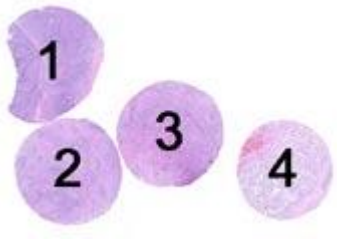
Assessment Run 31 2011
CD30

Material

The slide to be stained for CD30 comprised:

1. Tonsil, 2-3. Hodgkin's lymphoma classical type, NS.
4. Embryonal carcinoma



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD30 staining as optimal included:

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

172 laboratories participated in this assessment. 77 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD30, run 31**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	104 5 2 1 1 1 1	Dako NeoMarkers Cell Marque IDlabs Master Diagnostica Signet Laboratories Zytomed	38	54	18	5	80 %	84 %
mAb clone **1G12**	7	Leica/Novocastra	1	5	1	0	86 %	100 %
mAb clone **JCM182**	2	Leica/Novocastra	1	1	0	0	-	-
mAb clone **15B3**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **CON6D/B5**	1	Biocare	1	0	0	0	-	-
mAb clone **HRS4**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **Ber-H2, 790-2926**	23	Ventana	3	10	10	0	57 %	100 %
mAb clone **Ber-H2, IR602**	16	Dako	4	8	4	0	75 %	80 %
mAb clone **Ber-H2, 130M-97**	1	Cell Marque	0	1	0	0	-	-
mAb clone **Ber-H2, MS-361-R7**	1	NeoMarkers	0	1	0	0	-	-
mAb clone **1G12, PA0153**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **JCM182, PA0790**	1	Leica/Novocastra	1	0	0	0	-	-
mAb clone **CON6D/B5, PM346**	1	Biocare	0	0	1	0	-	-
Total	172		50	83	34	5	-	-

Proportion			29 %	48 %	20 %	3 %	77 %	-

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 either Tris-EDTA/EGTA pH 9 (7/18)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (9/21), TRS pH 9 (Dako) (1/10), TRS pH 6.1 (Dako) (1/6), Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (3/8), Cell Conditioning 1 (CC1; BenchMark, Ventana) (13/38), Diva Decloaker (Biocare) (2/2), Borg Decloaker (Biocare)(1/1), EDTA/EGTA pH8 (1/4) or Citrate pH 6 (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 80 out of 95 (84 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clone **1G12**: The protocol giving an optimal result was based HIER using BERS2 (Bond, Leica) (1/5) as the retrieval buffer. The mAb was diluted in 1:40. Using this protocol setting all of 5 (100 %) laboratories produced a sufficient staining.

mAb clone **JCM182**: The protocol giving an optimal result was based on HIER using BERS2 (Bond, Leica) (1/1) as the retrieval buffer. The mAb was diluted 1:30.

mAb clone **15B3**: The protocol giving an optimal result was based HIER using TRS pH 9 (Dako) (1/1) as the retrieval buffer. The mAb was diluted 1:50.

mAb clone **CON6D/B5**: The protocol giving an optimal result was based HIER using TRS pH 6.1 (Dako) (1/1) as the retrieval buffer. The mAb was diluted 1:50.

Ready-To-Use Abs
mAb clone **Ber-H2** (prod. no. 790-2926, Ventana): The protocols giving an optimal result were based on HIER using standard CC1, an incubation time of 60-92 min in the primary Ab and UltraView (760-500) as the detection system. Using these protocol settings all of 4 (100 %) laboratories produced a sufficient staining staining.

mAb clone **Ber-H2** (product no. IR602, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) and an incubation time of 20-30 min in the primary Ab and EnVision Flex (K8000/K8002) as the detection system. Using these protocol settings 12 out of 15 (80 %) laboratories produced a sufficient staining.

mAb clone **JCM182** (product no. PA0790, Leica/Novocastra): The protocol giving an optimal result was based on HIER using BERS1 (Bond, Leica), an incubation time of 15 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 - too short efficient heating time
- Inappropriate epitope retrieval - proteolytic pre-treatment

In this assessment and in concordance to the previous assessment of CD30 in NordiQC the prevalent feature of the insufficient results was a false negative or a too weak staining of the cells expected to be demonstrated. In 90 % of the insufficient results (35 out of 39) a too weak or a false negative staining was observed, and in the remaining 10 % both a too weak and a false positive staining was seen.
The too weak staining was in virtually all cases characterized by a weak or equivocal diffuse membranous staining in the Hodgkin cells, the embryonal carcinoma and the activated B- and T-cells in the tonsil. A too low concentration of the primary Ab and/or a too short HIER time were the main reasons for the insufficient stains. The false positive staining reactions appeared most likely to be caused by a too high titre of the primary Ab and/or inadequate washing in buffer. In a couple of cases contamination with another primary Ab occurred.

The tonsil is a reliable positive control for CD30: The activated inter- and perifollicular B- & T-cells must show 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 two Hodgkin lymphomas and the embryonal carcinoma also were negative or only showed an equivocal reaction.
Proteolytic pre-treatment should not be used, as both the number of positive cells is reduced (compared to the

result based on HIER), and the morphology is impaired, as the cell membranes are impaired by the enzymatic digestion.

This was the 3rd NordiQC assessment of CD30. Virtually the same pass rates have been achieved in the two latest runs (see table 2), despite the large number of new laboratories participating for the first time.

Table 2. **Proportion of sufficient results for CD30 in the three NordiQC runs performed**

	Run 11 2004	Run 25 2009	Run 31 2011
Participants, n=	74	126	172
Sufficient results	92 %	78 %	77 %

Only a slight difference in the pass rates was observed for the laboratories participating in the CD30 assessment for the first time compared to the laboratories also participating in the latest assessment run 25, 2009. For the laboratories participating for the first time the pass rate was 71 % (37 out of 52 laboratories), whereas the pass rate was 80 % (96 out of 120) for the laboratories participating in both runs.

Conclusion
The mAbs clones BER-H2, 1G12, 15B3, CON6D/B5 and JCM182 are all useful for the detection of CD30.
HIER, preferable in an alkaline buffer, is mandatory to provide an optimal staining reaction for CD30. Tonsil is recommended as positive control: The activated interfollicular and perifollicular B- & T-cells must show a moderate to strong, distinct membranous staining reaction.

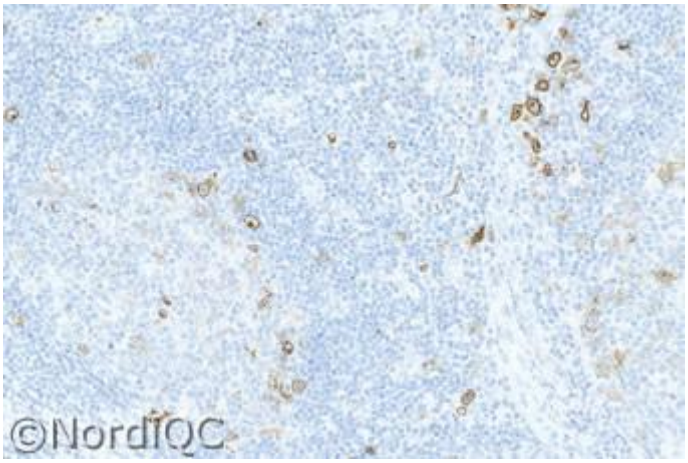


Fig. 1a
Optimal CD30 staining of the tonsil using the mAb clone Ber-H2 optimally calibrated and with HIER in an alkaline buffer. The activated B- and T-cells, particularly located at the periphery of the germinal centres, show a strong predominantly membranous staining reaction.

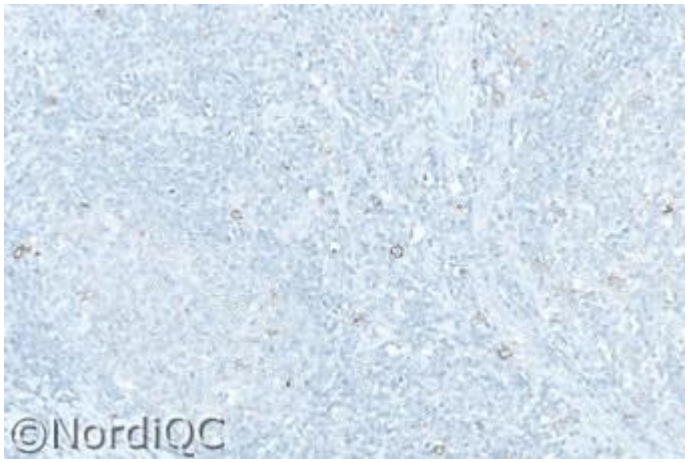


Fig. 1b
Insufficient CD30 staining of the tonsil using the mAb clone Ber-H2 too diluted – same field as in Fig. 1a. Only scattered activated B- and T-cells show a weak and diffuse membranous staining reaction. Also compare with Figs. 2b and 3b – same protocol.

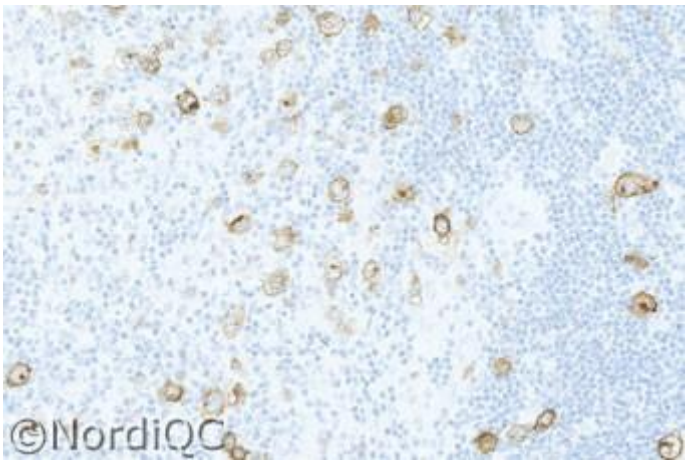


Fig. 2a
Optimal CD30 staining of the classical Hodgkin lymphoma NS (no. 2) using same protocol as in Fig. 1a. The Reed-Sternberg and the Hodgkin cells show a moderate to strong membranous and focally also a dot-like staining reaction.

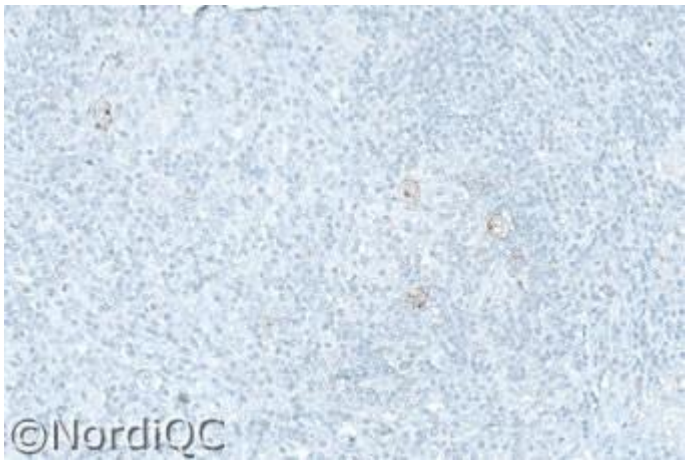


Fig. 2b
Insufficient CD30 staining of the classical Hodgkin lymphoma NS (no. 2) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak and equivocal staining reaction - same field as in Fig. 2a.

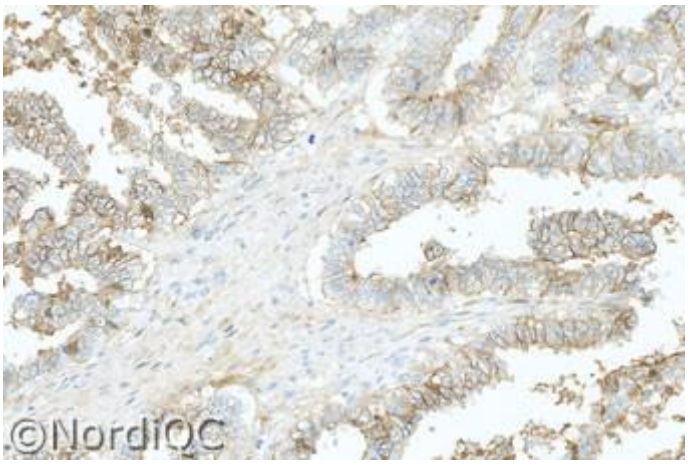


Fig. 3a
Optimal CD30 staining of the embryonal carcinoma using same protocol as in Fig. 1a & 2a. The majority of the neoplastic cells show a moderate and distinct membranous staining reaction. A weak background reaction due to necrosis is seen.

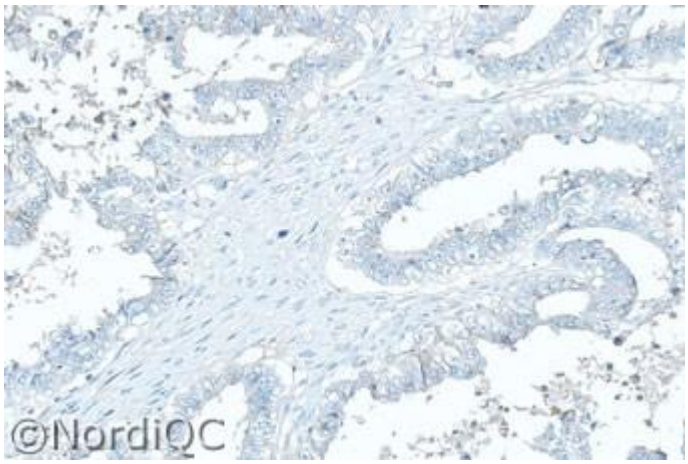


Fig. 3b
Insufficient CD30 staining of the embryonal carcinoma using same protocol as in Figs. 1b & 2b. The neoplastic cells are virtually negative – same field as in Fig. 3a.

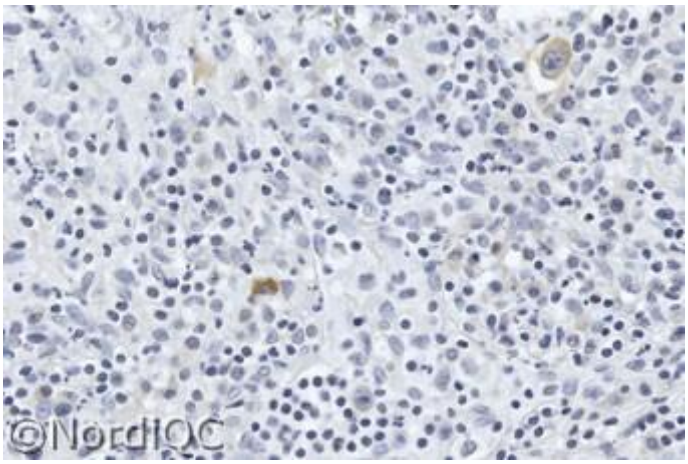


Fig. 4a
Insufficient CD30 staining of the classical Hodgkin lymphoma NS (no. 2) using the mAb clone Ber-H2 with proteolytic pre-treatment. The Reed-Sternberg and Hodgkin cells show a weak equivocal staining reaction, while the plasma cells reveal a moderate cytoplasmic staining reaction.

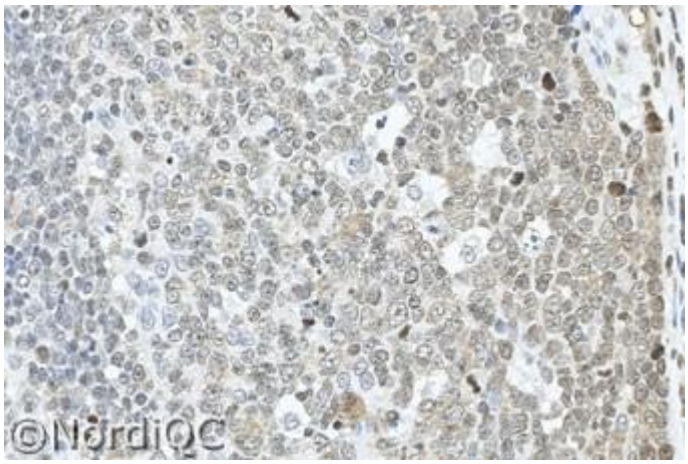


Fig. 4b
Insufficient CD30 staining of the tonsil.
A moderate background and aberrant nuclear staining reaction compromises the interpretation of the specific membranous staining reaction of the activated B- and T-cells. A too high concentration of the mAb clone Ber-H2 and/or inadequate washing in buffer may be the reason for the poor signal-to-noise ratio.

SN/MV/LE 7-4-2011