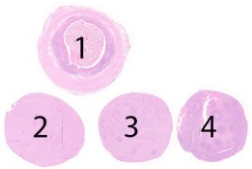
Assessment Run 76 2026
PAX5 (BSAP)

Purpose
Evaluation of the technical performance and the level of analytical sensitivity and specificity of the immunohistochemical (IHC) assays used by NordiQC participants for PAX5. The focus of the assessment was identification of Hodgkin and B-cell lymphomas in characterisation of hematological cancers. Relevant normal and neoplastic clinical tissues were selected to represent a broad range of PAX5 antigen densities (see below).

Material
The slide to be stained for PAX5 comprised:

1. Appendix, 2. Diffuse large B-cell lymphoma (DLBCL), 3. Classical Hodgkin lymphoma, 4. Tonsil.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing PAX5 staining as optimal included:

- A distinct, moderate to strong nuclear staining reaction of normal B-cells (all tissue cores), but especially of mantle zone and germinal centre B-cells in the tonsil.
- An at least weak to moderate, but distinct nuclear staining reaction of virtually all Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma.
- A strong, distinct nuclear staining reaction of all neoplastic cells in the DLBCL.
- No staining reaction of other cells, including T-cells, squamous epithelial cells of the tonsil and columnar epithelial cells of the appendix.

For some clones, aberrant cytoplasmic staining was observed in endothelial and neural cells. This reaction was considered acceptable provided that the staining intensity did not interfere with overall interpretation.

KEY POINTS FOR PAX5 IMMUNOASSAYS
- mAb clone DAK-PAX5 provided the highest proportion of optimal results both as concentrated format and RTU systems.
- rmAb clone SP34 was the most popular clone used by 44% of all laboratories. However, an aberrant staining reaction was observed in 51% of all laboratories affecting the overall pass rate.
- mAb clones 1EW, 24 and the rmAb clone SP34 were often challenged by low analytical sensitivity and/or poor signal-to-noise ratio hindering interpretation of the specific signal for PAX5

Participation

Number of laboratories registered for PAX5, run 76	406
Number of laboratories returning slides	335 (82%)

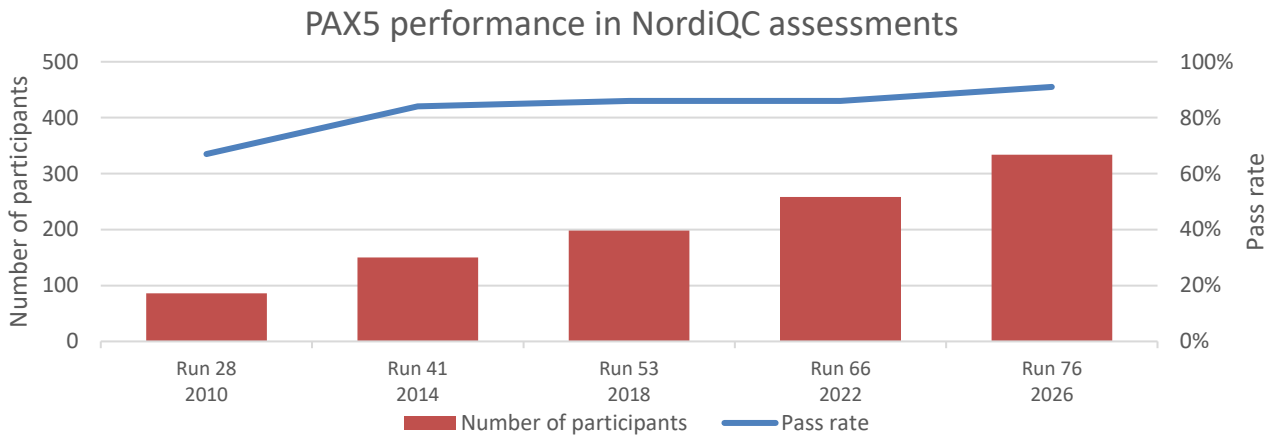
At the time of assessment, 82% of the participants had returned the circulated NordiQC slides.
In this assessment, run 76, general issues with the Danish postal service affected the distribution and return of slides to/from participants, resulting in a lower number of returned slides compared to previous assessments.
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
335 laboratories participated in this assessment and 91% achieved a sufficient mark (optimal or good) see Table 1a (see page 3). 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:
- Less successful and challenging primary antibodies e.g., mAb clones 1EW and 24.
- False positive staining reaction or poor signal-to-noise ratio in assays based on the rmAb SP34.
- Too short HIER used in combination with other low sensitivity protocol parameters.

Performance history
This was the fifth NordiQC assessment of PAX5. The pass rate increased to 91% in this assessment compared to the previous runs 66 and run 53 (86%) as shown in Graph 1.

Graph 1**. Proportion of sufficient results for PAX5 in the five NordiQC runs performed.**



Controls
Tonsil and appendix are recommended as positive and negative tissue controls for PAX5. In the tonsil, protocols must be calibrated to provide a distinct and strong nuclear staining reaction in virtually all mantle zone B-cells, germinal centre B-cells and interfollicular B-cells. In appendix dispersed B-cells in lamina propria must be strongly stained. A weak cytoplasmic staining reaction in B-cells must be accepted. No staining reaction must be seen in other tissue structures including T-cells, stromal cells, epithelial cells of the tonsil and appendix. As a supplement to tonsil and appendix, it is recommended to include a Hodgkin lymphoma, classical subtype, which often displays a weak to moderate nuclear expression in the neoplastic cells.

Conclusion
The mouse monoclonal antibody (mAb) clones **24**, **1EW**, **MX017**, **DAK-PAX5** and the rmAb clones **SP34, IHC115, RBT-PAX5, GR001, C12A5** could all be used to obtain optimal staining results for PAX5. Irrespective of the clone applied, efficient HIER, use of a sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. The mAb clone DAK-PAX5 was found very robust and the Ready-To-Use (RTU) systems from Dako/Agilent based on this clone (IR650 and GA650) provided superior results, when applied by the vendor recommended settings. Protocols based on the mAb clones 1EW, 24 and the rmAb clone SP34 were often challenged by low analytical sensitivity and/or poor signal-to-noise ratio hindering interpretation of the specific signal for PAX5.

Table 1a. **Overall results for PAX5, run 76**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	50	27	16	7	-	86%	54%
Ready-To-Use antibodies	285	170	93	21	1	92%	60%
Total	335	197	109	28	1		
Proportion		59%	32%	8%	1%	91%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for PAX5, run 76**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **DAK-PAX5**	24	Dako/Agilent	15	5	4	-	83%	63%
mAb clone **1EW**	10	Leica Biosystems	4	3	3	-	70%	40%
mAb clone **24**	3	BD Biosciences	3	-	-	-	-	-
	1	Cell Marque	-	1	-	-		
rmAb clone **SP34**	5	Cell Marque	1	4	-	-	100%	14%
	1	Thermo Fisher	-	1	-	-		
	1	Zytomed Systems	-	1	-	-		
rmAb clone **ZR268**	1	Zeta Corporation	1	-	-	-	-	-
rmAb clone **EPR3730(2)**	1	Abcam	-	1	-	-	-	-
rmAb clone **BPM6172**	1	Biolynx Biotechnology	1	-	-	-	-	-
rmAb clone **IHC115**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **RBT-PAX5**	1	BioSB	1	-	-	-	-	-
Total	50		27	16	7	-		
Proportion			54%	32%	14%	0%	86%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for PAX5, run 76**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **SP34 790-4420**[3]	41	Ventana/Roche	22	17	2	-	95%	54%
rmAb clone **SP34 790-4420**[4]	96	Ventana/Roche	46	39	11	-	89%	48%
mAb clone **DAK-PAX5 GA650**[3]	53	Dako/Agilent	44	9	-	-	100%	83%
mAb clone **DAK-PAX5 GA650**[4]	26	Dako/Agilent	25	1	-	-	100%	96%
mAb clone **DAK-PAX5 IR650**[3]	5	Dako/Agilent	5	-	-	-	100%	100%
mAb clone **DAK-PAX5 IR650**[4]	15	Dako/Agilent	9	5	1	-	93%	60%
mAb clone **1EW PA0552**[3]	14	Leica Biosystems	4	7	3	-	79%	29%
mAb clone **1EW PA0552**[4]	12	Leica Biosystems	3	8	1	-	92%	25%
rmAb clone **EP156 8500-C010**	3	Sakura Finetek	3	-	-	-	-	-
rmAb clone **SP34 312R-18**	3	Cell Marque	1	2	-	-	-	-
rmAb clone **JRMR-74 BL6011**	1	Guangzhou Biotron	1	-	-	-	-	-
rmAb clone **517B5E6 PA107**	1	Abcarta	1	-	-	-	-	-
rmAb clone **GR001 GT2096**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **C12A5 CPM-0244**	1	Celnovte	1	-	-	-	-	-
rmAb clone **EP156 PR064**	1	PathNSitu	-	1	-	-	-	-
rmAb clone **DY49712 4910392**	1	Dakewe	-	1	-	-	-	-
mAb clone **ZM26 Z2354MP**	1	Zeta Corporation	1	-	-	-	-	-
mAb clone **24 PM207**	1	Biocare Medical	-	-	-	1	-	-
mAb clone **24 312M-18**	2	Cell Marque	-	-	2	-	-	-
mAb clone **24 701175**	1	Monosan	-	-	1	-	-	-
mAb clone **BY169 BFM-0492**	1	Bioin Biotechnology	-	1	-	-	-	-
mAb clone **MX017 MAB-0706**	1	Fuzhou Maixin	1	-	-	-	-	-
mAb clone **MX017 MAD-000694QD**	4	Master Diagnostica/ Vitro S.A	2	2	-	-	-	-
Total	285		170	93	21	1		
Proportion			60%	32%	7%	1%	92%	

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).

Detailed analysis of PAX5, Run 76

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **DAK-PAX5**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (4/5)*, Cell Conditioning 1 (CC1; Ventana/Roche) (7/15), Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (3/3) or Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 18 of 19 (95%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **1EW**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (1/1) or BERS2 (Leica Biosystems) (3/7). The mAb was diluted in the range of 1:40-1:80 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 7 (86%) laboratories produced a sufficient staining result.

rmAb clone **SP34**: Only one protocol produced an optimal result. This protocol was based on HIER using BERS2 (Leica Biosystems) (1/1). The rmAb was diluted 1:75 and Bond Refine (Leica Biosystems) was used as the detection system.

Table 2. **Proportion of optimal results for PAX5 for the most commonly used antibody concentrates on the four main IHC systems by optimal 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	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **DAK-PAX5**	-	-	4/4	-	50% (6/12)	-	1/1	2/2
mAb clone **1EW**	1/1	-	-	-	-	-	50% (3/6)	-
rmAb clone **SP34**	-	-	-	-	-	-	1/1	-

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

Ready-To-Use antibodies and corresponding systems

rmAb clone **SP34**, product no. **790-4420**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Optimal protocols using UltraView (760-500)/UltraView with amplification (760-500 + 760-080) as detection system were typically based on HIER using CC1 (efficient heating time 36-64 min.) and 16-36 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 32-64 min.) and 16-32 min. incubation of the primary Ab.
Using these protocol settings, 102 of 109 (94%) laboratories produced a sufficient staining result.
The product was used by 2 laboratories on a non-intended platform. These data are not included.

mAb clone **DAK-PAX5**, product no. **GA650**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C), 15-30 min. incubation of the primary Ab and EnVision FLEX or Flex+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 74 of 74 (100%) laboratories produced a sufficient staining result.
The product was used by 3 laboratories on a non-intended platform. These data are not included here.

mAb clone **DAK-PAX5**, product no. **IR650**, Dako/Agilent, Autostainer:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 6.1 (3-in-1) or TRS pH 9 (3-in-1) (efficient heating time 15-20 min. at 97°C), 20-40 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002+K8022/K8021) as detection systems. Using these protocol settings, 12 of 12 (100%) laboratories produced an optimal staining result.
The product was used by 8 laboratories on a non-intended platform. These data are not included here.

mAb clone **1EW**, product no. **PA0552**, Leica Biosystems, Bond III, Prime, Max:
Protocols with optimal results were based on HIER using BERS2 pH 9 (efficient heating time 20-30 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 20 of 24 (83%) laboratories produced a sufficient staining result.

Table 3 (see page 6) summarizes 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 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 3. **Proportion of sufficient and optimal results for PAX5 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS BenchMark rmAb SP34 **790-4420** **UltraView DAB**	95% (39/41)	54% (22/41)	87% (32/37)	49% (18/37)
VMS BenchMark rmAb SP34 **790-4420** **OptiView DAB**			90% (51/57)	47% (27/57)
Dako Omnis mAb DAK-PAX5 **GA650**	100% (53/53)	83% (44/53)	100% (23/23)	87% (20/23)
Dako AS mAb DAK-PAX5 **IR650/IS650**	100% (5/5)	100% (5/5)	100% (7/7)	71% (5/7)
Leica BOND mAb 1EW **PA0552**	79% (11/14)	29% (4/14)	92% (11/12)	25% (3/12)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant 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 fifth NordiQC assessment for PAX5, the prevalent features of insufficient staining results were characterised either by a generally too weak/false negative staining reaction of the cells expected to be demonstrated or a poor-signal-to noise ratio (primarily observed by the use of the rmAb clone SP34 or mAb clone 1EW). Too weak or false negative staining results were observed in 76% of the insufficient results (22/29). Virtually all laboratories were able to detect PAX5 in high-level antigen expressing cells (normal B-cells and neoplastic cells in the DLBCL) whereas demonstration of PAX5 in low-level antigen expressing cells as Reed-Sternberg cells of the Hodgkin lymphoma was more challenging and could only be obtained with optimally calibrated protocols.
Poor-signal-to noise ratio or false positive staining results were observed in 24% of the insufficient results (7/29). In general, the use of the rmAb clone SP34, both within a laboratory developed (LD) and Ready-To-Use (RTU) assay, provided an aberrant cytoplasmic staining reaction in various stromal cells e.g., endothelial cells, lymphocytes and peripheral nerve cells. This aberrant reaction pattern was also observed in the previous runs for PAX5 and currently, no obvious parameters (e.g., lot numbers) have been identified to generate these deviating staining results. Some vendors of the SP34 clones describe this reaction pattern in the package insert as to be suspected.

The mAb clones **DAK-PAX5**, **1EW** and the rmAb clone **SP34** were the most widely used antibodies for demonstration of PAX5 and applied by 92% (307/335) of the laboratories (see Tables 1b-c). Used as concentrated format within laboratory developed (LD) assays the mAb clone DAK-PAX5 gave the highest proportion of optimal results - 63% (15/24).

The **mAb clone DAK-PAX5** provided optimal results on 3 of 4 main IHC platforms as shown in Table 2. The most common reason for an insufficient staining result was related to too diluted primary Ab causing a too weak expression. In this assessment, 15 protocols based on the concentrated format of DAK-PAX5 provided an optimal result. All used a 3-step detection system providing high analytical sensitivity. HIER could both be performed in high or low pH buffers. However, only protocols using acidic HIER on the Bond platforms were submitted in this assessment.

The **mAb clone 1EW** used within LD assays gave inferior results compared to protocols based on the mAb clone DAK-PAX5. 1EW provided a relatively low pass rate (70%) and proportion of optimal results (40%) (see Table 1b). The mAb clone 1EW was primarily used on the Leica Bond systems. The inferior results seemed to be mainly caused by either too low concentration of the primary Ab or by protocols with low analytical sensitivity resulting in a too weak PAX5 staining of especially the Reed-Sternberg cells of the Hodgkin lymphoma. In addition, it was noted that the 1EW antibody tended to give an aberrant cytoplasmic staining reaction of smooth muscle cells - especially lamina propria muscularis in the appendix. Careful calibration was essential for obtaining optimal results.
As mentioned in previous reports (e.g., run 41), the epitope recognized by 1EW is sensitive to endogenous peroxidase blocking and therefore, the blocking step must be performed after incubation of the primary Ab.

The use of **mAb clone 24** has decreased compared to earlier runs. Previous results indicated that staining performance for mAb clone 24 was dependent on the IHC platform, with reduced performance observed on BenchMark Ultra (Ventana/Roche) and Omnis (Dako/Agilent) compared to the performance on e.g. Autostainer (run 41). In the current run, only protocols performed on Autostainer or Bond III were submitted, and a 100% pass rate was achieved.

For both the mAb clones 1EW and 24, protocols seem challenging to optimise correctly as no conclusive parameters could be identified separating optimal and insufficient results. As this performance pattern has been observed in virtually all assessments for PAX5 (except for mAb clone 1EW in run 53), laboratories struggling to generate a sufficient staining result should consider changing to a more robust antibody (e.g. mAb clone DAK-PAX5).

The concentrated format of **rmAb clone SP34** provided an overall low proportion of optimal results (see Table 1b) and, as observed in previous runs, prone background staining characterised as cytoplasmic staining reaction in endothelial and nerve cells, hampering interpretation of the specific nuclear PAX5 signal. This aberrant reaction pattern was recorded in 57% (4/7) of the protocols and influenced the overall performance of the assays (see Table 1b). Use of the rmAb clone SP34, either within a LD-assay or as an RTU system, was challenging and an acceptable balance between background noise and specific signals was difficult to obtain. Therefore, and as suggested for the mAb clones 1EW and 24, laboratories might get better results selecting a more robust antibody clone.

85% (285/335) of the laboratories used an RTU format for detection of PAX5. This is a significant increase compared to the former run 66 in which 75% (193/258) of the participants applied an RTU format.

In this assessment, the RTU **rmAb clone SP34 (ref. no. 790-4420)**, developed for the BenchMark platform (Ventana/Roche), was the most commonly used RTU format, applied by 47% of laboratories (137/285). When vendor-recommended protocol settings were followed on the BenchMark system, the overall pass rate was 95% (39/41), although only 54% (22/41) were assessed as optimal (see Tables 1c and 3). The low level of optimal results was mainly an effect of the aberrant staining also observed with the concentrated SP34 format which occurred in 51% (70/137) of all protocols, including some that achieved optimal scores.
As shown in Table 4, using the 2-step UltraView detection system (recommended by vendor) in general resulted in a slightly higher pass rate (92%) and proportion of optimal results (51%) compared with OptiView or UltraView with amplification. However, using the more sensitive detection systems intensified the aberrant staining; with the 2-step system, 41% of protocols exhibited aberrant staining, increasing to 59% when a 3-step system was used.

Table 4. **Assessment marks for PAX5, run 76 Ready-To-Use rmAb clone SP34 (790-4420) on Ventana BenchMark and proportion of aberrant staining reaction for only 2-step or 3-step detection systems.**

Detection system	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]	Aberrant staining[3]
2-step UltraView DAB	61	31	25	5	-	92%	51%	41% (25/61)
3-step OptiView DAB UltraView+amp	71	34	30	7	-	90%	48%	59% (42/71)

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Aberrant staining was only registered in cases where the balance between unspecific (e.g. endothelial cells, lymphocytes and peripheral nerve cells) and specific PAX5 signals exceeded the acceptable limit.

OptiView with tyramide amplification was used in 3 cases with mixed results. In two of the cases, the Reed-Sternberg cells of the Hodgkin lymphoma exhibited overly intense staining, making differentiation from B-cells difficult along with intensifying the aberrant staining.
Optimisation of the protocols based on rmAb clone SP34 is challenging and as described above for the concentrated format of the rmAb clone SP34, the main problem seems to be related to the reaction pattern of the primary antibody itself. The protocol must be calibrated to provide the needed analytical sensitivity and staining intensity of cellular structures expected to be positive and at the same time avoid excessive background staining (poor signal-to-noise ratio).

In this assessment, the RTU systems from **Dako/Agilent IR650 and GA650** (both based on the mAb clone DAK-PAX5), developed for the platforms Autostainer and Omnis, respectively, provided the highest proportion of sufficient and optimal results being superior compared both to the level obtained using the

same clone as concentrate within a LD assay and to other RTU systems from the main vendors (see Tables 1b-c and 3).
For the RTU system IR650 designed for the Autostainer using vendor recommended protocol settings, all protocols (5/5) were assessed as optimal. In comparison using vendor recommended protocol settings, the RTU system GA650 for Omnis provided a slightly lower proportion of optimal results – 83% (44/53) but an overall high pass rate of 100%. For both RTU systems, IR650 and GA650, laboratory modified protocol settings (typically adjusting HIER, HIER buffer, incubation time of the primary Ab and/or choice of detection system) could be used with almost same success rate (see Table 3). Both RTU systems apply Envision FLEX+ as detection system. However, and as mentioned in the previous report run 66, the recommendation for HIER is different for the two RTU systems. Using the RTU system IR650 (Autostainer), the recommended HIER buffer is TRS Low pH, whereas for the RTU GA650 system (Omnis), the recommended HIER buffer is TRS High pH.

The RTU mAb clone **1EW product PA0552** (Leica Biosystems) was used by 26 laboratories. The pass rate was relatively low compared to the other main vendors. With the PA0552 product, the proportion of sufficient results was 79% (11/14) and 29% (4/14) optimal results using the vendor recommended protocol settings, where the blocking is preformed after primary Ab incubation.
Protocols using the PA0552 product was only submitted on the Leica Bond III and Bond Prime. On these two platforms, a big difference in number of optimal results was observed even though protocol settings, detection systems and buffers are the same. For the Bond III the overall pass rate was 77% (13/17), only 12% (2/17) being optimal compared to the Bond Prime providing a high pass rate of 100% (9/9) with 80% (5/9) being optimal.
The results assessed as good or borderline were based on protocol settings similar to protocols giving an optimal mark, and thus it is difficult for NordiQC to provide participants with solid recommendations regarding optimisation of the assays. As for the concentrate, it was also noticed that the RTU format had the same tendency to give an aberrant cytoplasmic staining reaction of smooth muscle cells and in the same tissue structures as described above. Laboratory modified protocol settings e.g., HIER in BERS1, prolonging HIER time and incubation time in primary antibody, did not improve the performance of the RTU assay.

Summary
This was the fifth assessment of PAX5 in NordiQC (see Graph 1). The pass rate increased compared to results obtained in the previous run for PAX5. In this assessment, the RTU systems IR/GA650 based on the mAb clone DAK-PAX5 from Dako/Agilent were superior to other RTU systems from the main vendors. Used within LD-assays, the mAb clone DAK-PAX5 also provided a high proportion of sufficient and optimal results. Several antibody clones could be used to obtain an optimal result (see Table 1). However, protocols based on the mAb clones 1EW, 24 and the rmAb clone SP34 were challenged by low analytical sensitivity and/or poor signal-to-noise ratio – often both at the same time.
Importantly, the primary Abs must be carefully calibrated according to the expected antigen level of the recommended control material.

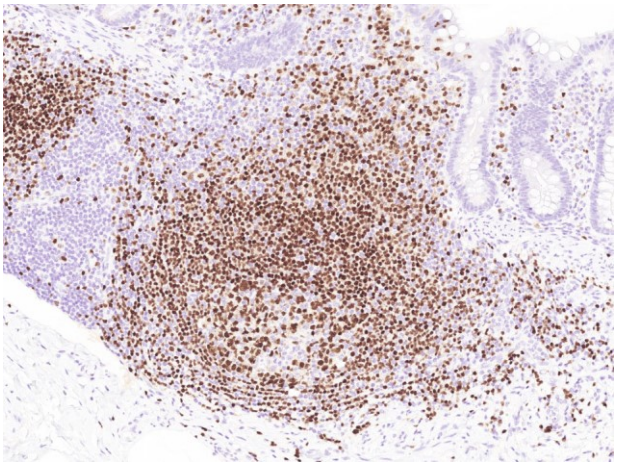


Fig. 1a (x100)
All mantle zone and germinal centre B-cells show a strong and distinct nuclear staining reaction. No staining reaction is observed in other cellular structures including T-cells and epithelial cells of the appendix. Same protocol used in Figs. 2a - 4a.

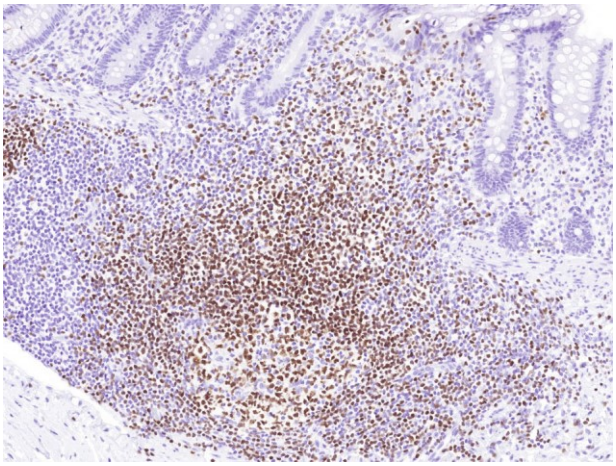


Fig. 1b (x100)
Staining reaction for PAX5 of the appendix using the same clone and protocol settings as used in Fig. 1a, but as a concentrate DAK-PAX5 (M7307, Dako/Agilent) in a dilution of 1:300 - same protocol used in Figs. 2b - 4b. Although the assay gave the expected reaction pattern in the appendix, a slight reduction was observed in the number of cells expected to be stained. The protocol provided too low analytical sensitivity in general - compare with Fig. 1a, same field

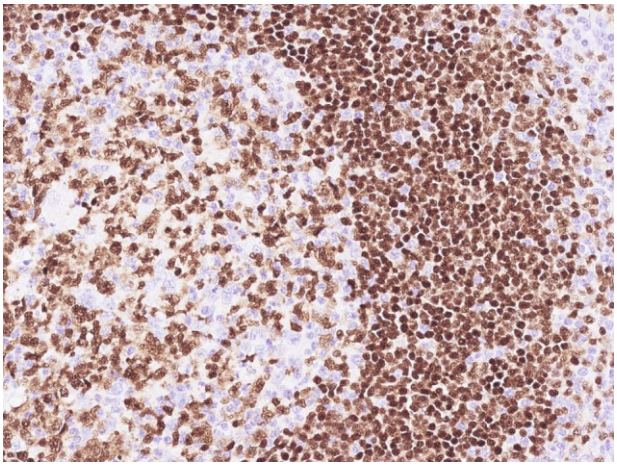


Fig. 2a (x200)
Optimal staining reaction for PAX5 of the tonsil using same protocol as in Fig. 1a. Virtually all B-cells show a strong and distinct nuclear staining reaction. Cytoplasmic staining reaction must be accepted.

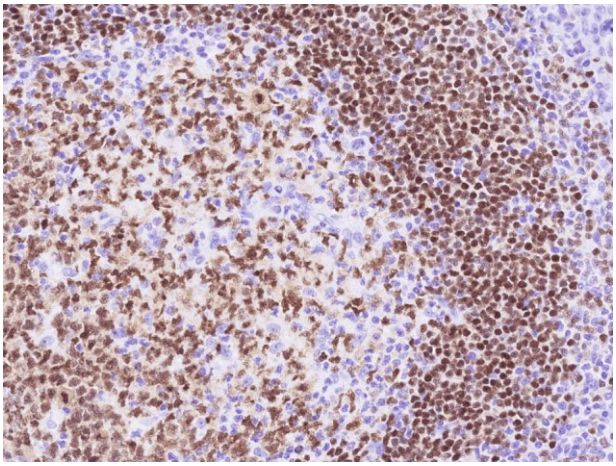


Fig. 2b (x200)
Insufficient staining reaction for PAX5 of the tonsil using same protocol as in Fig. 1b. The proportion and staining intensity of positive B-cells is reduced – compare with Fig. 2a, same field. The overall low analytical sensitivity of the protocol impacted the performance on neoplastic tissue, especially of the Hodgkin lymphoma as illustrated in Figs. 4a-4b.

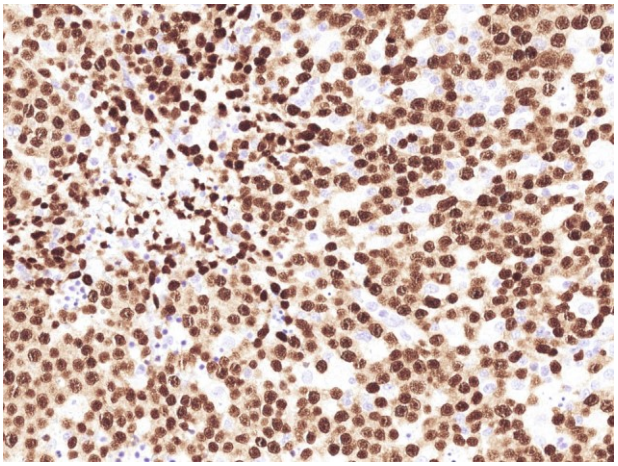


Fig. 3a (x200)
Optimal staining reaction for PAX5 of the DLBCL using same protocol as in Figs. 1a and 2a. All the neoplastic cells display a strong and distinct nuclear staining reaction.

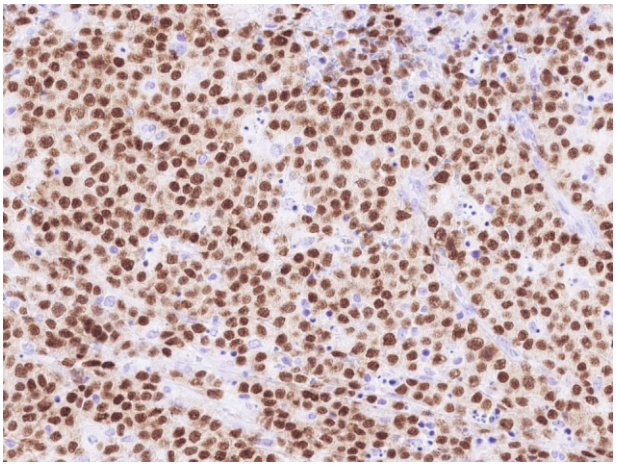


Fig. 3b (x200)
Staining reaction for PAX5 of the DLBCL using same insufficient protocol as in Figs. 1b and 2b. The nuclei of the neoplastic B-cells show a reduced staining intensity – compare with Fig. 3a.

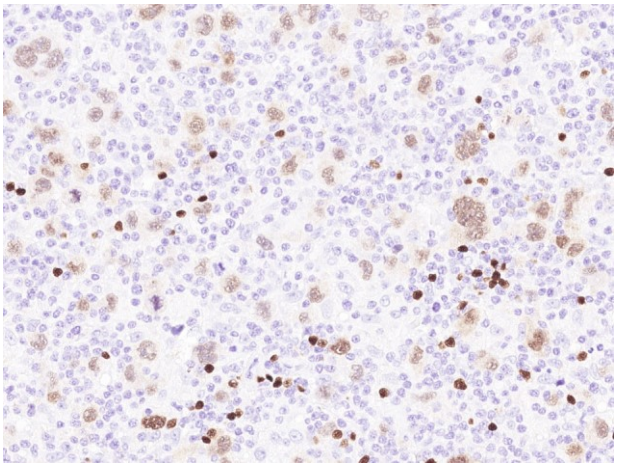


Fig. 4a (x200)
Optimal result for PAX5 of the Hodgkin lymphoma using same protocol as in Figs. 1a – 3a. Virtually all Hodgkin and Reed-Sternberg cells display a weak to moderate, but distinct nuclear staining reaction. Normal B-cells intermingling between the neoplastic cells are strongly stained.

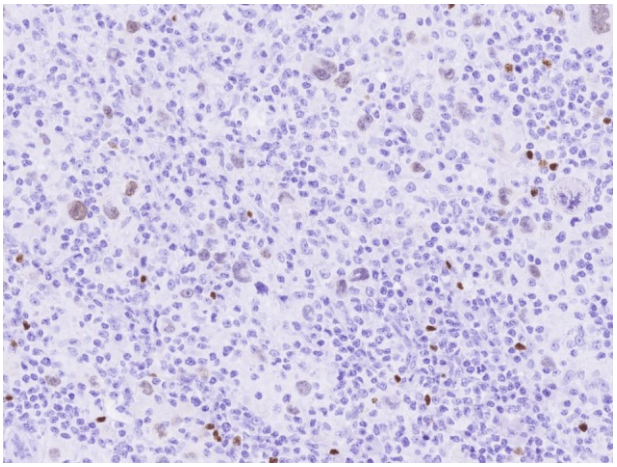


Fig. 4b (x200)
Insufficient staining reaction for PAX5 of the Hodgkin lymphoma, using same protocol as in Figs. 1b - 3b. Most of the neoplastic cells are false negative or only display a faint staining intensity. Normal B-cells intermingling between the neoplastic cells are strongly stained – compare with Fig. 4a.

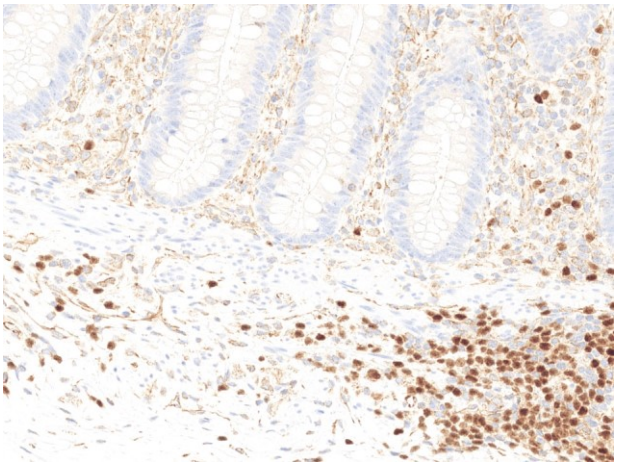


Fig. 5a (x200)
Sufficient staining reaction for PAX5 staining of the appendix using the rmAb clone SP34 (790-4420, Ventana/Roche) on a Ventana BenchMark Ultra. HIER was used in CC1 for 64 min. and incubation of the primary Ab for 36 min. using OptiView as detection system. The described aberrant reaction mainly seen with clone SP34 in endothelial cells, lymphocytes and peripheral nerve cells are clearly displayed in this protocol. This unwanted reaction of stromal cells compromised the interpretation of the specific signal - Also see Fig. 5b same protocol.

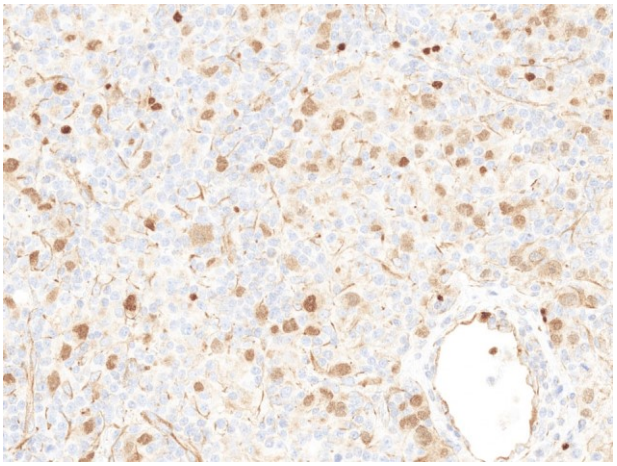


Fig. 5b (x200)
Sufficient staining reaction for PAX5 of the Hodgkin lymphoma using same protocol as in Fig. 5a. Interpretation is difficult due the unwanted reaction of stromal cells. In protocols with weaker expression of the Hodgkin and Reed-Sternberg cells the diagnostic read-out could be even more challenging.

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