Assessment Run 25 2009
CD30

The slide to be stained for CD30 comprised:
1. Tonsil, 2. Embryonal carcinoma, 3-4. Hodgkin lymphoma classical type, NS.
All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD30 staining as optimal included:

- An at least moderate and distinct membranous staining of the activated interfollicular and perifollicular B- & T-cells in the tonsil.
- An at least weak to moderate, distinct membranous and dot-like cytoplasmic staining of the majority of the Hodgkin cells in the two Hodgkin lymphomas.
- A moderate to strong, distinct membranous staining of majority of the neoplastic cells in the embryonal carcinoma.
- A strong cytoplasmic staining in the plasma cells in all specimens.
- No or only a minimal background reaction.

126 laboratories participated in the assessment. 78 % achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Abs and scores for CD30, run 25**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	98 3 1	Dako NeoMarkers Zymed	47	32	21	2	78 %	81 %
mAb clone **1G12**	3	Novocastra	2	1	0	0	-	-
mAb clone **15B3**	2	Novocastra	1	1	0	0	-	-
mAb clone **HRS4**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **Ber-H2**	12	Ventana	3	6	2	1	75 %	86 %
mAb clone **Ber-H2**	6	Dako	0	4	2	0	67 %	-
Total	126		53	45	25	3	-	-
Proportion			42 %	36 %	20 %	2 %	78 %	83 %

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

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **Ber-H2**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (22/45)*, Cell Conditioning 1 (BenchMark, Ventana) (6/16), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (5/10)*, Bond Epitope Retrieval Solution 2 (Bond, Leica) (5/8), Target Retrieval Solution pH 6.1 (Dako) (4/6), Bond Epitope Retrieval Solution 1 (Bond, Leica)(2/3), Citrate pH 6 (2/7) or EDTA/EGTA pH 8 (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:10 – 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 79 out of 97 (81 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **1G12**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Bond Epitope Retrieval Solution 2 (Bond, Leica)(1/2)* or Tris-EDTA/EGTA pH 9 (1/1)*. The mAb was diluted in the range of 1:10 – 1:40 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

mAb clone **15B3**: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using

Cell Conditioning 1 (BenchMark, Ventana). The Ab was diluted 1:30. Using similar protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **Ber-H2**, prod. no. 760-2926, Ventana: The protocols giving an optimal result were based on HIER in Cell Conditioning 1, standard, an incubation time of 32-48 min in the primary Ab and UltraView or iView as the detection system. Two laboratories used amplification kit. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER – too short heating time or use of citrate pH 6.0
- Proteolytic pre-treatment

In this assessment the prevalent feature of the insufficient results was a false negative or too weak staining in the cells expected to be demonstrated. This was especially observed in the Hodgkin lymphoma no. 4 and the embryonal carcinoma, whereas the staining was acceptable in the Hodgkin lymphoma no. 3 in the majority of the stains submitted.
Normal tonsil was found to be a reliable positive control for CD30, provided that the activated inter- and perifollicular B- & T-cells showed a distinct membranous staining and focally also a dot-like reaction. If these cells were negative or only weakly demonstrated, the neoplastic cells in the Hodgkin lymphomas and embryonal carcinoma also were negative or only showed an equivocal reaction. Occasionally a weak reaction was seen in serum in the vessels, especially when a highly sensitive protocol was applied (e.g., a 3-step polymer based detection system combined with HIER in an alkaline buffer). This was fully acceptable, as this reaction did not interfere with the interpretation of CD30. Proteolytic pre-treatment could not be used to obtain a sufficient result, as both the number of positive cells was reduced compared to the result based on HIER, but also due to an impaired morphology, as the membranes of the cells were extracted by the enzymatic digestion.

This was the second assessment of CD30 in NordiQC, and the proportion of sufficient results declined from 92 % in run 11, 2004 to 78 % in the current run. The lower pass rate is probably due to more challenging tissue material circulated.

Table 2. **Sufficient results with CD30 in two runs**

	Run 11 2004	Run 25 2009
Participants, n=	74	126
Sufficient results	92 %	78 %

Conclusion
The mAbs clones BER-H2, 1G12 and 15B3 are all useful markers for CD30.
HIER - preferable in an alkaline buffer - is mandatory to have an optimal reaction for CD30.
Tonsil is recommended as positive control: The activated interfollicular and perifollicular B- & T-cells shall show a moderate to strong and distinct membranous reaction.

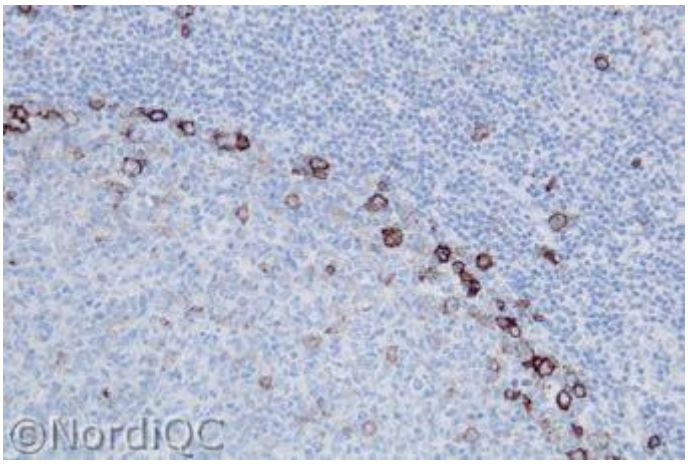

Fig. 1a
Optimal CD30 staining of the tonsil using the mAb clone Ber-H2 optimally calibrated and with HIER. The activated B- and T-cells, in particular located at the periphery of a germinal centre, show a strong predominantly membranous staining.

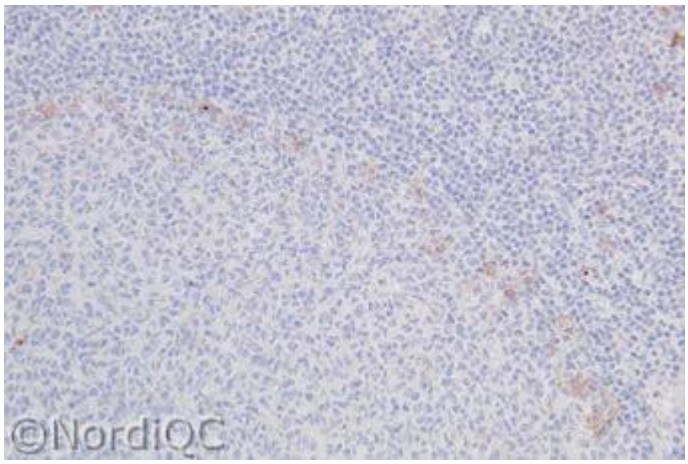

Fig. 1b
Insufficient CD30 staining of the tonsil using the mAb clone Ber-H2 too diluted – same field as in Fig. 1a. Only scattered activated B- and T-cells show a weak and diffuse membranous staining. Also compare with Fig. 2b and 3b – same protocol.

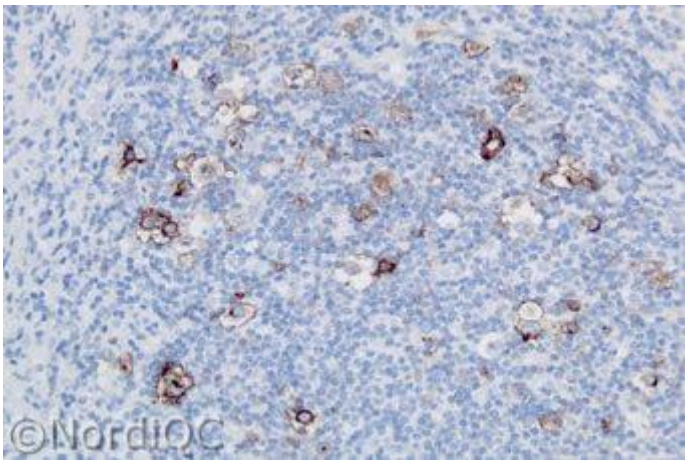

Fig. 2a
Optimal CD30 staining of the classical Hodgkin lymphoma (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a moderate to strong membranous staining and focally a dot-like positivity.

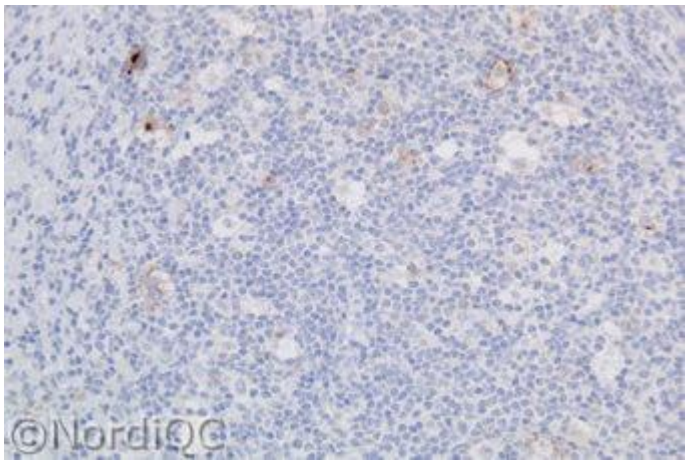

Fig. 2b
Insufficient CD30 staining of the classical Hodgkin lymphoma (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak and dubious staining - same field as in Fig. 2a.

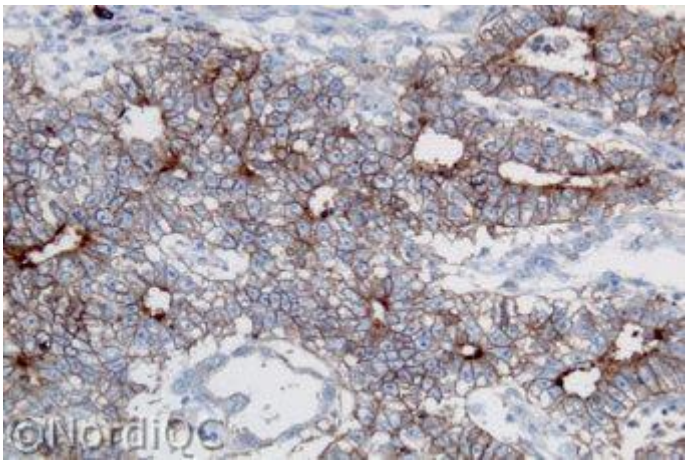

Fig. 3a
Optimal CD30 staining of the embryonal carcinoma using same protocol as in Fig. 1a & 2a. Virtually all the neoplastic cells show a distinct membranous staining and no background reaction is seen.

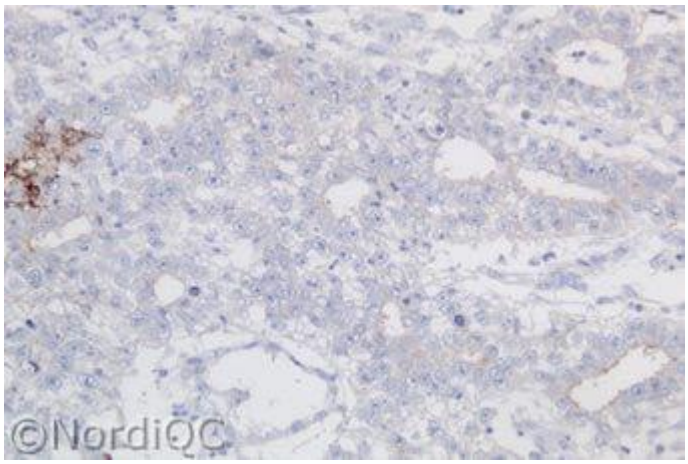

Fig. 3b
Insufficient CD30 staining of the embryonal carcinoma using same protocol as in Fig. 1b & 2b. The neoplastic cells are virtually negative and only a weak reaction is seen at the luminal surface of scattered cells – same field as in Fig. 3a.

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