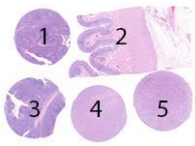
Assessment Run 53 2018

B-cell specific activator protein (BSAP, PAX5)

Material
The slide to be stained for BSAP comprised:



1. Tonsil, fixed 24h, 2. Colon, 3. Tonsil, fixed 48h, 4. Hodgkin Lymphoma, classical, 5. Diffuse large B-cell lymphoma (DLBCL).
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), especially interfollicullar, mantle zone and germinal centre B-cells in the tonsils.
- An at least weak to moderate, but distinct nuclear staining reaction of the vast majority of Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma.
- A strong, distinct nuclear staining reaction of virtually all neoplastic cells of the DLBCL.
- No staining reaction of other cells, including T-cells, squamous epithelial cells of the tonsils and columnar epithelial cells of the colon.

A weak, cytoplasmic staining reaction in cells with a strong nuclear staining reaction was accepted. In addition, a faint cytoplasmic staining reaction of endothelial cells was accepted as long as it did not interfere with the specific reaction for BSAP (this staining pattern was primarily seen with rabbit monoclonal antibody (rmAb) clone SP34).

Participation

Number of laboratories registered for BSAP, run 53	204
Number of laboratories returning slides	198 (97%)

Results
198 laboratories participated in this assessment. Of these, 171 (86%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- 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 third NordiQC assessment of BSAP. A minor increase in pass rate was seen compared to run 41, 2014 (see table 2).

Table 2. **Proportion of sufficient results for BSAP in the three NordiQC runs performed**

	Run 28 2010	Run 41 2014	Run 53 2018
Participants, n=	86	150	198
Sufficient results	67%	84%	86%

Conclusion
The mouse monoclonal antibody (mAb) clones **24**, **BC/24**, **1EW**, **MX017, DAK-Pax5** and the rmAb clones **SP34, EP156, BSR59, BV6** 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 is very robust and the Ready-To-Use (RTU) systems (IS/IR650 and GA650) from Dako provided the highest proportion of sufficient and optimal results. The majority of assays based on the rmAb clone SP34, both as concentrated formats and RTU systems, were challenged by poor signal-to-noise ratio hindering interpretation of the specific signal for BSAP.
Tonsil and appendix/colon 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 T-cells, squamous epithelial cells of the tonsil and columnar epithelial cells of the appendix/colon must be seen.

Table 1. **Antibodies and assessment marks for BSAP, run 53**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1EW**	9	Leica/Novocastra	7	2	0	0	-	-
mAb clone **24**	6 2	BD Biosciences Immunologic	3	2	1	2	-	-
mAb clone **BC/24**	2	Biocare Medical	0	2	0	0	-	-
mAb clone **MX017**	1	Immunologic	1	0	0	0	-	-
mAb clone **ZP007**	1	Biogenex	0	1	0	0	-	-
mAb clone **DAK-Pax5**	23	Agilent/Dako	15	7	0	1	96%	100%
rmAb clone **BSR59**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **BV6**	1	Diagnostic Biosystems	1	0	0	0	-	-
rmAb clone **EP156**	1	Cell marque	1	0	0	0	-	-
rmAb clone **SP34**	12 3 2	Cell Marque Thermo Scientific Spring Biosciences	4	11	2	0	88%	100%
pAb **RB-9406**	3	Thermo Scientific	0	0	1	2	-	-
Ready-To-Use antibodies								
mAb clone **1EW PA0552**	5	Leica/Novocastra	2	2	1	0	-	-
mAb clone **BC/24 PM207**	1	Biocare Medical	1	0	0	0	-	-
mAb clone **24 312M-18**	1	Cell marque	0	1	0	0	-	-
mAb clone **MX017 MAB-0706**	1	Maixin	1	0	0	0	-	-
mAb clone **MX017 MAD-000694QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **DAK-Pax5 IS/IR650**	23	Agilent/Dako	19	3	1	0	96%	100%
mAb clone **DAK-Pax5 IS/IR650**[3]	3	Agilent/Dako	3	0	0	0	-	-
mAb clone **DAK-Pax5 GA650**	24	Agilent/Dako	24	0	0	0	100%	100%
mAb clone **DAK-Pax5 GA650**[3]	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **EP156 8500-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **RBT-PAX5 BSB 5862**	1	BioSB	0	0	0	1	-	-
rmAb clone **SP34 790-4420**	33	Ventana	3	23	7	0	79%	75%
rmAb clone **SP34 312R-18**	35	Cell Marque	2	25	8	0	77%	100%
Total	198		92	79	21	6	-	
Proportion			46%	40%	11%	3%	86%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) Ready-to-use product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually.

Detailed analysis of BSAP, Run 53
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **1EW**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (3/4)*, Novocastra Epitope Retrieval Solution pH 9 (Leica) (1/1), Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/2), Tris-EDTA/EGTA pH 9 (1/1) or Bond Epitope Retrieval Solution 1 (BERS1; Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:50 depending on the total sensitivity of the protocol employed.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **24**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (1/2), TRS pH 9 (Dako) (1/1) or BERS2 (Leica) (1/3) 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.

mAb clone **MX017**: One protocol with an optimal result was based on HIER using Citrate pH 6.7 as retrieval buffer. The mAb was diluted 1:60 and BrightVision DPVO-HRP (Immunologic) was applied as detection system.

mAb clone **DAK-Pax5**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako) (3/3), Cell Conditioning 1 (CC1; Ventana) (6/13), BERS2 (Leica) (4/4) or BERS1 (Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 19 of 19 (100%) laboratories produced a sufficient staining result. One laboratory obtained an optimal result without performing any pre-treatment at all.

rmAb clone **BSR59**: One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted 1:200 and anti-Rabbit HRP polymer (Nordic Biosite) was applied as detection system.

rmAb clone **BV6**: One protocol with an optimal result was based on HIER using Montage citrate antigen retrieval solution (Diagnostic BioSystems). The mAb was diluted 1:10 and Montage PolyVue Plus Auto Detection System (Diagnostic BioSystems) was applied as detection system.

rmAb clone **EP156**: One protocol with an optimal result was based on HIER using TRS pH 9 (3-in-1) (Dako). The mAb was diluted 1:50 and Envision Flex+ (Dako) was applied as the detection system.

rmAb clone **SP34**: Protocols with optimal results were all based on HIER using CC1 (Ventana) (3/10) and TRS pH 9 (3-in-1) (Dako) (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, 10 of 10 (100%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for BSAP for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **DAK-Pax5**	2/2**	-	1/1	0/1	6/12 (50%)	-	4/4	1/1
rmAb clone **SP34**	0/1	-	1/1	-	3/9	-	0/1	-

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

Ready-To-Use antibodies and corresponding systems
mAb clone **1EW**, product no. **PA0552**, Leica, Bond-max/Bond-III:
Protocols with optimal results were based on HIER using BERS2 pH 9 (Leica) (efficient heating time 20 min. at 99-100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.

mAb clone **BC/24**, product no. **PM207**, Biocare, IntelliPATH:
One protocol with an optimal result was based on HIER using Borg Decloaker pH 9.5 (Biocare) (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH 4 Universal HRP-polymer (M4U534) as detection system.

mAb clone **MX017**, product no. **MAD-000694QD**, Master Diagnostica, MD-Stainer:
One protocol with an optimal result was based on HIER in a TRIS-EDTA/EGTA pH9 based buffer (Master Diagnostica) (efficient heating time 20 min. at 100°C), 10 min. incubation of the primary Ab and Master polymer plus (MAD-000230QP) as detection system.

mAb clone **DAK-Pax5**, product no. **IS650/IR650**, Dako, Autostainer+/Autostainer Link:
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 10-20 min. at 95-99°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient staining result.

mAb clone **DAK-Pax5**, product no. **GV650**, Dako, OMNIS:
Protocols with optimal results were typically based on HIER using TRS pH 6.1 (3-in-1) or 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+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 24 of 24 (100%) laboratories produced an optimal staining.

rmAb clone **EP156**, product no. **8500-C010**, Sakura Finetek, Tissue-Tek Genie:
Protocols with optimal results were based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (Sakura Finetek) (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro DAB kit (8826-K250) as detection system.

rmAb clone **SP34**, product no. **790-4420**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-90 min. at 95-100°C), 32-44 min. incubation of the primary Ab. and UltraView with amplification (760-500 + 760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 12 of 16 (75%) laboratories produced a sufficient staining result.

rmAb clone **SP34**, product no. **312R-18**, Cell Marque, Ventana BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-64 min. at 98-100°C), 16-20 min. incubation of the primary Ab. and OptiView (760-700) as detection systems. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result

Table 4. **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 **IS/IR650**	100% (7/7)	100% (7/7)	100% (15/15)	80% (12/15)
Dako Omnis mAb DAK-Pax5 **GA650**	100% (15/15)	100% (15/15)	100% (5/5)	100% (5/5)
VMS Ultra/XT/GX rmAb SP34 **790-4420**	3/4	0/4	79% (23/29)	10% (3/29)

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments
In this third 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 caused primarily by the use of the rmAb clone SP34. Too weak or false negative staining results were observed in 26% of the insufficient results (7 of 27). Virtually all laboratories were able to demonstrate BSAP in high-level antigen expressing cells, e.g., normal B-cells in tonsils and the neoplastic cells of the diffuse large B-cell lymphoma. In contrast, BSAP in low-level antigen expressing cells (e.g. Hodgkin and Reed-Sternberg cells) could only be demonstrated with an optimally calibrated protocol.
Poor-signal-to noise ratio or false positive staining results were observed in 63% of the insufficient results (17 of 27). In general, the use of the rmAb clone SP34, both within a laboratory developed (LD) and RTU assay, provided an aberrant cytoplasmic staining of most stromal cells (e.g. endothelial cells, lymphocytes and peripheral nerve cells). From NordiQC reference laboratories and internal studies it is well known, that assays based on the rmAb clone SP34 can cause problems and that the level of the aberrant cytoplasmic staining result may depend on lot to lot variations (see Fig. 5a and 5b).

In the remaining 11% of insufficient results, poor signal-to-noise ratio and/or false positive staining reaction in combination with a too weak staining reaction for BSAP were seen.

Used within an LD-assay, the mAb clones 1EW, 24, MX017 and DAK-Pax5, and the rmAb clones BSR59, BV6, EP156 and SP34 could all be used to obtain optimal staining results for BSAP (see Table 1). Irrespective of the clone used, the vast majority of laboratories (98%, 60 of 61) applied HIER in an alkaline buffer for optimal performance.
The mAb clone DAK-Pax5 and rmAb clone SP34 were the most widely used antibodies for demonstration of BSAP. Used as a concentrate, mAb clone DAK-Pax5 gave an overall pass rate of 96% (22 of 23). One laboratory that did not produced a sufficient result, used a protocol with too low sensitivity (HIER in TRS Low pH, too diluted antibody and the less sensitive detection system Envision Flex). As shown in Table 3, the mAb clone DAK-Pax5 is a robust primary Ab and optimal results could be obtained on all four main IHC platforms - Omnis (Dako), Autostainer (Dako), Bond (Leica) and BenchMark (Ventana).

The mAb clone 1EW used within LD assays provided pass rate of 100% (9 of 9) of which 78% (7 of 9) were assessed as optimal. In comparison to the last run 41, providing a pass rate of 78% (7 of 9) of which 44% (4 of 9) were optimal, this is an improvement in overall performance. As mentioned in the former report (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. Thus, the improvement in performance obtained in this assessment may well be a consequence of laboratories taking action on the problems related to endogenous peroxidase blocking described above.

Analogous to the mAb clone 1EW, performance of the mAb clone 24 is affected by the platform (IHC instrument). As specified in the former report (run 41), the selection of the clone must be tailored to the IHC system used. Staining for BSAP using the mAb clone 24 on the BenchMark IHC system (Ventana) is challenging compared to e.g. Autostainer system (Dako/LabVision). It is encouraging that laboratories using a Ventana Benchmark platform have taken note of this and changed the primary antibody to a more robust clone (e.g. DAK-Pax5). In this assessment, only one laboratory used the mAb clone 24 on the BenchMark IHC system (assessed as insufficient).

In the previous run, the concentrated format of the rmAb clone SP34 (Cell Marque, Spring Bioscience and NeoMarkers/Thermo) occasionally gave an aberrant staining pattern (most likely contaminated with CK20), where appendiceal epithelium (in addition to BSAP positive cells) was stained. This pattern was not observed in this assessment (colon epithelium). However, as mentioned above, the rmAb SP34 is prone to background staining hampering interpretation of the specific nuclear BSAP signal. Although the pass rate is relative high (88%), the proportion of optimal results is significantly lower (24%) compared to DAK-Pax5 or 1EW providing an optimal score rate of 65% (15 of 23) and 78% (7 of 9), respectively. 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. This had an impact on the overall performance for assays based on this clone.

66% (131 of 198) of the laboratories used an RTU system for detection of BSAP. In this assessment, the RTU systems from Dako (IS/IR650 and GA650) based on the mAb clone DAK-Pax5, provided high pass rate and proportion of optimal results (see Table 1). For the RTU system GA650 designed for the Omnis, all protocols (24 of 24) were assessed as optimal. Both vendor and laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could be used with same success rate (see Table 4). Both RTU systems (IS/IR650 and GA650) apply Envision Flex+ as the fundamental detection system. However, it was noted that vendor recommendations on HIER were different between the two RTU systems. Using the RTU system IS/IR650 (Autostainer), the recommended HIER buffer is TRS Low pH, whereas using the RTU GA650 (Omnis), the recommended HIER buffer is TRS High pH. For the RTU IS/IR650, all protocols (16 of 16) based on HIER in TRS Low pH were assessed as optimal, whereas only 43% (3 of 7) using HIER in TRS High pH were giving an optimal mark. The one laboratory that did not provide a sufficient staining result performed HIER in TRS High pH and used the less sensitive detection system Envision Flex.

52% (68 of 131) of the laboratories applying a RTU product used a system based on the rmAb clone SP34 (Ventana and Cell Marque). All were applied on a Ventana Benchmark platform. The pass rate was 78% (53 of 68) but only 7% (5 of 68) were assessed as optimal. The main problem (poor signal-to-noise ratio) seems to be related to the nature of rmAb clone SP34 itself, since no other protocol parameter could be identified unravelling the difference in performance. Protocols providing optimal results were similar to protocols assessed as insufficient. As shown in Table 4, the majority of participants used laboratory modified protocol settings, and one may speculate if the purpose of these "optimization-steps" of an RTU product in general was to calibrate for the problem with background staining encountered by laboratories using rmAb clone SP34 in their assays. In protocols assessed as optimal, only a faint cytoplasmic staining

reaction of endothelial cells was observed and all other stromal cells (except normal B-cells) displayed as expected a negative reaction for BSAP.

This was the third assessment of BSAP in NordiQC (see Table 2). A minor improvement in pass rate was obtained compared to the latest run 41, 2014. The extended use of robust BSAP clones (e.g., DAK-Pax5), both as a Conc format and an RTU system tailored to a specific IHC platform may account for the overall increase in sufficient results. Also, an increasing number of laboratories seems to follow the general recommendations given by NordiQC, typically applying HIER (preferable in alkaline buffer for most markers) and use of a sensitive 3-step detection system (e.g., OptiView/Ventana). Importantly, the primary Abs must be careful calibrated according to the expected antigen level of the recommended control material (see below). Finally, there are difficulties with the rmAb clone SP34 that require attention as background staining was seen in virtually all protocols based on this primary Ab.

Controls

Tonsil and colon/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 colon/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 cells including T-cells, stromal cells, epithelial cells of the tonsil and colon/appendix. As a supplement to tonsil and colon/appendix, especially in the technical calibration phase, it is recommended to verify the protocol on Hodgkin lymphoma, classical subtype, which often displays a weak to moderate nuclear expression in the neoplastic cells.

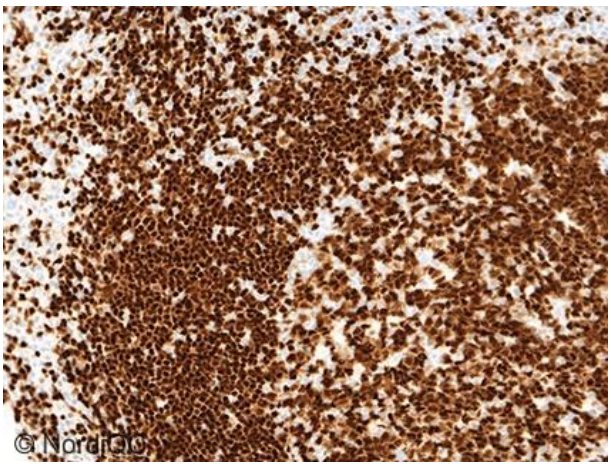


Fig. 1a (x200)
Optimal BSAP staining reaction of the tonsil using the mAb clone DAK-Pax5, optimally calibrated, HIER in TRS (3-1) pH 9 (Dako) and a 3-step polymer based detection system (Flex+/Dako).
All mantle zone and germinal centre B-cells show a strong and distinct nuclear staining reaction. Cytoplasmic staining reaction in positive B-cells must be accepted. No staining reaction is observed in other cellular structures including T-cells. Same protocol used in Figs. 2a - 4a.

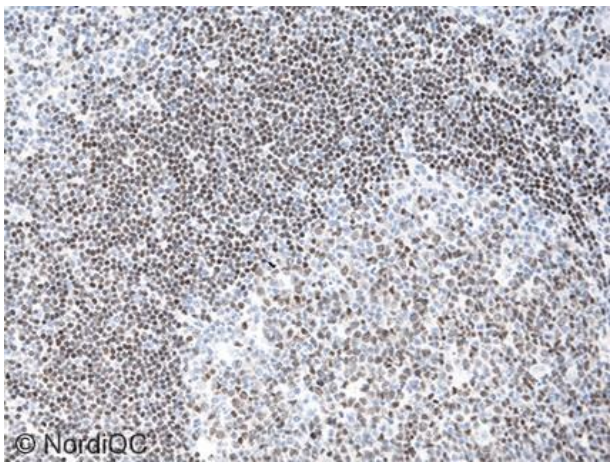


Fig. 1b (x200)
Insufficient staining of BSAP in the tonsil using the mAb clone DAK-Pax5, too diluted, HIER in TRS (3-1) pH 6 (Dako) and the less sensitive detection system Flex (Dako) – same field as in Fig. 1a.
B-cells of the mantle zone and germinal centres only display a weak to moderate staining intensity of the nuclei (compare with Fig. 1a). Same protocol used in Figs. 2b - 4b.

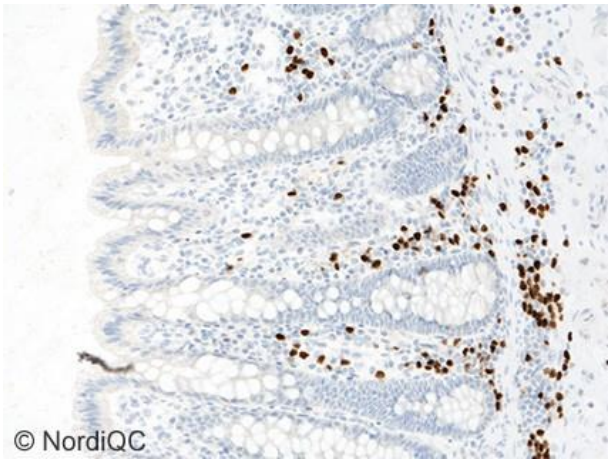


Fig. 2a (x200)
Optimal staining of BSAP in the colon using same protocol as in Fig. 1a. Dispersed B-cells in lamina propria mucosa show a strong and distinct nuclear staining reaction.

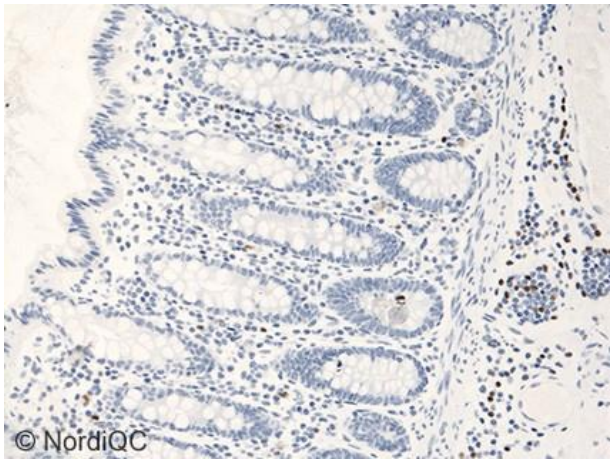


Fig. 2b (x200)
Insufficient staining of BSAP in the colon using same protocol as in Fig. 1b – same field as in Fig. 2a.
The staining intensity and proportion of positive B-cells is significantly reduced and virtually no positive cells can be identified in lamina propria.

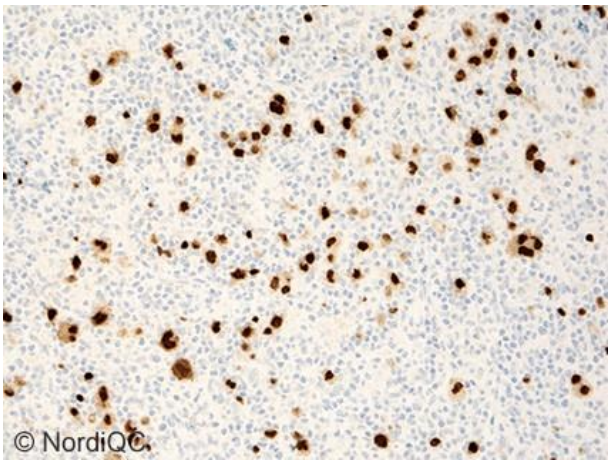


Fig. 3a (x200)
Optimal BSAP staining of the Hodgkin Lymphoma (classical type) using same protocol as in Figs. 1a and 2a. The vast majority of Hodgkin and Reed-Sternberg cells, intermingling between B-cells (strong positive staining reaction) and T-cells (negative staining reaction), show a moderate to strong, distinct nuclear staining reaction.

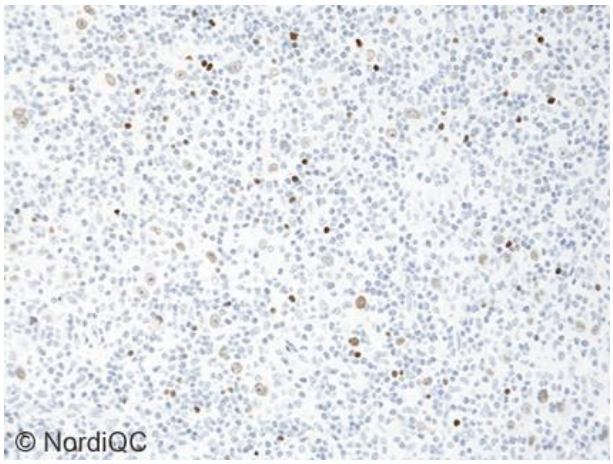


Fig. 3b (x200)
Insufficient BSAP staining of the Hodgkin Lymphoma (classical type) using same protocol as in Figs. 1b and 2b - same field as in Fig. 3a. The Hodgkin and Reed-Sternberg cells are only faintly demonstrated, or false negative.

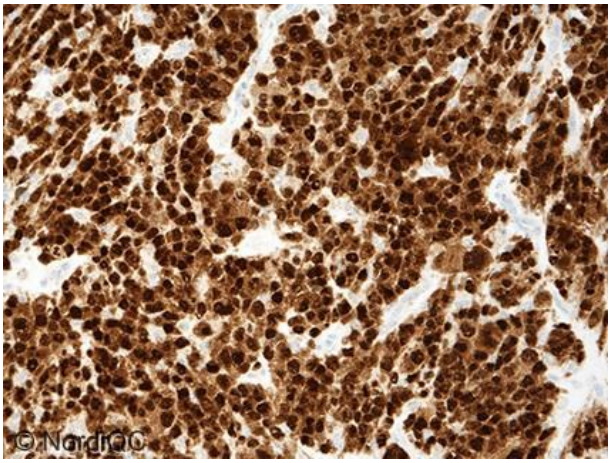


Fig. 4a (x200)
Optimal BSAP staining of the DLBCL using same protocol as in Figs. 1a – 3a. All the neoplastic cells display a strong and distinct nuclear staining reaction. Cytoplasmic staining reaction of the neoplastic cells must be accepted.

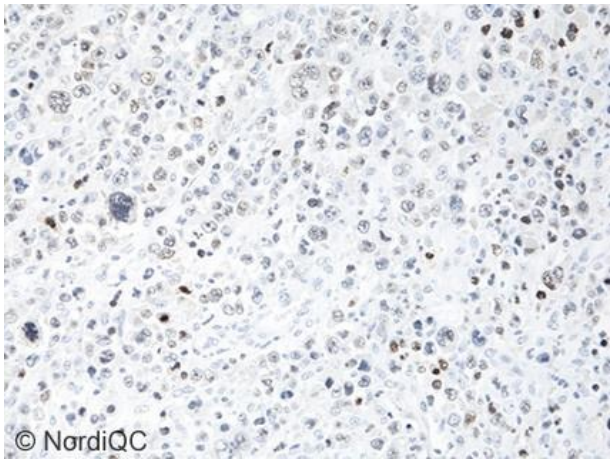


Fig. 4b (x200)
Insufficient BSAP staining of the DLBCL using same protocol as in Figs. 1b and 3b - same field as in Fig. 4a. The staining reaction of the nuclei in the neoplastic cells is barely visible or abscent.

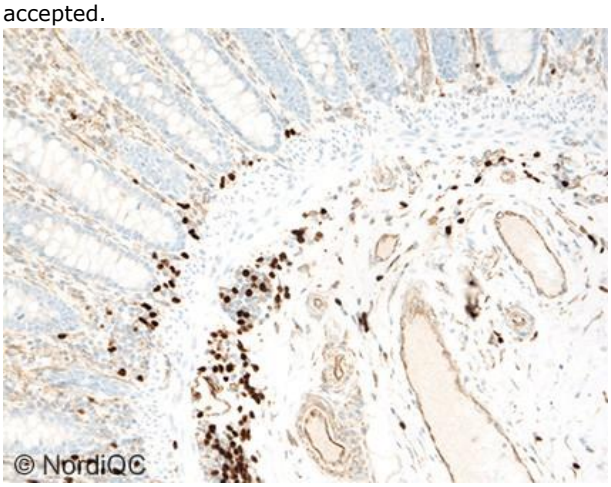


Fig. 5a (x200)
Insufficient BSAP staining of the colon. The protocol was based on the rmAb clone SP34 as RTU format (790-4420, lot. no. Y18596, Ventana), HIER in CC1 and OptiView (Ventana) as the detection system– same protocol used in Fig. 5b, but with a different lot. no. (both slides stained in a NQC reference laboratory). Typical reaction pattern seen with the rmAb SP34. The B-cells show the expected nuclear staining reaction, but the vast majority of stromal cells (e.g. endothelial cells) displays an unacceptable aberrant cytoplasmic staining reaction providing a poor signal-to-noise ratio.

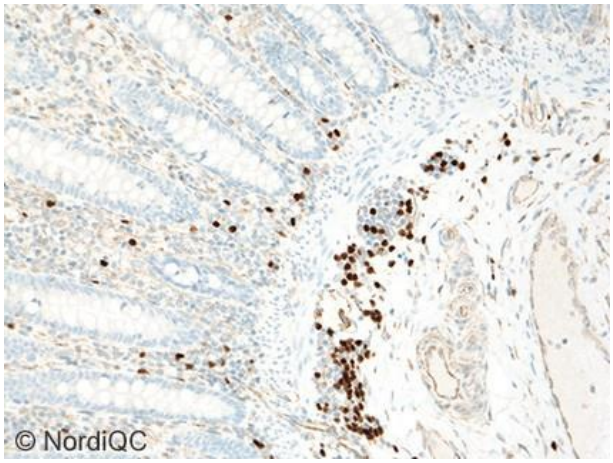


Fig. 5b (x200)
Sufficient BSAP staining (good) of the colon using the same protocol as in Fig. 5a, but with lot.no. Y05958 (primary Ab). It has been observed from NQC reference labs, but also seen in this assessment that there are lot-to-lot variation of concentrates/RTUs based on the rmAb SP34. Only a faint to weak aberrant cytoplasmic staining reaction of stromal cells is seen – compare with Fig. 5a. For optimal performance of assays based on the rmAb SP34, and provided that the specific nuclear reaction for BSAP was present at the expected level in all tissue cores (see Fig.1a - 4a), only faint cytoplasmic staining of endothelial cells was accepted.

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