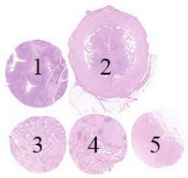
Assessment Run 65 2022

BAP1 (BRCA1-Associated Protein 1)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for BAP1, identifying malignant mesothelioma.
Relevant clinical tissues, both normal and neoplastic, were selected to display a spectrum of antigen densities for BAP1 (see below).

Material
The slide to be stained for BAP1 comprised:



1. Tonsil, 2. Appendix, 3. Lung adenocarcinoma, 4-5. Malignant mesothelioma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a BAP1 staining as optimal included:

- An at least weak nuclear staining reaction in the majority of mantle zone lymphocytes in tonsil.
- A weak to moderate nuclear staining reaction in virtually all germinal centre lymphocytes.
- An at least weak to moderate nuclear staining reaction in virtually all epithelial cells in appendix.
- An at least weak to moderate nuclear staining reaction in the neoplastic cells in the lung adenocarcinoma.
- An at least weak to moderate nuclear staining reaction of most stromal cells in both malignant mesotheliomas.
- No nuclear staining of neoplastic cells in both malignant mesotheliomas. A weak cytoplasmic staining reaction was accepted.

Participation

Number of laboratories registered for BAP1, run 65	180
Number of laboratories returning slides	163 (91%)

At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. 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.

Results
163 laboratories participated in this assessment. 113 (69%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less sensitive detection system
- Insufficient HIER (too low temperature and/or too short efficient heating time)

Performance history
This was the first NordiQC assessment of BAP1 and the overall pass rate was 69% (see Table 2).

Table 2. **Proportion of sufficient results for BAP1 in the first NordiQC run performed**

	Run 65 2022
Participants, n=	163
Sufficient results	69%

Conclusion
The mAb clones **C-4** and **BSB-109** and the rmAb clone **EPR22826-65** could all provide an optimal result for the demonstration of BAP1. HIER in alkaline buffer, precise calibration of the primary Ab and in particular use of a 3-step polymer or multimer based detection system were the main prerequisites for an optimal result. Protocols based on pAbs produced inferior results.
In total, 81% (132/163) of all protocols were based on the mAb clone C-4. The vast majority of laboratories (92%) used 3-step polymer/multimer based detection systems obtaining a superior pass rate

of 74% (45% being optimal) compared to 2-step detection systems giving only a pass rate of 9%, all optimal.
Importantly, laboratories should use a robust Ab, calibrate the protocols correctly and stain accordingly to the expected antigen level of the recommended control materials (see below).

Table 1. **Antibodies and assessment marks for BAP1, run 65**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb **BSB-109**	14 3	BioSB Gennova	6	7	4	0	77%	35%
mAb **C-4**	5 2 103 12	Nordic Biosite Immunologic Santa Cruz Zeta Corporation	52	31	25	14	68%	43%
rmAb **EPR22826-65**	1	Abcam	1	0	0	0	-	-
pAb	1	Abcam	0	0	0	1	-	-
pAb	1	Biocare Medical	0	0	0	1	-	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb **BSB-109** **BSB 3300/3301/3302**	10	BioSB	6	2	1	1	80%	60%
mAb **C-4** **AZC-E0R3F3**	2	Nordic Biosite	2	0	0	0	-	-
mAb **C-4** **PDM595**	1	Diagnostic BioSystems	0	0	0	1	-	-
mAb **C-4** **Z2318MP**	7	Zeta Corporation	2	4	0	1	86%	29%
pAb **API 3247 AA**	1	Biocare Medical	0	0	0	1	-	-
Total	163		69	44	30	20		
Proportion			42%	27%	19%	12%	69%	

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

Detailed analysis of BAP1 Run 65
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **BSB-109**: Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana/Roche) (4/8)*, or Target Retrieval Solution (TRS) pH high (Dako/Agilent) (3/7) as retrieval buffer. The mAb was typically diluted in the range of 1:50-150 and using a 3-layer polymer/multimer-based detection system. Using these protocol settings, 12/16 (75%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **C-4**: Protocols with optimal results were typically based on HIER using CC1 (Ventana/Roche) (32/74), TRS pH high (Dako/Agilent) (7/14), TRS pH low (Dako/Agilent) (1/1), TRS pH 9 (3-in-1) (Dako/Agilent) (2/12) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (10/15) as retrieval buffer. The mAb was typically diluted in the range of 1:25-500. Using these protocol settings, 83/117 (71%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for BAP1 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / 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	BERS2 pH 9.0	BERS1 pH 6.0
mAb **BSB-109**	-	-	3/7** (43%)	-	3/8 (38%)	-	0/1	-
mAb **C-4**	2/12 (17%)	-	7/17 (41%)	1/1	32/71 (45%)	-	10/15 (67%)	-

*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
No Ready-To-Use Abs with a corresponding system were giving optimal results in this assessment.

Comments
In this first assessment of BAP1, a pass rate of 69% was obtained.

The prevalent feature of an insufficient result in this first assessment of BAP1 was characterized by too weak or completely false negative results seen in 56% of the insufficient results (28/50). The majority of all laboratories were able to demonstrate BAP1 in the neoplastic cells of the lung adenocarcinoma, but only carefully calibrated protocols with high analytical sensitivity were able to demonstrate BAP1 in the stromal cells serving as an important internal positive tissue control in the malignant mesotheliomas with loss of BAP1 in the neoplastic cells. In 30% (15/50) of the insufficient result, a poor signal-to-noise ratio or an excessive background staining complicating the interpretation was observed. False positive staining reaction was seen in the neoplastic cells in 12% (6/50) and 2% (1/50) of the insufficient results was caused by both a false negative and false positive staining reaction.

87% (142/163) of the laboratories used Abs as concentrated format within laboratory developed (LD) tests for BAP1. The mAb clone C-4 was the most widely used clone, being applied by 86% (122/142) with a pass rate of 62%, 43% optimal (see Table 1). Optimal results could be obtained on the fully automated systems on the three main platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems (see Table 2). The main prerequisites for a sufficient staining with mAb clone C-4 were efficient HIER in an alkaline buffer, careful calibration of the titre of the primary Ab and use of a sensitive detection system, preferably a 3-step polymer/multimer based detection system. The proportion of sufficient staining results for 3-step and 2-step polymer/multimer based systems was 73% and 11%, respectively. 48% (58/122) of the participants used OptiView (Ventana/Roche) as detection system and performed the assay on BenchMark (Ventana/Roche). If OptiView with amplification was applied, a pass rate of 79% (30/38), 61% optimal, was seen compared to 60% (12/20), 25% optimal, without the amplification step. However, it is well-known from previously NordiQC assessments, that assays based on OptiView with amplification kit (tyramide based) can be challenging to calibrate and frequently will provide a binary result as either negative or positive and not giving a "normal" dynamic range of antigen expression levels from low to high. The "lack" of dynamic range and the binary pattern can compromise the demonstration of low-level antigen expressing structures and at the same time also induce a risk of aberrant granular precipitation of the chromogen in structures expected to be negative. Nevertheless, the data observed in this assessment provided both a higher pass rate and proportion of optimal results for protocols based on OptiView with amplification kit compared to standard OptiView detection system. Similar observation was also observed in this runs assessment for CD30.

17% (27/163) of the laboratories used the mAb clone BSB-109 as either a concentrate or RTU, with an overall pass rate of 77% (21/27), 44% optimal. The RTU format was found to be slightly superior to the concentrated format giving a higher proportion of optimal results as shown in Table 1. For both the concentrated and RTU format, efficient HIER in combination with a sensitive 3-step detection system were the main prerequisites for an optimal result. For the concentrate in addition, a careful calibration of the titre must be addressed.

Controls
At present and according to publications and preliminary data generated in this NordiQC assessment, appendix and/or tonsil are recommended as external positive tissue controls for BAP1. In appendix, virtually all epithelial cells should show an at least moderate nuclear staining reaction, and an at least weak nuclear staining reaction must be seen in virtually all lymphocytes and stromal cells. In tonsil, an at least weak nuclear staining reaction in most mantle zone lymphocytes must be seen, whereas a weak to

moderate nuclear staining reaction should be seen in virtually all germinal centre lymphocytes. In addition to external controls, it is of most importance that stromal cells within the tumour tissues tested exhibit a distinct nuclear staining reaction serving as internal positive tissue control (see Fig. 4a and 4b).

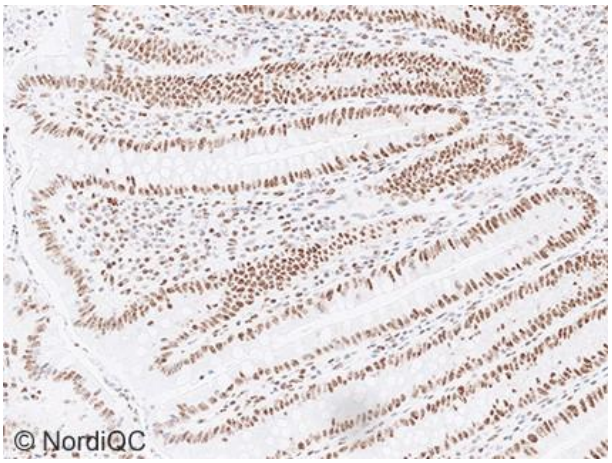


Fig. 1a
Optimal BAP1 staining of appendix using the mAb clone C-4 - diluted, 1:25 (40 min. incubation), epitope retrieval using HIER in CC1 (32 min.), a 3-step multimer based detection system (OptiView) with thyramide amplification (OptiView Amplification) and performed on BenchMark (Ventana/Roche).
Virtually all epithelial cells display a moderate nuclear staining reaction, and the vast majority of lymphocytes/stromal cells show a weak nuclear staining reaction. Same protocol used in Figs. 2a-4a.

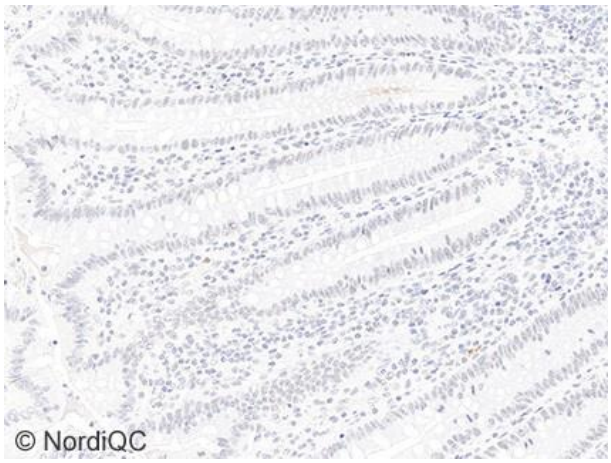


Fig. 1b
Insufficient BAP1 staining of the appendix using same clone and similar protocol settings as in Fig. 1a, but with a less sensitive detection system (UltraView).
Only scattered epithelial cells show a faint nuclear staining reaction. Virtually all lymphocytes/stromal cells are negative.
Same protocol used in Figs. 2b-4b.

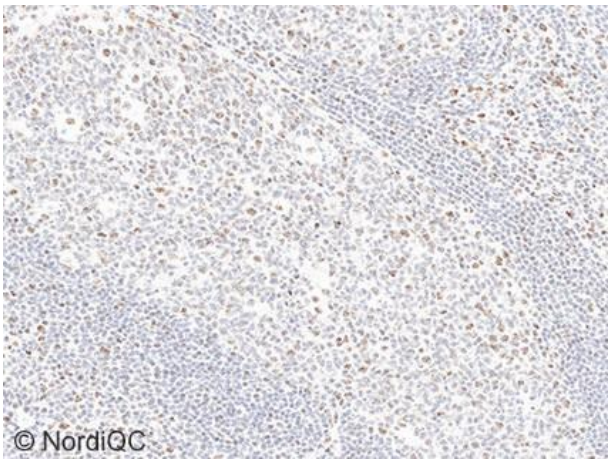


Fig. 2a
Optimal BAP1 staining of tonsil using same protocol as in Fig. 1a. A weak nuclear staining reaction is seen in most mantle zone lymphocytes. Virtually all germinal centre lymphocytes show a weak to moderate nuclear staining reaction.

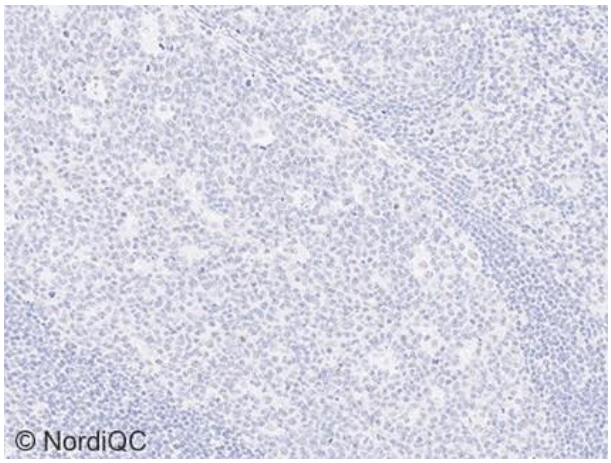


Fig. 2b
Insufficient BAP1 staining of tonsil using same protocol as in Fig. 1b. Virtually all lymphocytes are negative.

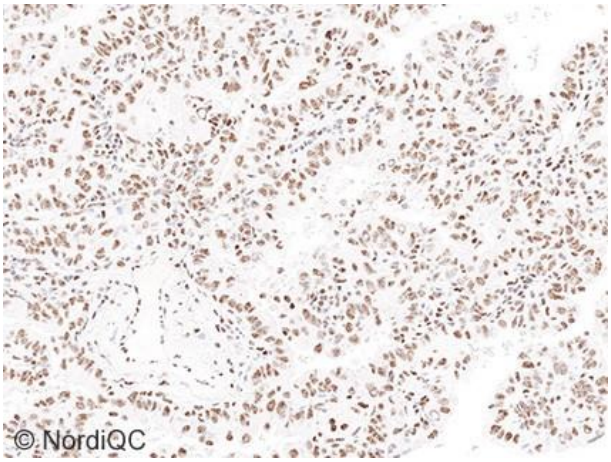


Fig. 3a
Optimal BAP1 staining of the lung carcinoma, tissue core no. 3, using same protocol as in Figs. 1a and 2a. The vast majority of the neoplastic and stomal cells display a weak to moderate nuclear staining reaction.

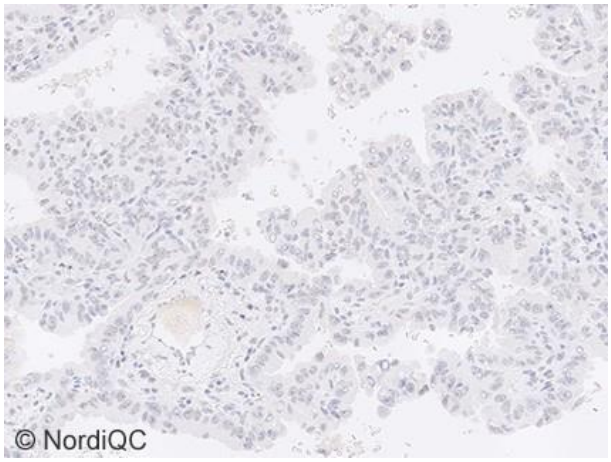


Fig. 3b
Insufficient BAP1 staining of the lung carcinoma, tissue core no. 3, using same protocol as in Figs. 1b and 2b. The neoplastic cells are negative and only a dubious dot-like reaction is seen in a few cells.

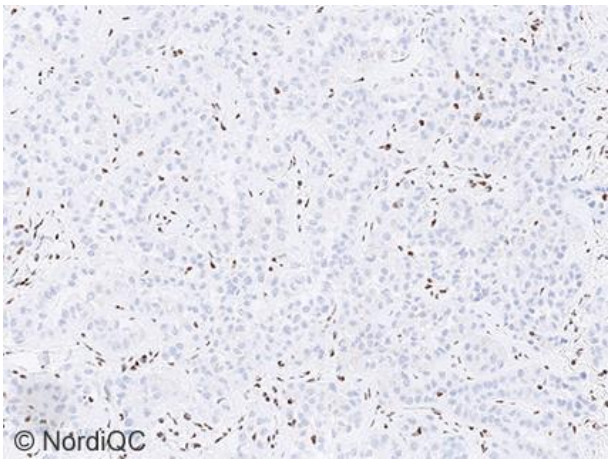


Fig. 4a
Optimal BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1a – 3a. All neoplastic cells are negative, whereas stromal cells show a distinct, weak to moderate nuclear staining reaction.

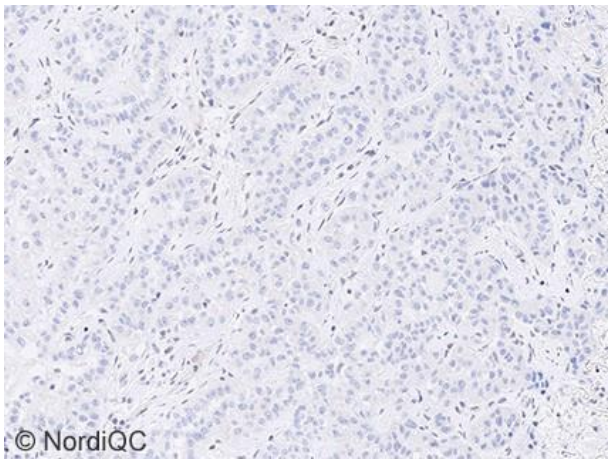


Fig. 4b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The neoplastic cells are negative as expected. However, also the stromal cells, expected to be positive serving as internal positive tissue control, are negative.

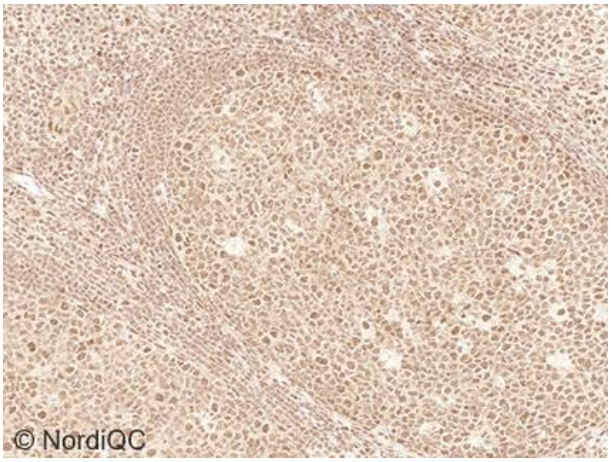


Fig. 5a
Insufficient BAP1 staining of tonsil using the mAb clone C-4, diluted 1:400 (60 min. incubation), epitope retrieval using HIER in BERS2 (30 min.), a 3-step polymer based detection system (BOND Polymer Refine Detection) and performed on BOND (Leica Biosystems). Same protocol used in Fig. 5b.
Virtually all lymphocytes show a moderate nuclear staining reaction but also an excessive cytoplasmic reaction is seen – compare with optimal result in Fig. 2a.

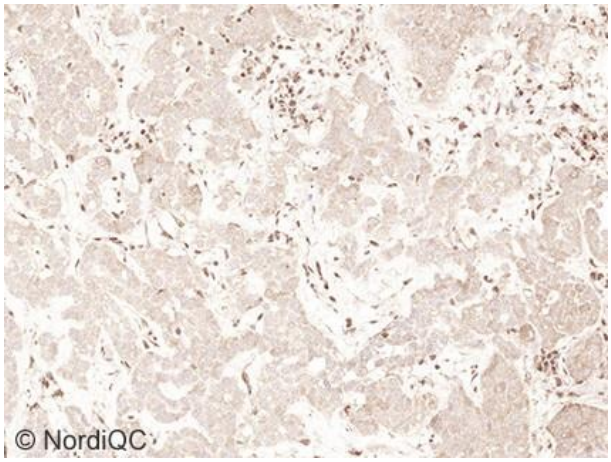


Fig. 5b
Insufficient BAP1 staining of the malignant mesothelioma, tissue core no. 4, using same protocol as in Fig. 5a. In general, a poor signal-to-noise ratio is seen, complicating the interpretation. The neoplastic cells show an aberrant, weak predominantly cytoplasmic staining reaction with a faint aberrant nuclear staining reaction in scattered neoplastic cells.
Compare with optimal result in Fig. 4a.

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