The slide to be stained for SOX11 comprised:

1. Tonsil, 24h fixation, 2. Tonsil, 48h fixation, 3. B-CLL, 4-5. Mantle cell lymphoma

All tissues were fixed in 10% neutral buffered formalin.
Criteria for assessing SOX11 staining as optimal included:

- A moderate to strong, nuclear staining reaction of virtually all neoplastic cells in the mantle cell lymphoma core no. 5.
- An at least weak to moderate nuclear staining reaction of the majority of neoplastic cells in the mantle cell lymphoma core no. 4.
- No nuclear staining reaction of the neoplastic cells in the B-CLL.
- No staining of other cells.

A weak, cytoplasmic staining reaction in cells with a strong nuclear staining reaction was accepted.

Participation

Number of laboratories registered for SOX11, run 47	89
Number of laboratories returning slides	79 (89%)

Results
79 laboratories participated in this assessment. 52 (66%) achieved a sufficient mark.
Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of polyclonal SOX11 antibodies
- Inappropriate calibration of the primary Ab
- Insufficient HIER - too short efficient HIER time and/or use of non-alkaline buffers
- Use of detection systems with low sensitivity

Performance history
This was the first NordiQC assessment of SOX11 and a pass rate of 66% was observed.

Table 2. **Proportion of sufficient results for SOX11 in the first NordiQC run performed**

	Run 47 2016
Participants, n=	79
Sufficient results	66%

Conclusion
The mAb clones **MRQ-58** and **SOX11-C1** were the two most successful antibodies for SOX11. mAb clone MRQ-58 was used by the majority of laboratories and optimal results was obtained both within a laboratory developed (LD) assay on the main IHC platforms and as Ready-To-Use format (Ventana). Within LD assay, efficient HIER in an alkaline buffer and use of a sensitive and specific 3-step polymer / multimer based detection system gave the highest proportion of optimal results.
Use of polyconal antibodies was less successful providing a poor signal-to-noise ratio.
At present, no accessible normal tissue expressing SOX11 has been identified and mantle cell lymphoma seems to be the preferred recommendable positive tissue control.The vast majority of neoplastic cells must show an as strong as possible nuclear staining reaction (a minimal cytoplasmic staining reaction must be accepted), while other cells must be negative. In addition tonsil can be used as negative tissue control, in which no nuclear staining reaction must be seen. A weak diffuse background staining can be expected.

Table 1. **Antibodies and assessment marks for SOX11, run 47**

Concentrated antibodies:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CL0142**	1	Abcam	0	0	1	0	-	-
mAb clone **CL0143**	1	Atlas	0	1	0	0	0	-
mAb clone **MRQ-58**	38 1 1	Cell Marque ImPath Zeta	13	17	7	3	75%	80%
mAb clone **SOX11-C1**	5 1	Affymetrix/eBioscience Biocare Medical	3	1	2	0	67%	100%
mAb clone **ZSX11**	1	Zytomed	0	0	1	0	-	-
Polyclonal	4 1	Sigma Atlas	0	1	1	3	20%	-
Ready-To-Use antibodies:								
mAb clone **MRQ-58 760-4888**	16	Ventana/Cell Marque	3	7	4	2	63%	100%
mAb clone **MRQ-58 382M-18**	5	Cell Marque	0	2	3	0	40%	-
mAb clone **MRQ-58 MAB-0699**	2	Maixin	1	1	0	0	-	-
mAb clone **MRQ-58 MAD-000581QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **SOX11-C1 API3120**	1	Biocare Medical	0	1	0	0	-	-
Total	79		21	31	19	8	-	
Proportion			27%	39%	24%	10%	66 %	

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

Detailed analysis of SOX11, Run 47
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
rmAb clone **MRQ-58**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/3)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (1/6), Target Retrieval Solution (TRS) pH 9 (Dako) (3/4), Target Retrieval Solution BERS2 (Leica) (3/6) or Cell Conditioning 1 (BenchMark, Ventana) (5/16) as retrieval buffer. The rmAb was typically diluted in the range of 1:25– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 28 of 35 (78%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **SOX11-C1**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (Dako) (2/2) or Cell Conditioning 1 (BenchMark, Ventana) (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. Using these protocol settings 3 of 3 (100%) laboratories produced a sufficient staining.

Table 3. **Proportion of optimal results for SOX11 for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic / Omnis		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
	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 **MRQ-58**	4/11** (36%)	-	5/22 (22%)	-	3/6 (50%)	-
rmAb clone **SOX11-C1**	2/2	0/1	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
rmAb clone **MRQ-58**, product no. **790-4888**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using Cell Conditioning 1 (efficient heating time 64 min.) and 4-16 min. incubation of the primary Ab. OptiView (760-700) was used as detection system. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-58**, product no. **MAB-0699**, Maixin, manual staining:
One protocol with an optimal result was based on HIER using Tris-EDTA pH 9 in a waterbath (efficient heating time 20 min. at 100°C), 60 min. incubation of the primary Ab and KIT-5230 (Maixin) as detection system.

mAb clone **MRQ-58**, product no. **MAD-000581QD**, Master Diagnostica:
One protocol with an optimal result was based on HIER using EDTA/EGTA pH 8 in PT module (efficient heating time for 20 min. at 95°C), 10 min. incubation of the primary Ab at room temperature and MAD-000237QK as detection system.

Comments
In this first NordiQC assessment of SOX11, the prevalent features of an insufficient staining result were either a generally too weak staining reaction of cells expected to be demonstrated and/or a poor signal-to-noise ratio compromising the interpretation.
Too weak or false negative staining reaction was seen in 41% of the insufficient results (11 of 27). The majority of the laboratories were able to demonstrate SOX11 in the neoplastic cells in the mantle cell lymphoma, tissue core no. 5, whereas the mantle cell lymphoma, tissue core no. 4, was slightly more challenging and displayed a lower level of SOX11 expression. Too weak staining was most frequently caused by use of protocol settings providing too low technical sensitivity for an otherwise successful primary Ab. In 59% of the insufficient results a poor signal-to-noise ratio or excessive background staining compromised the interpretation. This was mainly caused by pAbs and use of Ready-To-Use (RTU) antibodies not being calibrated for the protocol settings applied by the laboratories, e.g. use of Cell Marque RTU format of MRQ-58 on a Bond IHC platform, Leica.

The mAb clone MRQ-58 was the most widely used concentrated antibody for demonstration of SOX11 within a laboratory developed (LD) assay. Optimal results could be obtained on all main IHC systems, see table 3. The main prerequisites for obtaining sufficient and optimal results were use of efficient HIER in alkaline buffer and use of a 3-step polymer / multimer based detection system. Using mAb clone MRQ-58 as concentrate, HIER in an alkaline buffer in combination with a 3-step detection system, a pass rate of 79% (15 of 19 laboratories) was observed and 47% optimal. If a 2-step system was used, the pass rate was 65% (11 of 17 laboratories) and 12% optimal.
The mAb clone SOX11-C1 was used by few laboratories (n=6), but also provided optimal results with LD assays based on HIER in alkaline buffers and 3-step detection systems.

The Ventana RTU system based on mAb clone MRQ-58 (**760-4888)** was the most widely used RTU system (n=16). Only 4 laboratories used the RTU format accordingly to the protocol settings recommended by Ventana (HIER in CC1 for 64 min., 32 min. incubation in primary Ab and UltraView as detection kit). Of these, 2 laboratories obtained the score good, 1 borderline and 1 poor. Optimal results could only be obtained by laboratory modified protocol settings, primarily using OptiView as detection system and reduced incubation time in the primary Ab compared to the recommendations provided by Ventana/Cell Marque.

Controls
At present, no accessible normal tissue expressing SOX11 has been identified and mantle cell lymphoma seems to be the preferred recommendable positive tissue control.The vast majority of neoplastic cells must show an as strong as possible nuclear staining reaction (a minimal cytoplasmic staining reaction

must be accepted), while other cells must be negative. In addition tonsil can be used as negative tissue control, in which no nuclear staining reaction must be seen. A weak diffuse background staining can be expected.
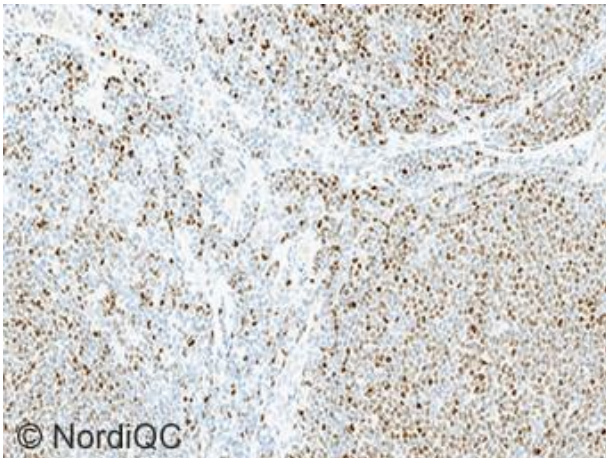

Fig. 1a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 4, using the mAb clone SOX-C11 diluted 1:25, HIER in CC1, a 3-step multimer based detection kit (OptiView) and performed on BenchMark Ultra, Ventana. The vast majority of neoplastic cells show a moderate, distinct, nuclear staining reaction. No background reaction is seen. Also compare with Figs. 2a – 4a, same protocol.
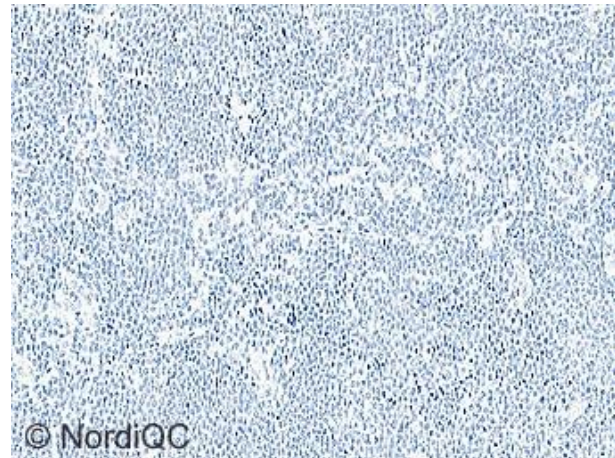
Fig. 1b
Insufficient SOX11 staining of the mantle cell lymphoma, tissue core no. 4, using the mAb clone SOX-C11 with a protocol providing a too low sensitivity.
The Ab was used at 1:200, HIER in TRS pH 6,1, a 3-step polymer based detection system, FLEX+ (Dako) and performed on Autostainer Link 48, Dako. Only few cells show a faint nuclear staining reaction. Compare with Fig. 1a – same field.
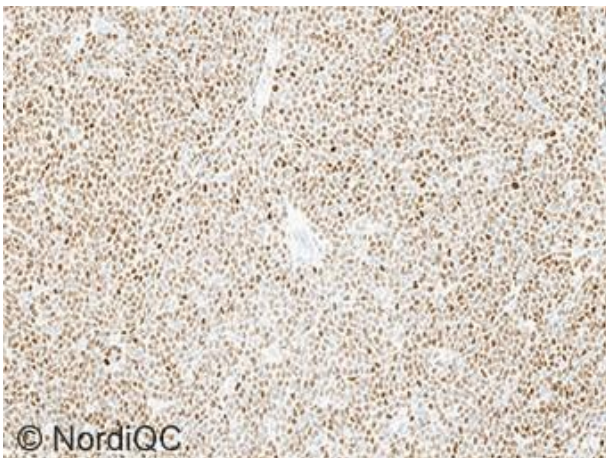Also compare with Figs. 2b – 3b – same protocol

Fig. 2a
Optimal SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. No background reaction is seen.
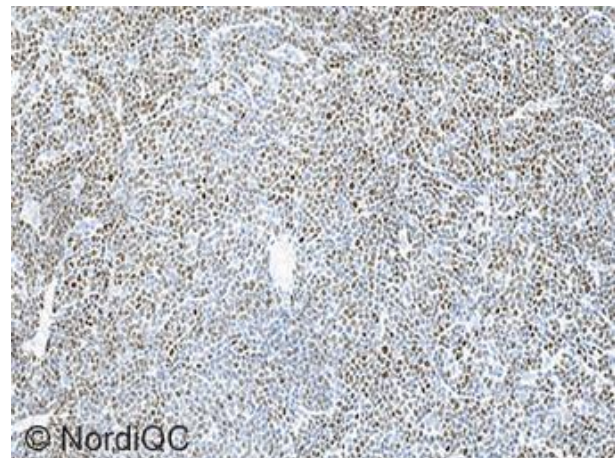

Fig. 2b
SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 1b – same field as in Fig. 2b. The majority of neoplastic cells are demonstrated, but the proportion and intensity is reduced compared to the level expected.
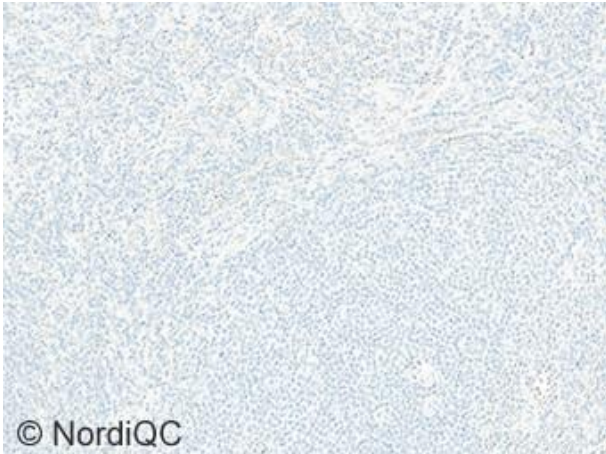
Fig. 3a
Optimal SOX11 staining of the B-CLL using same protocol as in Figs. 1a and 2a. No staining is seen.
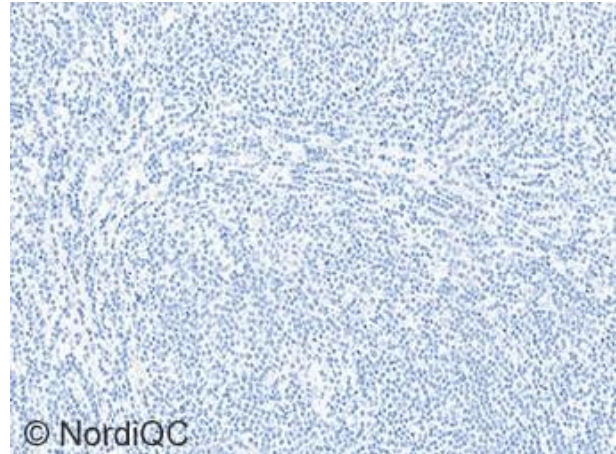

Fig. 3b
SOX11 staining of the B-CLL using same protocol as in Figs. 1b and 2b. No staining is seen.

Fig. 4a
Optimal SOX11 staining of the tonsil using same protocol as in Figs. 1a - 3a.
No staining is seen and the staining reaction of the tonsil confirms an adequate level of signal-to-noise ratio.
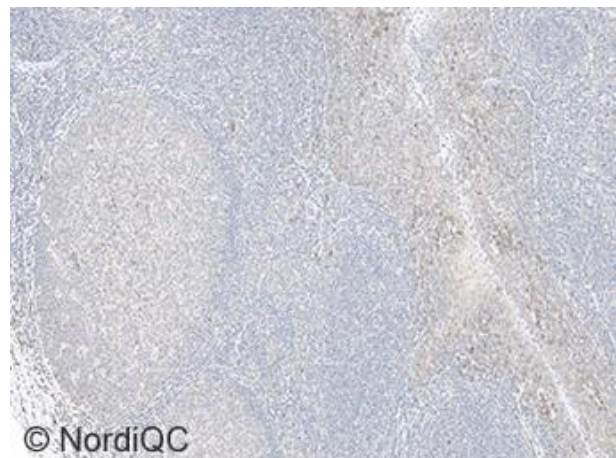Compare with Fig. 4b.

Fig. 4b
Insufficient SOX11 staining of the tonsil using a pAb providing an insufficient result characterized by a poor signal-to-noise ratio. In the tonsil a general background staining is seen and in e.g. plasma cells and squamous epithelial cells a moderate aberrant cytoplasmic staining reaction is seen. Also compare with Figs. 5a and 5b, same protocol.
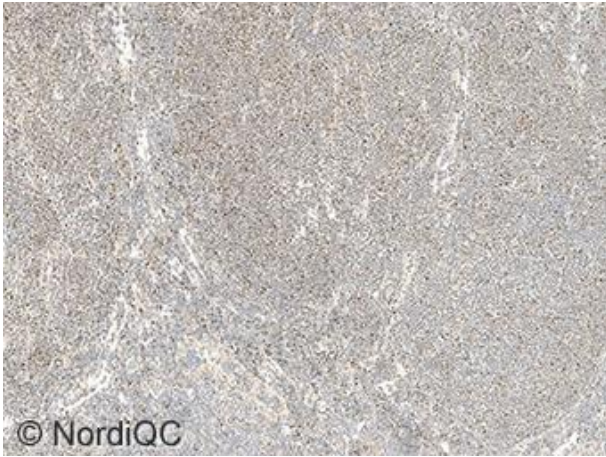
Fig. 5a
SOX11 staining of the mantle cell lymphoma, tissue core no. 5, using same protocol as in Fig. 4b. Many neoplastic cells show a weak to moderate nuclear staining reaction, but simultaneously a general background staining is seen compromising the interpretation. The intensity and proportion of cells demonstrated is reduced compared to the level expected and obtained in Fig. 2a. Also compare with Fig. 5b, same protocol.
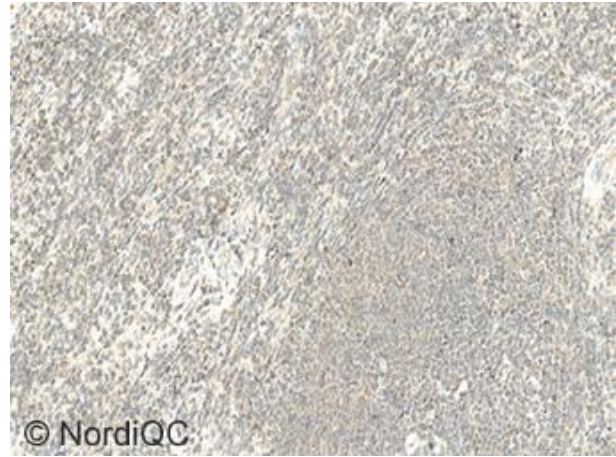

Fig. 5b
Insufficient SOX11 staining of the B-CLL. A poor signal-to-noise ratio is seen and the aberrant background staining complicates the interpretation of SOX11 in the neoplastic cells.

JLL/SN/LE/MV/RR 11.06.2016