic reaction almost obscures the specific nuclear reaction compromising the interpretation. Compare with Fig. 3a – same field.

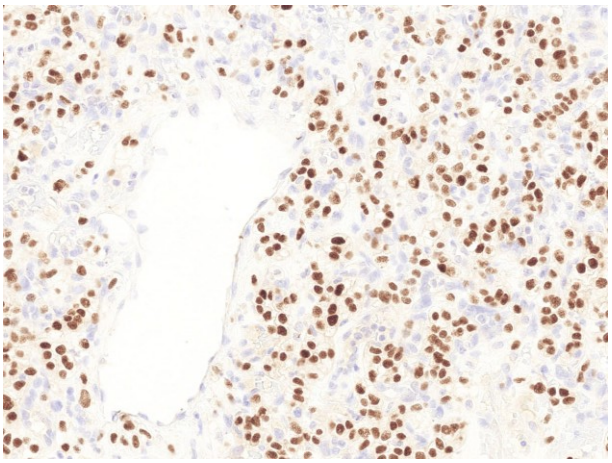


Fig. 4a x200
Optimal PAX8 staining reaction of the ccRCC using the same protocol as in Figs. 1a-3a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. Compare with Fig. 4b.

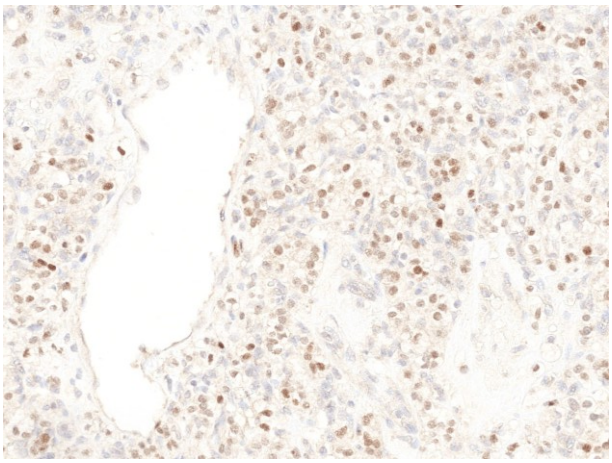


Fig. 4b x200
Insufficient PAX8 staining reaction of the ccRCC using the same protocol as in Figs. 1b-3b.
A reduced number of positive cells is seen and the staining intensity is also reduced compared with Fig. 4a. – same field.

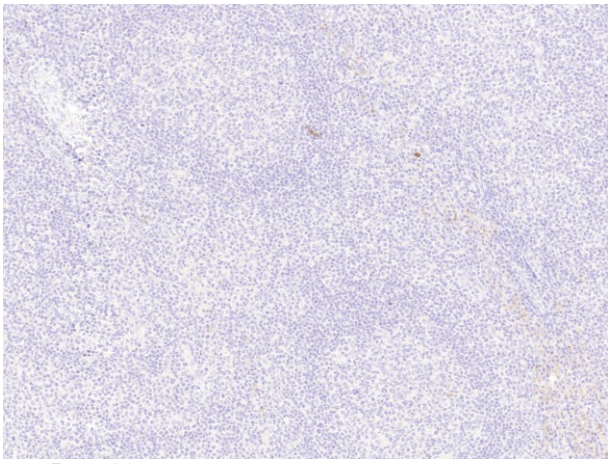


Fig. 5a x100
PAX8 result without PAX5 cross reactivity. PAX8 staining reaction in tonsil using the same protocol as in Figs. 1a-4a. The rmAb clone SP348 does not cross-react with PAX5, leaving the B-cells unstained. Compare with Fig. 5b.

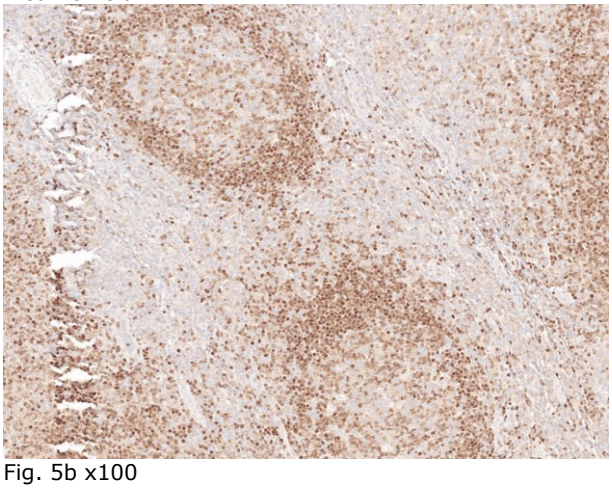


Fig. 5b x100
PAX8 result with PAX5 cross reactivity. PAX8 staining reaction in tonsil using the same protocol as in Figs. 1b-4b. The mAb clone MRQ-50 cross-reacts with PAX5 resulting in a nuclear staining reaction in virtually all B-cells. Compare with Fig. 5a – same field.

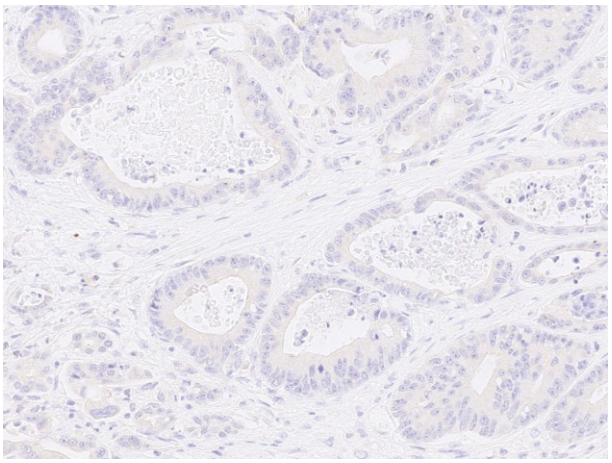


Fig. 6a x200
Optimal PAX8 staining reaction of the colon adenocarcinoma using the same protocol as in Figs. 1a-5a. All the neoplastic cells are, as expected, negative. Compare with Fig. 6b.

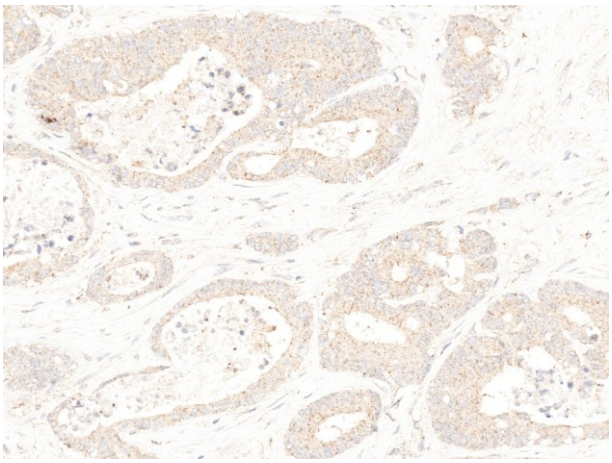


Fig. 6b x200
Insufficient PAX8 staining reaction of the colon adenocarcinoma using the mAb clone MRQ-50 (Ventana/Cell Marque) as RTU within a laboratory developed assay by adding tyramide amplification to the detection kit in order to increase analytical sensitivity on the Ventana BenchMark system. The nuclei of the neoplastic cells remain negative, however, a diffuse granular cytoplasmic reaction is interfering with the interpretation. Compare with the optimal result in Fig. 6a. Also see Fig. 7a, same protocol settings giving an improper balance of analytical sensitivity and specificity.

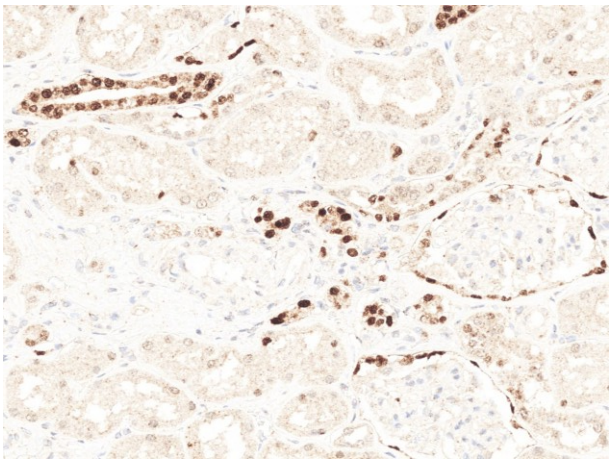


Fig. 7a x200
Insufficient PAX8 staining reaction of the kidney, using the same protocol settings as in Fig. 6b. The tyramide amplification increases the background signal, but the nuclei of the low expressing tubules remain negative.

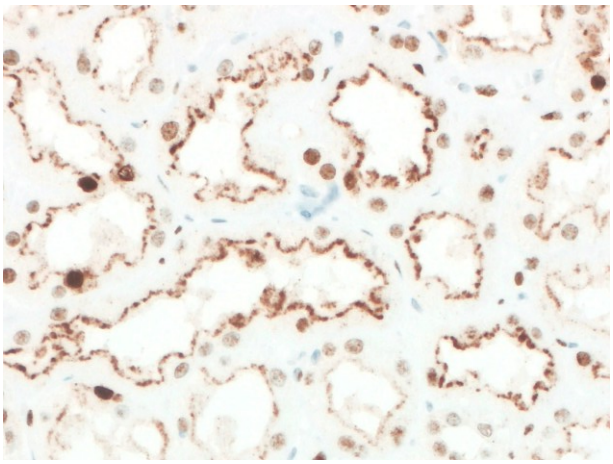


Fig. 7b x200
Staining result for PAX8 of the kidney using an insufficient protocol based on the rmAb clone EP331 as RTU (760-6077, Ventana/Cell Marque) within a laboratory developed assay, using HIER in an alkaline buffer as pretreatment, a 3-step multimer based detection system (OptiView, Ventana/Roche) and performed on the Ventana BenchMark system. Compared to the optimal result of kidney shown in Fig. 1a, this protocol provides a granulated cytoplasmic staining reaction and a strong staining of the glycocalyx in the proximal tubules and at the same time a reduced specific nuclear staining reaction in the cells expected to be distinctively demonstrated.

TJU/SN/KBA/RR 07.07.2026

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*.