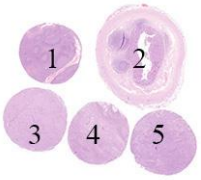
Assessment Run 66 2022

B-cell specific activator protein (BSAP, PAX5)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for BSAP (PAX5), typically identifying classical Hodgkin`s lymphoma and B-cell lymphomas in the diagnostic work-up of hematological neoplasms. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for PAX5 (see below).

Material
The slide to be stained for BSAP comprised:



1. Tonsil, 2. Appendix, 3. Diffuse large B-cell lymphoma (DLBCL), 4-5. Hodgkin's Lymphomas, classical subtypes (CHL).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a BSAP 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's and Reed-Sternberg cells in the Hodgkin's lymphomas (CHL).
- 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.

A weak, cytoplasmic staining reaction in cells with a strong nuclear staining reaction was accepted. Also, a faint cytoplasmic staining reaction of stromal cells (e.g., endothelium) was accepted, providing that interpretation of the specific reactions for BSAP was not compromised (this aberrant staining pattern was primarily seen with rabbit monoclonal antibody (rmAb) clone SP34).

Participation

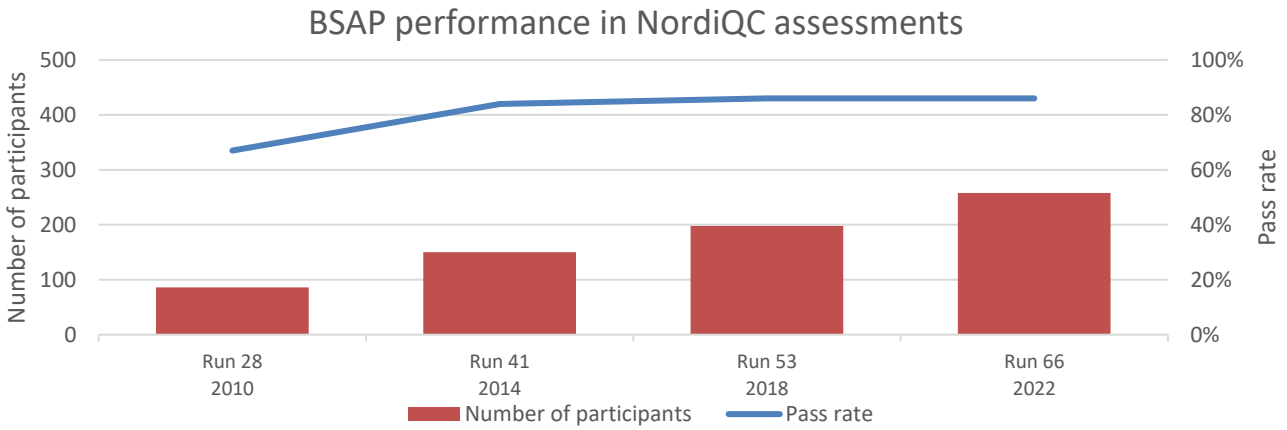
Number of laboratories registered for BSAP, run 66	283
Number of laboratories returning slides	259 (92%)

Results
One laboratory applied an inappropriate antibody and thus, 258 laboratories participated in this assessment. Of these, 221 (86%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 3).
All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

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 of assays based on the rmAb SP34
- Use of low sensitivity detection systems
- Technical issues

Performance history
This was the fourth NordiQC assessment of BSAP. The pass rate was similar to the results obtained in run 41, 2014 and run 53, 2018 (see Graph 1).

Graph 1. **Proportion of sufficient results for BSAP in the four NordiQC runs performed**



Conclusion
The mouse monoclonal antibody (mAb) clones **24**, **1EW**, **MX017, BPM6172**, **DAK-Pax5** and the rmAb clones **SP34, IHC115, RBT-PAX5, QR056, GR001, C12A5** could all be used to obtain optimal staining results for BSAP. Irrespective of the clone applied, efficient HIER, use of 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 BSAP.
Tonsil and appendix are recommended as positive and negative tissue controls: Virtually all B-cells, except plasma cells, must show an as strong as possible nuclear staining reaction (a weak cytoplasmic staining reaction must be accepted). No staining reaction of other tissue structures including T-cells, squamous epithelial cells of the tonsil and columnar epithelial cells of the appendix should be seen.
A Hodgkin's lymphoma (classical type) could be included as control of low level demonstration, in which the neoplastic cells often display a weak to moderate nuclear staining reaction and to unravel if the protocol provides appropriate analytical sensitivity for routine purpose.

Table 1. **Antibodies and assessment marks for BSAP, run 66**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1EW**	9	Leica Biosystems	2	5	1	1	78%	22%
mAb clone **24**	5 2 1	BD Biosciences Biocare Medical Immunologic	1	4	2	1	63%	13%
mAb clone **DAK-Pax5**	31	Dako/Agilent	16	12	3	0	90%	52%
mAb clone **BPM6172**	1	Biolynx tech.	1	0	0	0	-	-
rmAb clone **IHC115**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **RBT-PAX5**	1	Bio SB	1	0	0	0	-	-
rmAb clone **D7H5X**	1	Cell Signaling Tech.	0	1	0	0	-	-
rmAb clone **SP34**	9 1 1 1	Cell Marque Thermo Fisher Zytomed Systems DCS	4	3	4	1	58%	33%
rmAb **clone QR056**	1	Quartett	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **1EW PA0552**[3]	8	Leica Biosystems	2	5	1	0	88%	25%
mAb clone **1EW PA0552**[4]	5	Leica Biosytems	0	3	1	1	60%	0%
mAb clone **BC/24 PM207**	1	Biocare Medical	0	1	0	0	-	-
mAb clone **24/Pax-5 AM967**	1	BioGenex	0	1	0	0	-	-
mAb clone **MX017 MAB-0706**	1	Maixin	1	0	0	0	-	-
mAb clone **MX017 MAD-000694QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb clone **DAK-Pax5 IR650**[3]	10	Dako/Agilent	10	0	0	0	100%	100%
mAb clone **DAK-Pax5 IR650**[4]	10	Dako/Agilent	7	1	2	0	80%	70%
mAb clone **DAK-Pax5 GA650**[3]	40	Dako/Agilent	33	6	1	0	98%	83%
mAb clone **DAK-Pax5 GA650**[4]	21	Dako/Agilent	17	2	2	0	90%	81%
rmAb clone **EP156 8500-C010**	1	Sakura Finetek	0	0	1	0	-	-
rmAb clone **SP34 790-4420**[3]	17	Ventana/Roche	5	9	3	0	82%	29%
rmAb clone **SP34 790-4420**[4]	69	Ventana/Roche	27	34	8	0	88%	39%
rmAb clone **SP34 312R-18**	3	Cell Marque	1	0	2	0	-	-
rmAb clone **517B5E6 PA107**	1	Abcarta	0	0	0	1	-	-
rmAb clone **GR001 GT2096**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **C12A5 CPM-0244**	1	Celnovte	1	0	0	0	-	-
pAb **BRB027**	1	Zytomed Systems	0	0	0	1	-	-
Total	258		133	88	31	6	-	
Proportion			52%	34%	12%	2%	86%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols)

Detailed analysis of BSAP, Run 66
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) (4/8)*, Cell Conditioning 1 (CC1; Ventana/Roche) (7/17), HIER buffer H (Epredia) (1/1) or Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (3/3) 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, 22 of 24 (92%) laboratories produced a sufficient staining result. One laboratory obtained an optimal result without performing any pre-treatment at all.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **1EW**: Protocols with optimal results were based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (2/5). The mAb was diluted in the range of 1:40-1:80 and Bond Refine (Leica Biosystems) was used as the detection system.

mAb clone **24**: One protocol with an optimal result was based on HIER using BERS2 (1/3) as retrieval buffer. The mAb was diluted 1:75 and Bond Refine was used as the detection system.

mAb clone **BPM6172**: One protocol with an optimal result was based on HIER using TRS pH 9 (3-in-1) as retrieval buffer. The mAb was diluted 1:1600 and Envision FLEX+ (Dako/Agilent) was used as the detection system.

rmAb clone **SP34**: Protocols with optimal results were based on HIER using CC1 (2/6), TRS pH 9 (3-in-1) (1/2) and BERS1 (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 7 of 9 (78%) laboratories produced a sufficient staining result.

rmAb clone **RBT-PAX5**: One protocol with an optimal result was based on HIER using CC1 as retrieval buffer. The mAb was diluted 1:50 and OptiView (Ventana/Roche) was used as the detection system.

rmAb clone **QR056**: One protocol with an optimal result was based on HIER using BERS1. The rmAb was diluted 1:75 and Bond Refine was applied as detection system.

Table 2. **Proportion of optimal results for BSAP for the three most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark Ultra**		**Leica Biosystems Bond III**	
	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
mAb clone **DAK-Pax5**	1/3**	-	3/3	-	5/15 (33%)	-	0/1	2/2
mAb clone **1EW**	-	-	-	-	-	-	2/4	-
rmAb clone **SP34**	-	-	1/2	-	2/5 (40%)	-	-	1/1

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **1EW**, product no. **PA0552**, Leica Biosystems, Bond-III:
Protocols with optimal results were based on HIER using BERS2 pH 9 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 7 of 8 (88%) laboratories produced a sufficient staining result.

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 20 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002+K8022/K8021) as detection systems. Using these protocol settings, 9 of 9 (100%) laboratories produced an optimal staining result.

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 30 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 39 of 40 (98%) laboratories produced a sufficient staining result.

mAb clone **MX017**, product no. **MAB-0706**, Fuzhou Maixin, Titan-Stainer:
One protocol with an optimal result was based on HIER in DNS buffer (efficient heating time 18 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super (TT-0805) as detection system.

rmAb clone **SP34**, product no. **790-4420**, Ventana/Roche, BenchMark Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab. and UltraView (760-500)/UltraView with amplification (760-500 + 760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 52 of 56 (93%) laboratories produced a sufficient staining result.

Table 3 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 according 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 BSAP for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb DAK-Pax5 **IR650**	100% (10/10)	100% (10/10)	100% (7/7)	86% (6/7)
Dako Omnis mAb DAK-Pax5 **GA650**	98% (39/40)	83% (33/40)	89% (16/18)	78% (14/18)
Leica BOND III mAb 1EW **PA0552**	88% (7/8)	25% (2/8)	75% (3/4)	0% (0/4)
VMS Ultra/XT/GX rmAb SP34 **790-4420**	82% (14/17)	29% (5/17)	88% (60/68)	40% (27/68)

* 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 are integrated.

Comments
In this fouth NordiQC assessment for BSAP, the prevalent features of insufficient staining results were characterized 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). Too weak or false negative staining results were observed in 62% of the insufficient results (23/37). Virtually all laboratories were able to detect BSAP in high-level antigen expressing cells, e.g., normal B-cells in the tonsil and of the neoplastic cells in the diffuse large B-cell lymphoma, whereas demonstration of BSAP in low-level antigen expressing cells as Reed-Sternberg cells of the Hodgkin's lymphomas (classical type) 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 35% of the insufficient results (13/37). 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 run 53 for BSAP and currently, no obvious parameters (e.g., lot numbers) have been identified to generate these deviating staining results. One protocol assessed as insufficient provided a too weak staining result in combination with excessive background staining.

The mAb clones Dak-Pax5, 1EW and the rmAb clone SP34 were the most widely used antibodies for demonstration of BSAP and applied by 91% (235/258) of the laboratories (see Table 1). Used as concentrated format within laboratory developed (LD) assays, and compared to other clones, the mAb clone DAK-Pax5 gave the highest proportion of sufficient and optimal results, 90% (28/31) and 52% (16/31), respectively. As shown in Table 2, the mAb clone DAK-Pax5 provided optimal results on all main IHC platforms. The most common reasons for an insufficient staining result were primarily related to technical issues e.g., impaired morphology, granular precipitation of the chromogen compromising the interpretation (OptiView with tyramide signal amplification) and excessive background. In this assessment, 16 protocols based on the DAK-Pax5 as concentrate provided an optimal result and the protocol settings typically providing high analytical sensitivity as e.g., efficient HIER in an alkaline buffer (75%, 12/16), a relative high working concentration (dilution range 1:20-1:50) of the primary antibody (81%, 13/16) and the use of a 3-step polymer/multimer detection system (94%, 15/16). Using the same protocol settings, except for substituting a 3-step polymer/multimer with a 2-step polymer/multimer detection system, the proportion of optimal results was only 20% (1/5). However, optimal results could also be obtained using the acidic HIER buffer BERS1 (Leica Biosystems) on the BOND platform as long as laboratories (3/3) calibrated the titer of the primary antibody carefully (dilution range between 1:30-1:100).

The mAb clones 1EW and 24 used within LD assays gave inferior results compared to protocols based on the mAb clone DAK-PAX5. These two clones provided a relatively low pass rate and most importantly a very low proportion of optimal results (see Table 1). Grouped together, 65% (11/17) of the laboratories used one of these two clones on the BOND III platform (Leica Biosystems) and obtained an acceptable overall pass rate of 91% (10/11) however only 27% (3/11) being optimal. In comparison, and using other platforms than BOND III, the pass rate was only 33% (2/6) - none being optimal. In addition, and for users of the mAb clone 1EW, it was noted that this primary antibody tended to give an aberrant cytoplasmic staining reaction of smooth muscle cells - in particular of lamina propria muscularis in the appendix. This was seen in 33% (3/9) of the protocols and although data is limited, related to use of the following lot numbers: 6094449, 6092057 and 6060913.
As mentioned in previous reports (e.g., run 41), the epitope to 1EW is sensitive to endogenous peroxidase blocking and therefore, the blocking step must be performed after incubation of the primary Ab.
Also, the performance of mAb clone 24 is affected by the chosen IHC platform and a decline in staining performance can be seen on e.g., the Benchmark Ultra (Ventana/Roche) or Omnis (Dako/Agilent) compared to the performance on e.g. Autostainer (BSAP NordiQC run 41). Therefore, and due to the low proportion of optimal results, protocols based on these two antibodies seems challenging to optimize correctly as no conclusive parameters could be identified separating an optimal from an insufficient result. As this performance pattern have been observed in virtually all assessments for BSAP (except for mAb clone 1EW in run 53), laboratories struggling to generate an appropriate staining result using mAb clone 1EW and 24, should consider changing to a more robust antibody as e.g., mAb clone DAK-Pax5.

The concentrated format of rmAb clone SP34 also provided an overall low pass rate and proportion of optimal results (see Table 1) and as observed in previous runs, prone to give background staining characterized as cytoplasmic staining reaction in e.g., endothelial and nerve cells, hampering interpretation of the specific nuclear BSAP signal. This aberrant reaction pattern was recorded in 50% (6/12) of the protocols and influenced the overall performance of the assays (see Table 1). The majority of protocols were applied on the Benchmark Ultra (7/12) providing 57% (4/7) sufficient results and 29% (2/7) being optimal. Use of the rmAb clone SP34, either within a LD-assay or as 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.

75% (193/258) of the laboratories used an RTU format for detection of BSAP. This is a significant increase compared to the former run 53 in which 66% (131/198) of the participants applied a RTU format. 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 1 and 3).
For the RTU system IR650 designed for the Autostainer, and using vendor recommended protocol settings, all protocols (10/10) were assessed as optimal. In comparison, and using vendor recommended protocol settings, the RTU system GA650 for Omnis provided a slightly lower proportion of optimal results – 83% (33/40)- but a high overall pass rate of 98%. 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 the fundamental detection system. However, and as mentioned in the previous report run 53, 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. In three of the five protocols assessed as insufficient HIER was based on TRS Low pH on the Omnis (not recommended by the vendor), one laboratory used the RTU format IR650 on the Benchmark Ultra (not validated for this particular platform) and one laboratory, although applying optimal protocol settings for the RTU system GA650, were downgraded due to unexplained technical issues.

Within the RTU family for BSAP, 7% (13/193) of the laboratories used the RTU product PA0552 based on the mAb clone 1EW (Leica Biosystems). As shown in Table 3, the proportion of sufficient results was relatively high (88%) but the proportion of optimal results was low (25%) applying vendor recommended protocol settings. The results assessed as good or borderline were based on protocol settings completely similar to protocols giving an optimal mark, and thus it is difficult for NordiQC to provide participants with solid recommendations regarding optimization 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. This was seen in 58% (7/12) of the protocols using the following lot numbers; 71976, 71840, 71027 and 69644 but not seen with the lot numbers 68332 and 72567, both giving optimal results. 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.

Within RTU formats, 45% (85/193) of the laboratories applied the RTU system 790-4420 based on the rmAb clone SP34 and developed for the BenchMark platform (Ventana/Roche). One laboratory used the RTU product on a non-validated platform (BOND III, Leica Biosystems). Applying vendor recommended protocol settings on BenchMark, the pass rate was 82% (14/17) but only 29% (5/17) were assessed as optimal (see Table 3). For all results assessed as optimal, both based on vendor and laboratory modified protocol settings, the following conditions were observed; 81% (26/32) used a 3-step multimer based detection system (Optiview or UltraView with amplification), the median HIER time in CC1 was 52 min. (range 24-64 min. at 95-100ºC) and the median incubation time in primary antibody was 28 min. (range 16-60 min. at 36-37 ºC). In comparison, and for results assessed as insufficient, the following protocol parameters was observed; 55% (6/11) of the laboratories used a 3-step multimer based detection system (Optiview or UltraView with amplification), the median HIER time in CC1 was 47 min. (range 8-64 min. at 95-100ºC) and the median incubation time in primary antibody was 29 min. (range 16-40 min. at 36-37 ºC). These data indicate, that the most critical and successful parameter discriminating an optimal result from an insufficient result was related to the application of a 3-step multimer based detection system, whereas HIER time and primary Ab incubation time was of less importance. However, optimization 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). As described above the rmAb clone SP34 typically gave an extensive cytoplasmic staining reaction in non-BSAP expressing cells as endothelial cells and stromal cells compromising the read-out (see Figs. 5b and 6b). This problem was seen in 36% (4/11) of the insufficient results, but also seen in 56% (24/43) results assessed as good.

This was the fourth assessment of BSAP in NordiQC (see Graph 1). The pass rate was similar compared to results obtained in the previous run for BSAP. 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 careful calibrated according to the expected antigen level of the recommended control material (see below).

Controls
Tonsil and appendix are recommended as positive and negative tissue controls for BSAP. 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's lymphoma, classical subtype, which often displays a weak to moderate nuclear expression in the neoplastic cells.

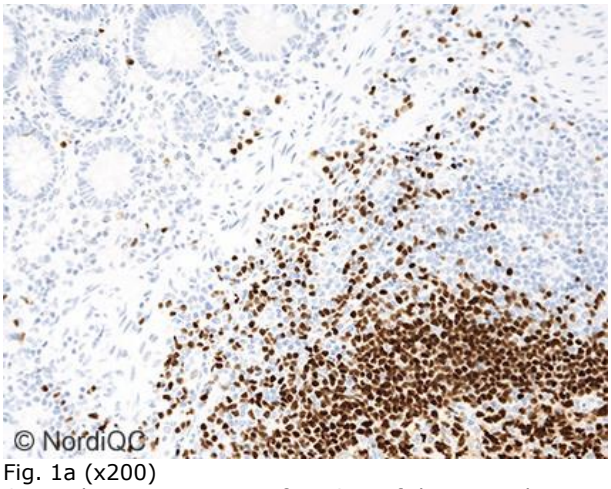


Fig. 1a (x200)
Optimal staining reaction for BSAP of the appendix using the RTU assay GA650 (Omnis, Dako/Agilent) based on the mAb clone DAK-Pax5, following the vendor recommended protocol settings based on HIER in TRS (3-in-1) pH 9 and Envision FLEX+ as detection system. All mantle zone and germinal 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.

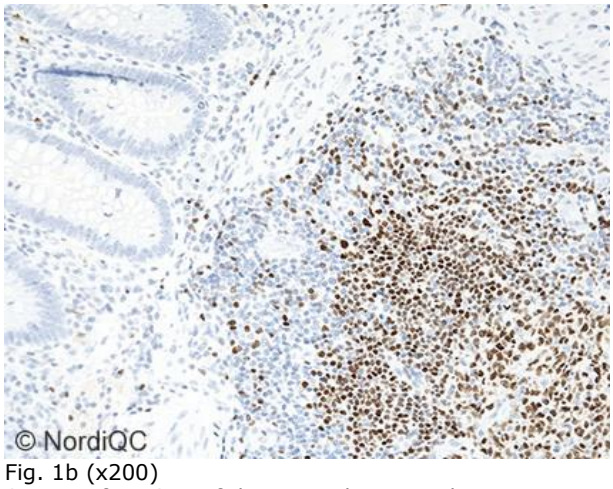


Fig. 1b (x200)
Staining for BSAP of the appendix using the same RTU system as in Fig. 1a, but with laboratory modified protocol settings: HIER in TRS (3-in-1) pH 6.1 and the less sensitive detection system Envision FLEX - same protocol used in Figs. 2b - 4b.
Although the assay gave the expected reaction pattern in the appendix, the protocol provided too low analytical sensitivity - compare with Fig. 2a-4b.

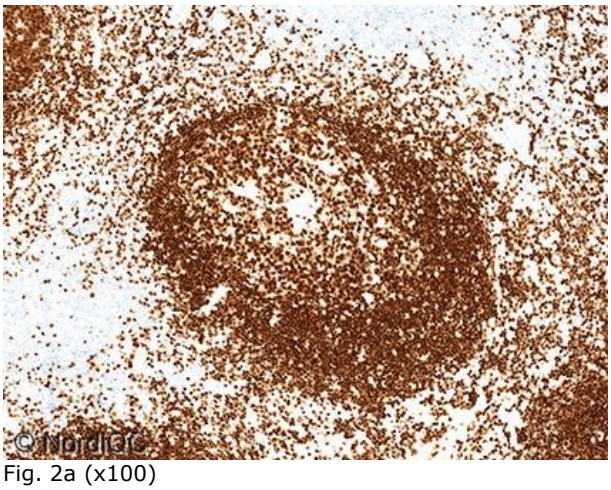


Fig. 2a (x100)
Optimal staining reaction for BSAP of the tonsil using same protocol as in Fig. 1a. Virtually all B-cells show a strong and distinct nuclear staining reaction. Cytoplasmic staining reaction must be accepted.

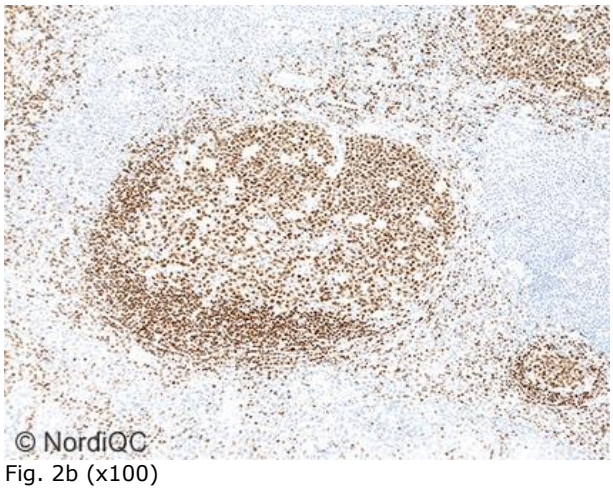
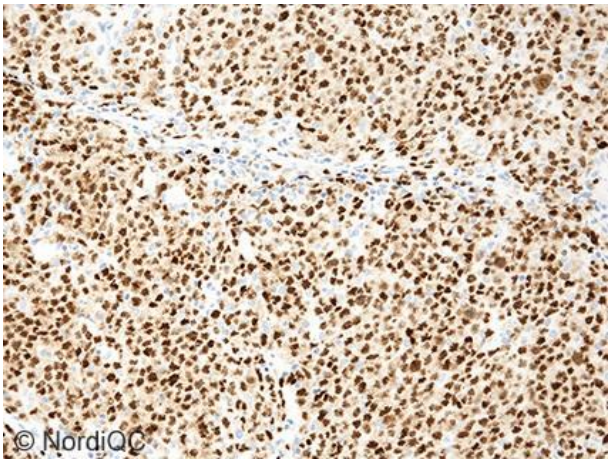


Fig. 2b (x100)
Insufficient staining reaction for BSAP of the tonsil using same protocol as in Fig. 1b. The proportion and staining intensity of positive B-cells is significantly reduced – compare with Fig. 2a. The overall low analytical sensitivity of the protocol impacted the performance on neoplastic tissue, especially of the Hodgkin's lymphomas, classical subtypes, as illustrated in Figs. 4a-4b.



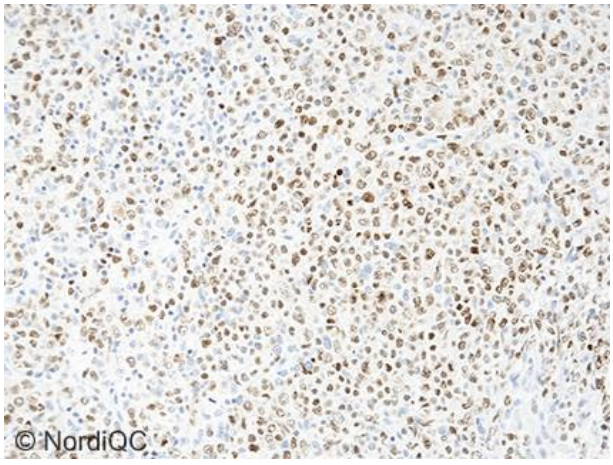


Fig. 3a (x200)
Optimal staining reaction for BSAP staining of the DLBCL using same protocol as in Figs. 1a and 2a. All the neoplastic cells display a strong and distinct nuclear staining reaction.

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

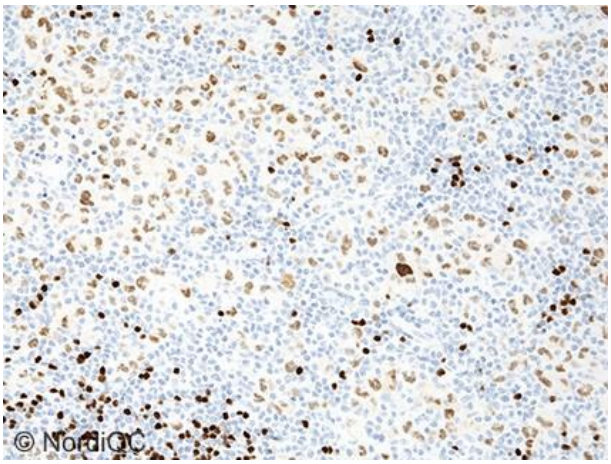


Fig. 4a (x200)
Optimal result for BSAP of the Hodgkin's lymphoma, classical subtype (tissue core 5), using same protocol as in Figs. 1a – 3a. Virtually all Hodgkin and Reed-Sternberg cells display a weak to moderate, but distinct nuclear staining reaction. Normal B-cells intermingling between the neoplastic cells are strongly stained.

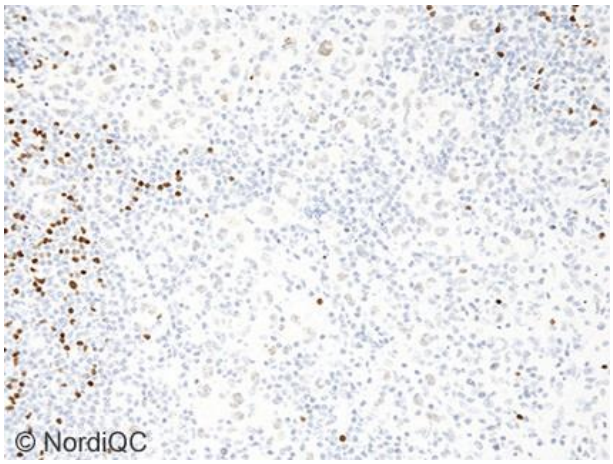


Fig. 4b (x200)
Insufficient staining reaction for BSAP of the Hodgkin's lymphoma, classical subtype (tissue core 5), using same protocol as in Figs. 1b - 3b. The vast majority of the neoplastic cells are false negative or only display a faint staining intensity.

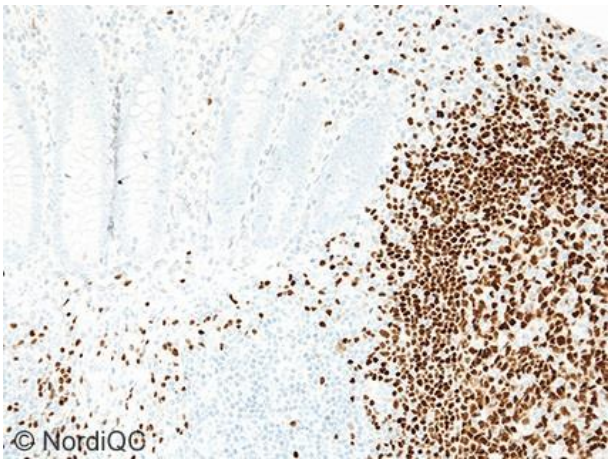


Fig. 5a (x200)
Optimal staining reaction for BSAP of the appendix using the RTU assay 790-4420 based on the rmAb clone SP34. The protocol was carefully calibrated using HIER in CC1 (32 min. at 100°C), incubation time in primary Ab for 24 min. and use of OptiView as the detection system. By doing so, the laboratory avoided problems with an unacceptable aberrant cytoplasmic staining reaction providing a poor signal-to-noise ratio, typically seen in stromal cells as e.g., endothelial and nerve cells using this RTU product (see description above).

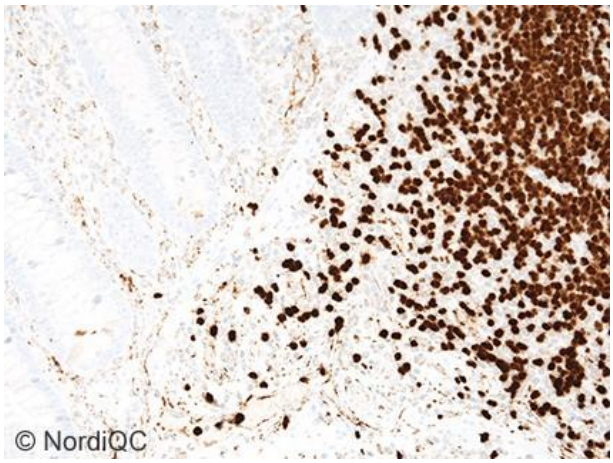


Fig. 5b (x200)
Insufficient staining reaction for BSAP staining of the appendix using the same RTU product as in Fig. 5a, but with extended HIER time in CC1 (48 min. at 95°C) extended incubation time in primary Ab (32 min.) and OptiView with amplification. These protocol settings (relative high analytical sensitivity), enhanced the staining intensity not only of B-cells expected to be demonstrated, but also of the unwanted reaction of stromal cells compromising interpretation of the specific signal – compare with Figs. 5a-6b.

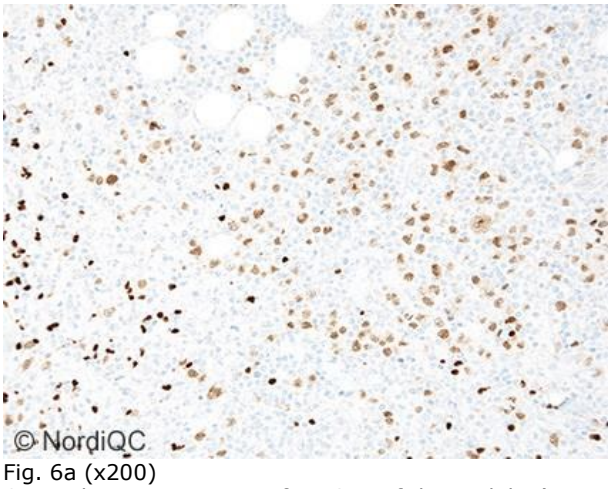


Fig. 6a (x200)
Optimal staining reaction for BSAP of the Hodgkin's Lymphoma, classical subtype (tissue core 4), using same protocol as in Fig. 5A. The protocol gave the expected reaction pattern and a good contrast/balance of staining intensity between neoplastic cells and normal B-cells was obtained. In addition, the aberrant staining reaction of stromal calls was attained to a minimal level and thus, did not hinder interpretation of the specific signal.

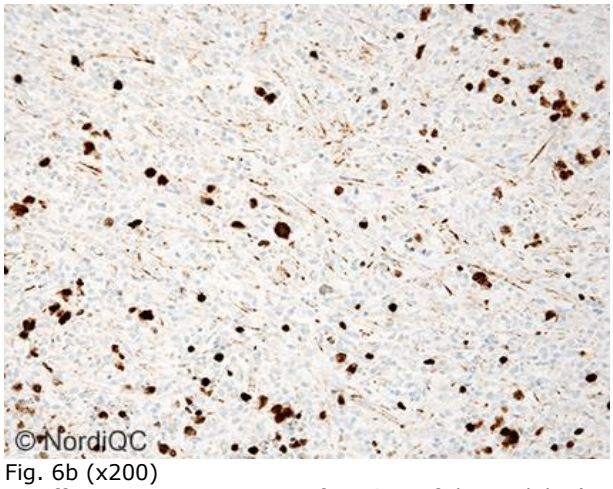


Fig. 6b (x200)
Insufficient staining reaction for BSAP of the Hodgkin's Lymphoma, classical subtype (tissue core 4), using same protocol as in Fig. 5b. Interpretation is difficult due to poor contrast between Hodgkin and Reed-Sternberg cells and normal B-cells. Also, reaction in stromal cells is evident in this case, making the diagnostic read-out more challenging – compare with Fig. 6a.

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