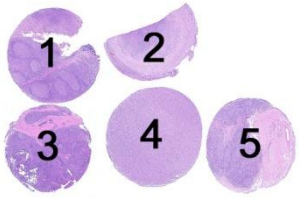
Assessment Run 28 2010

B-cell specific activator protein (BSAP, Pax5)

The slide to be stained for BSAP comprised:
1. Tonsil fixed 24 h., 2. Appendix, 3. Tonsil fixed 48 h., 4. Diffuse large B-cell lymphoma, 5. Hodgkin lymphoma, NS.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a BSAP staining as optimal included:

- A moderate to strong, nuclear staining of virtually all the mantle zone B-cells, the germinal centre B-cells and the interfollicular peripheral B-cells in the tonsils and the appendix.
- A moderate to strong nuclear staining of virtually all the neoplastic cells of the diffuse large B-cell lymphoma.
- An at least weak but distinct nuclear staining of the majority of the Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma.

A weak cytoplasmic reaction in cells with strong nuclear staining was accepted. All other cells should be negative.

87 laboratories participated in this assessment. 1 lab used an inappropriate Ab. Of the remaining 86 labs 67 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for BSAP, run 28**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **24**	22 5 1 1	BD Bioscience Immunologic Abcam Zeta	6	13	5	5	66 %	74 %
mAb clone **1EW**	11	Novocastra	3	5	1	2	73 %	80 %
mAb clone **BC/24**	5	Biocare	0	4	1	0	80 %	-
mAb clone **DAK-Pax5**	5	Dako	4	1	0	0	100 %	100 %
rmAb clone **SP34**	1 1 1	Cell Marque NeoMarkers Spring Bioscience	1	1	0	1	-	-
pAb **RB-9406**	14	NeoMarkers	4	4	2	4	57 %	100 %
Ready-To-Use Abs								
mAb clone **DAK-Pax5, IR650**	3	Dako	3	0	0	0	-	-
rmAb clone **SP34, 790-4420**	4	Ventana/Cell Marque	2	2	0	0	-	-
mAb clone **24, 760-4270**	5	Ventana/Cell Marque	0	0	4	1		-
mAb clone **24, 312M-17, CMA462**	3	Cell Marque	0	1	0	2	-	-
mAb clone **1EW, PA0552**	2	Leica	1	1	0	0	-	-
mAb clone **BC/24, PM027**	1	Biocare	0	1	0	0	-	-
mAb clone **ZP007, ZM0466**	1	Zymed	0	0	1	0	-	-
Total	86		24	33	14	15	-	-
Proportion			28 %	39 %	16 %	17 %	67 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **24**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using one of the following buffers: Tris-EDTA/EGTA pH 9 (3/10)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/7) or Citrate pH 6 (2/6). The mAb was typically diluted in the range of 1:10– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 19 (74 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **1EW**: The protocols giving an optimal result were all based on HIER using one of the following buffers: Tris-EDTA/EGTA pH 9 (1/6), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/2) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2).
The mAb was typically diluted in the range of 1:40– 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 5 (80 %) laboratories produced a sufficient staining.

mAb clone **DAK-Pax5**: The protocols giving an optimal result were all based on HIER using one of the following buffers: Tris-EDTA/EGTA pH 9 (1/1), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/1), Target Retrieval Solution pH 6 (EnVision FLEX TRS low pH, Dako) (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/1). The mAb was typically diluted in the range of 1:15– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 4 laboratories produced an optimal staining.

rmAb clone **SP34**: The protocol giving an optimal result was based on HIER used Cell Conditioning 1 (BenchMark, Ventana) (1/2) as the retrieval buffer. The mAb was diluted 1:50. Using these protocol settings 1 out of 2 laboratories produced a sufficient staining.

pAb **RB-9406**: The protocols giving an optimal result were all based on HIER using either EDTA/EGTA pH 8 (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (3/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:5– 1:20 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 4 laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **DAK-Pax5** (prod. no IR650, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) or Target Retrieval Solution pH 6 (EnVision FLEX TRS low pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 3 laboratories produced an optimal staining.

rmAb clone **SP34** (prod. no. 790-4420, Ventana/Cell Marque): The protocols giving an optimal result were based on HIER using standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 32 min in the primary Ab and Ultra View (760-500) as the detection system. 1 lab used amplification kit. With these protocol settings all of 3 laboratories produced a sufficient staining.

mAb clone **1EW** (prod. no. PA0552, Leica): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica), an incubation time of 30 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Insufficient HIER
- Less successful ready-to-use mAb clone 24
- Omission of HIER

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. Virtually all laboratories were able to demonstrate BSAP in high antigen expressing cells, e.g., the normal peripheral B-cells in the tonsils and the neoplastic cells of the diffuse large B-cell, whereas BSAP in low antigen expressing cells such as the Hodgkin and Reed-Sternberg cells could only be demonstrated with an optimally calibrated protocol.
In this assessment the newly launched Ab for BSAP, mAb clone DAK-Pax5, both as a concentrate and as a Ready-To-Use (RTU) format, gave a higher pass rate and a higher proportion of optimal results than all other Abs (see Table 1).
The newly launched rmAb clone SP34 in RTU format for the Ventana BenchMark XT and Ultra platform gave a superior performance compared to the old mAb clone 24 in RTU format (see Table 1).

From the assessment it can be difficult to identify a robust and reliable control for BSAP as most normal peripheral B-cells have a high expression of BSAP, which complicates a correct calibration of the protocol to secure a correct BSAP demonstration in neoplastic cells with a weak expression, as seen in Hodgkin's lymphoma. At present the best recommendation is to use tonsil and to calibrate the primary Ab to give an as strong as possible nuclear reaction in the B-cells. A weak cytoplasmic reaction in the B-cells must be accepted, where as no reaction should be seen in the squamous epithelial cells, smooth muscle cells and endothelial cells.

Conclusion

The mAbs clones 24, 1EW, DAK-Pax5, rmAb clone SP34 and the pAb RB-9406 can all be used to obtain an optimal staining for BSAP. The mAb clone DAK-Pax5 and the rmAb clone SP34 were in this assessment the most robust markers. HIER is mandatory to obtain an optimal staining.

Tonsil is a recommended positive control: Virtually all the B-cells must show an as strong as posible nuclear reaction (a weak cytoplasmic reaction is acceptable). No staining reaction should be seen in the squamous epithelial cells and smooth muscle cells.

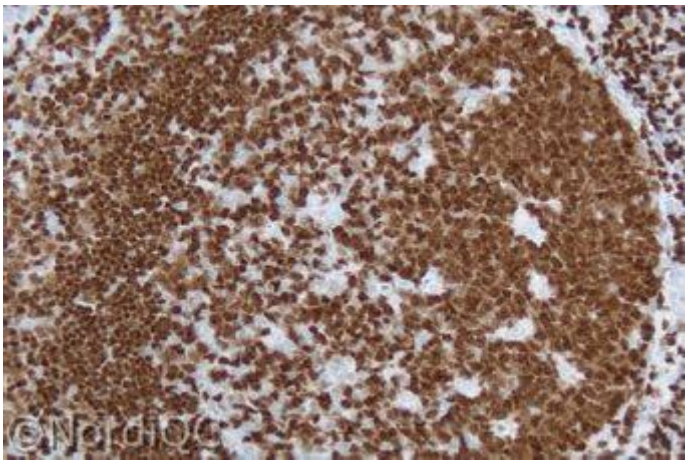


Fig. 1a (X200)
Optimal BSAP staining of the tonsil using the rmAb clone SP34 optimally calibrated and with HIER in an alkaline buffer. Virtually all the mantle zone and germinal centre B-cells show a strong nuclear staining. A weak cytoplasmic staining but no background staining is seen – compare with Figs. 2a & 3a, same protocol.

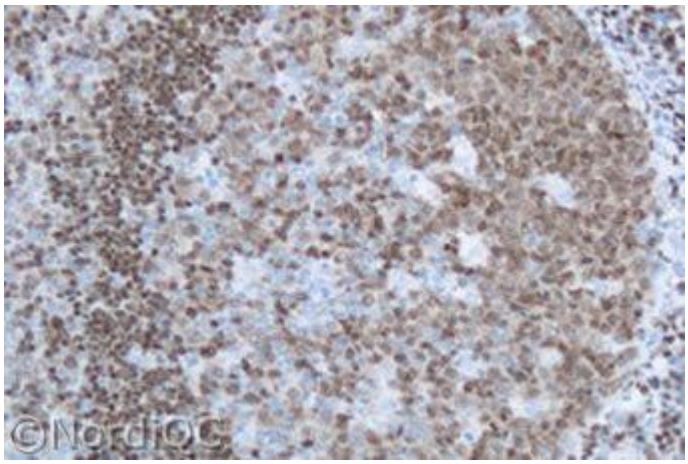


Fig. 1b (X200)
Insufficient BSAP staining of the tonsil using the mAb clone 24 too diluted - same field as in Fig. 1a. The majority of the mantle zone and germinal centre B-cells are demonstrated, but the intensity is significantly reduced – also compare with Figs. 2b & 3b, same protocol.

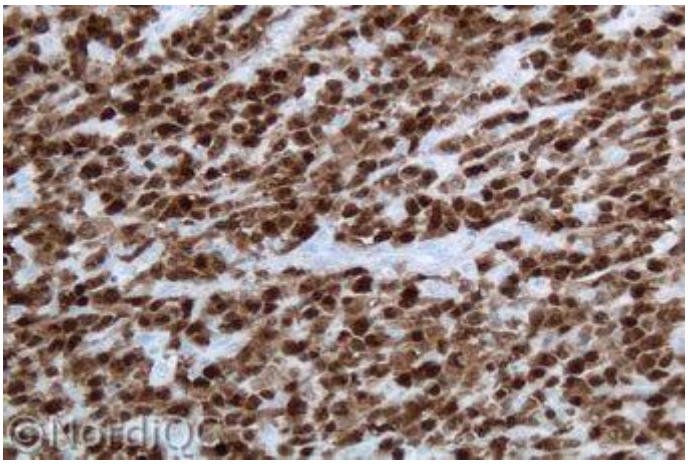


Fig. 2a (X200)
Optimal BSAP staining of the diffuse large B-cell lymphoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong nuclear staining. A weak to moderate cytoplasmic staining, but no background staining is seen.

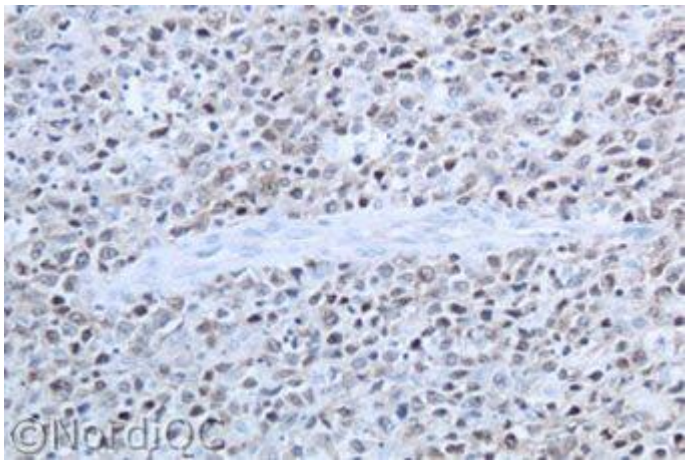


Fig. 2b (X200)
Insufficient BSAP staining of the diffuse large B-cell lymphoma using same protocol as in Fig. 1b - same field as in Fig 2a. Only scattered neoplastic cells only show a weak and equivocal staining.

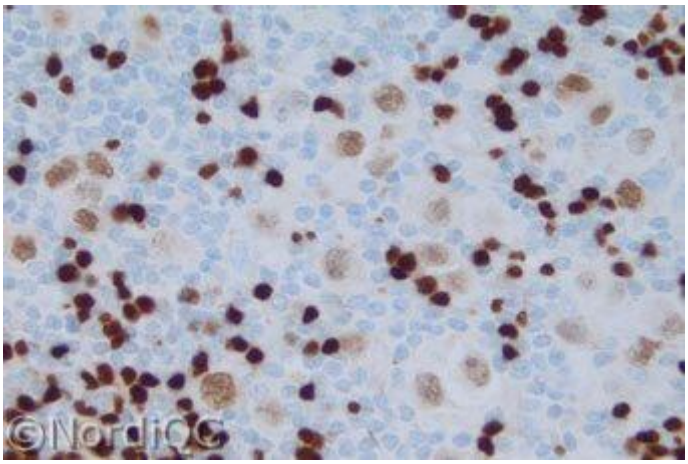


Fig. 3a (X400)
Optimal BSAP staining of the Hodgkin lymphoma using same protocol as in Figs. 1a & 2a. The peripheral B-cells show a strong nuclear staining while the Hodgkin and Reed-Sternberg cells show a weak but distinct nuclear staining.

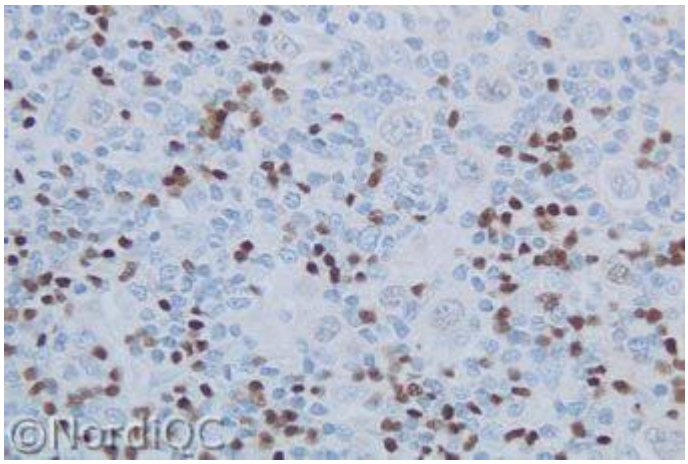


Fig. 3b (X400)
Insufficient BSAP staining of the Hodgkin lymphoma using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. Only the peripheral B-cells are demonstrated, while the Hodgkin and Reed-Sternberg cells are negative.

SN/MV/LE 7-4-2010