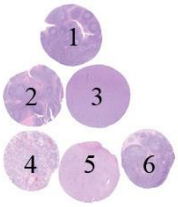
Assessment Run 51 2017
CD30

Material
The slide to be stained for CD30 comprised:



1-2. Tonsil, 3. Anaplastic large cell lymphoma (ALCL), 4. Embryonal carcinoma, 5-6. Hodgkin's lymphoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD30 staining as optimal included:

- An at least weak to moderate, distinct membranous staining reaction of interfollicular activated B- and T-cells and a subpopulation of activated B-cells primarily located in the rim of the germinal centres in the two tonsils.
- A strong, predominantly membranous staining reaction of all neoplastic cells in the ALCL.
- A moderate to strong, predominantly membranous staining reaction of all neoplastic cells in the embryonal carcinoma - a weak background staining due to necrosis was accepted.
- A moderate to strong, predominantly membranous staining reaction of all the neoplastic cells in the Hodgkin lymphoma (tissue core no 5).
- An at least moderate, predominantly membranous and dot-like (Golgi zone) cytoplasmic staining reaction of the vast majority of Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma (tissue core no 6).
- No or only a weak background staining. Cytoplasmic staining of the plasma cells was accepted.

Participation

Number of laboratories registered for CD30, run 51	302
Number of laboratories returning slides	282 (93%)

Results
282 laboratories participated in this assessment. 234 (83%) achieved a sufficient mark (optimal or good). Table 1 summarizes the used antibodies (Abs) and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Insufficient HIER (too short heating time or too low temperature)
- Too low concentration of the primary antibody
- Use of low sensitivity detection systems
- Unexplained technical issues

Performance history
This was the fifth NordiQC assessment of CD30. The overall pass rate increased compared to run 43, 2015 (see Table 2).

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

	Run 11 2004	Run 25 2009	Run 31 2011	Run 43 2015	Run 51 2017
Participants, n=	74	126	172	252	282
Sufficient results	92%	78%	77%	71%	83%

Conclusion
The mAb clones **Ber-H2**, **CON6D/5** and **JCM182** could all be used to obtain optimal staining results for CD30. The mAb clone **Ber-H2** was the most frequently used antibody for CD30 both in laboratory develop assays and in Ready-To-Use systems. Efficient HIER, preferable in an alkaline buffer or modified low pH buffer, in combination with a sensitive and specific IHC system were the main prerequisites for optimal performance. Weak or false negative staining reactions were the most prominent characteristics of insufficient staining results.
Tonsil is recommended as positive and negative tissue control: Interfollicular activated B- and T-cells, and activated B-cells primarily located in the rim of the germinal centres must at least display a weak to moderate but distinct membranous staining reaction. Virtually all other cells must be negative.

Table 1. **Antibodies and assessment marks for CD30, run 51**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	94 10 2 2 2 2 1	Agilent/Dako Cell Marque Thermo S./Neomarkers Diagnostic Biosystems Immunologic Zytomed Systems Nordic Biosite	53	41	13	6	83%	84%
mAb clone **JCM182**	10	Leica/Novocastra	6	2	1	1	80%	100%
mAb clone **1G12**	6	Leica/Novocastra	0	4	1	1	67%	-
mAb clone **CON6D/5**	5	Biocare Medical	4	0	1	0	80%	100%
mAb clone **HRS4**	1	Thermo Scientific	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **Ber-H2 IS/IR602**	30	Agilent/Dako	18	11	1	0	97%	96%
mAb clone **Ber-H2 IS/IR602**[3]	21	Agilent/Dako	15	4	1	1	90%	-
mAb clone **Ber-H2 790-4858**	75	Roche/Ventana	34	27	8	6	81%	87%
mAb **Ber-H2 MAD-002045QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb **Ber-H2 130M-XX**	2	Cell Marque	0	0	0	2	-	-
mAb clone **Ber-H2 MS-361-R7**	1	Thermo S. /Neomarkers	1	0	0	0	-	-
mAb clone **Ber-H2 MAB-0023**	1	Maxin	0	1	0	0	-	-
mAb clone **JCM182 PA0790**	10	Leica/Novocastra	7	2	1	0	90%	90%
mAb clone **1G12 PA0153**	3	Leica/Novocastra	0	1	2	0	-	-
mAb clone **HRS4 AM351-5/10**	1	BioGenex	0	1	0	0	-	-
mAb clone **unknown 8265-C010**	1	Sakura Finetek USA	0	0	1	0	-	-
Total	282		140	94	31	17	-	
Proportion			50%	33%	11%	6%	83%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Agilent/Dako semi-automated systems (Autostainer) but used by laboratories on the Omnis (Agilent/Dako), Ventana Benchmark XT/Ultra or manually.

Detailed analysis of CD30, Run 51

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **Ber-H2**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Agilent/Dako) (6/18)*, TRS pH 9 (Agilent/Dako) (1/2), TRS pH 6.1 (Agilent/Dako) (6/8), Cell Conditioning 1 (CC1, Ventana) (32/58), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (6/10), DSB Montage EDTA solution (Diagnostic Biosystem) (1/1) or Tris-EDTA/EGTA pH 9 (1/5) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 75 of 89 (84%) laboratories produced a sufficient staining result (optimal or good). One laboratory obtained an optimal result without performing any antigen retrieval at all.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **JCM182**: Protocols with optimal results were all based on HIER using BERS2 (Leica) (4/4) or Bond Epitope Retrieval Solution 1 (BERS1, Leica) (2/4) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 7 of 7 (100%) laboratories produced a sufficient staining result.

mAb clone **CON6D/5**: Protocols with optimal results were all based on HIER using TRS pH 6.1 (Agilent/Dako) (4/4) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 4 of 4 (100%) laboratories produced an optimal staining result.

Table 3. **Proportion of optimal results for CD30 with the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer / Omnis**		**Ventana BenchMark GX /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 **Ber-H2**	4/18** (22%)	4/8 (50%)	28/51 (55%)	-	6/10 (60%)	-

** 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
mAb clone **Ber-H2**, product no. **IS602/IR602**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1), TRS pH 9 or TRS pH 6.1 (efficient heating time 10-20 min. at 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 24 of 25 (96%) laboratories produced a sufficient staining result (optimal or good). Two laboratories obtained an optimal mark without performing any antigen retrieval at all.

mAb clone **Ber-H2**, product no. **790-4858**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1) (efficient heating time 8-92 min. at 90-100°C), 24-120 min. incubation of the primary Ab. and UltraView (760-500) with amplification or OptiView (760-700) with or without amplification as detection systems. Using these protocol settings, 53 of 61 (86%) laboratories produced a sufficient staining result. One laboratory obtained an optimal mark without performing any antigen retrieval at all.

mAb clone **JCM182,** product no. **PA0790**, Leica, BOND-max/BOND-III:
Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2) or Bond Epitope Retrieval Solution 1 (BERS1) (efficient heating time 20-30 min. at 95-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 9 of 10 (90%) laboratories produced a sufficient staining result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the specific IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for CD30 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb Ber-H2 **IS/IR602**	100% (3/3)	100% (3/3)	96% (23/24)	50% (12/24)
VMS Ultra/XT mAb Ber-H2 **790-4858**	66% (6/9)	22% (2/9)	96% (23/24)	49% (11/24)
Leica BOND MAX/III mAb JCM182 **PA0790**	83% (5/6)	50% (3/6)	100% (4/4)	100% (4/4)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments
In this fifth NordiQC assessment for CD30, and in concordance to the previous runs 25, 31 and 43, the prevalent features of an insufficient staining result were a generally too weak or completely false negative staining reaction of the cells expected to be demonstrated. This was observed in 96% of the insufficient results (46 of 48). The remaining insufficient results were caused by a poor signal-to-noise ratio compromising the interpretation.
Virtually all laboratories were able to demonstrate CD30 in high-level antigen expressing cells, such as neoplastic cells of the anaplastic large cell lymphoma and neoplastic cells of the Hodgkin lymphoma (tissue core no 5). However, demonstration of CD30 in low-level antigen expressing cells as activated B- and T-cells in the tonsil and neoplastic cells of the Hodgkin lymphoma (tissue core no 6) was more challenging and required optimally calibrated protocols.
Optimal staining results were seen with the mAb clones Ber-H2, JCM182 and CON6D/5 (see Table 1). The mAb clone JCM182 from Leica/Novocastra gave an unexpected staining reaction of both endothelial cells and a subpopulation of macrophages (see Fig. 7). This aberrant staining pattern was accepted, as it did not compromise interpretation of the specific reaction patterns for CD30 in this run.

The mAb clone Ber-H2 was the most widely used antibody for demonstration of CD30 and provided optimal results on all three main IHC platforms from Agilent/Dako, Leica and Roche/Ventana (see Table 3). Used as a concentrate within a laboratory developed (LD) assay, mAb clone Ber-H2 gave a pass rate of 83% (94 of 113) out of which 47% were optimal. Efficient HIER in an alkaline buffer or a modified low pH buffer (Target Retrieval Solutions pH 6.1, Dako), careful calibration of the primary antibody and use of a sensitive 3-step polymer/multimer based detection system gave the highest proportion of optimal results. The overall pass rate for participants using a 3-step polymer/multimer based detection system (e.g. Bond Refine (Leica), Envision Flex+ (Dako) and OptiView (Ventana)) was 87% (78 of 88) of which 53% (47 of 88) were assessed as optimal. In comparison and for laboratories using a 2-step polymer/multimer based detection system (e.g. Envision Flex (Dako) and UltraView (Ventana)), the overall pass rate was only 59% (13 of 22) of which 18% (4 of 22) were assessed as optimal.
The proportion of optimal results were also affected by the choice of HIER buffer. Using the mAb clone Ber-H2 within a LD-assay, any dilution range of the primary Ab and EnVision Flex/Flex+ (Dako) as the detection systems and HIER in TRS pH 9 (Dako), provided a pass rate of 84% (16 of 19) but only 21% (4 of 19) were assessed as optimal. If same protocol settings were applied using HIER in TRS pH 6.1 (Dako), a pass rate of 90% (9 of 10 protocols) was seen out of which 60% were optimal. Laboratories should be cautious, as standard low pH buffers will not provide the same result as the modified low pH buffer (TRS pH 6.1) from Agilent/Dako.

In this context, and as noted in the previous run for CD30, the mAb clone CON6D/5 within a LD-assay requires HIER in a modified low pH buffer for optimal performance. Using this buffer, 100% (4 of 4) were assessed as optimal. The primary Ab was typically diluted 1:25-1:100 and used in combination with a 3-step polymer detection system (Envision Flex+, Dako). One protocol assessed as insufficient used HIER in Diva Decloaker retrieval solution (Biocare), which have the same demasking ability as the modified low pH buffer from Agilent/Dako, but applied a protocol providing too low sensitivity due to low concentration of the primary Ab. In addition, an excessive antigen retrieval procedure (pressure cooker for 20 min. at 110°C) was used, damaging morphology and as a result, reducing the expected level of the specific antigen (CD30) – see Fig. 1a-5b.

Using the mAb clone JCM182 within a LD assay, the overall pass rate was 80% (8 of 10) out of which 60% were assessed as optimal. The mAb was mainly used by laboratories on the BOND MAX/III (8 of 10) using efficient HIER in BERS2, dilution range 1:50-1:200 of the primary Ab and BOND refine as the detection system. All protocols (4 of 4) were assessed as optimal.

In this assessment, the Ready-to-use (RTU) system IR/IS602 (Dako Autostainer) based on the mAb Ber-H2 was the most successful assay for detection of CD30. Optimal results could be obtained with both vendor and laboratory modified protocol settings (see Table 4). Applying optimal protocol settings (see description above) and using Envision Flex+ as the detection system, 100% (12 of 12) of the protocols were assessed as sufficient and 83% gave an optimal result. Using exactly the same conditions but with Envision Flex as the detection system, 92% (12 of 13) of the protocols were sufficient but only 31% (4 of 13) were assessed as optimal. In comparison, the official recommendation for RTU system IR/IS602 is based on HIER in TRS low pH for 20 min. at 95-97°C, 20 min. incubation of the primary Ab and EnVision Flex as the detection system. Using these conditions, all protocols (3 of 3) were assessed as optimal. As noted for the LD-assays based on the mAb Ber-H2, the use of the modified low pH buffer (TRS pH 6.1, Dako) can significantly alter outcome of an assay towards better and stronger staining of cells expected to be positive, and may explain why the use of a detection system with a generally lower sensitivity (Envision Flex) can work in diagnostic settings.

The RTU system 790-4858 (Ventana Benchmark GX/XT/Ulta) based on the mAb Ber-H2, optimized and developed for the use of OptiView or UltraView with amplification as the detection systems, could provide sufficient and optimal results using both vendor and laboratory modified protocol settings (see Table 4). However, the proportion of sufficient and optimal results was higher using laboratory modified protocol settings typically adjusting parameters such as HIER time, incubation time of the primary antibody and choice of detection system. Compared with the previous assessment for CD30, using vendor recommended protocol settings, only 22% (2 of 9) of the protocols were assessed as optimal in this run, whereas 86% (6 of 7) of the protocols in run 43 gave an optimal result. Currently, there is no good explanation for the discrepancy in performance between the two runs, as laboratories used almost identical protocol settings. However, in this run, the choice of detection system influenced not only the overall pass rate but also the proportion of optimal results. For participants applying optimal protocol settings as described above and using OptiView as the basic/solitary detection system, 79% (15 of 19) of the laboratories produced a sufficient result of which 26% (5 of 19) were assessed as optimal. If participants used exactly the same conditions, but applied OptiView with amplification as the detection system, 100% (27 of 27) of the laboratories produced a sufficient result of which 89% (24 of 27) were assessed as optimal (see Fig. 6a-6c).

The RTU system PA0790 (Leica, BOND MAX/III) based on the mAb JCM182 provided significantly better results using laboratory modified protocol settings typically adjusting parameters such as the HIER buffer (BERS2 instead of BERS1), HIER time (extended) and/or incubation time of the primary antibody (extended). Using these conditions, all protocols (4 of 4) were assessed as optimal.

This was the fifth assessment of CD30 in NordiQC (see Table 2). A pass rate of 83% was obtained, which is a significant increase compared to the Run 43, 2015. In this run, the performance of RTU system IS/IR602 based on the mAb Ber-H2 (Dako/Agilent) was the most successful assay for detection of CD30. Grouped together, the RTU system from the three main suppliers (Agilent/Dako, Roche/Ventana and Leica) provided an overall pass rate of 89% (99 of 115) and was in this assessment marginally better compared to LD-assays based on the same clones (mAb Ber-H2 and mAb JCM182), giving an overall pass rate of 82% (102 of 123).

Controls
Tonsil is recommended as positive and negative tissue control for CD30. The protocol must be calibrated to provide a weak to moderate but distinct membranous staining reaction of interfollicular activated B- and T-cells, and activated B-cells primarily located in the rim of the germinal centres. Virtually all other cells must be negative. Plasma cells, macrophages and endothelial cells may be positive depending on the primary antibody clone applied (e.g. plasma cells can be positive using the mAb Ber-H2, endothelial cells and macrophages can be positive using the mAb JCM182).

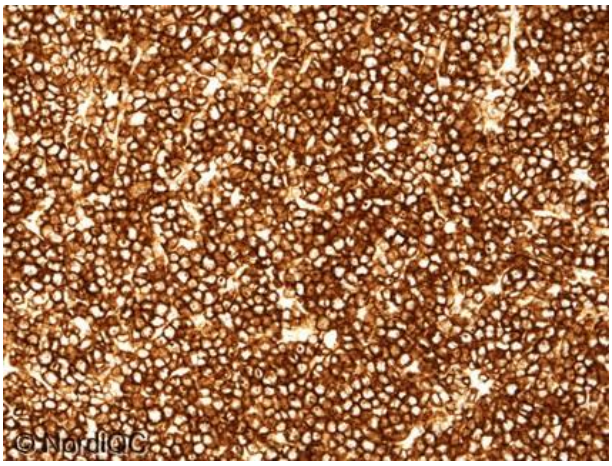


Fig. 1a (x200)
Optimal CD30 staining of the ALCL using the mAb clone CON6D/5 as concentrate, HIER in an modified low pH buffer (TRS pH 6.1, Dako) and a 3-step polymer based detection system (Flex+, Dako Omnis). Same protocol used in Figs. 2a - 5a. All neoplastic cells show a strong predominantly membranous staining reaction - compare with Fig. 1b.

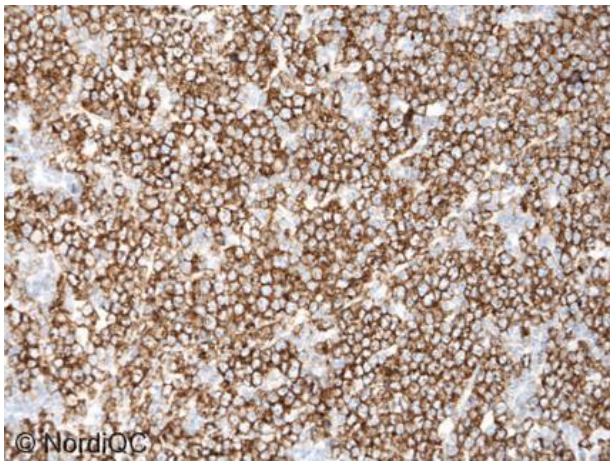


Fig. 1b (x200)
Insufficient staining for CD30 of the ALCL using the mAb clone CON6D/5 as concentrate (too diluted), HIER in Diva Decloaker solution pH 6.2 (excessive) and MACH1 (Biocare) as detection system – same protocol used in Figs. 2b – 6b. Staining intensity of the neoplastic cells are reduced - compare with Fig. 1a (same field), but also with Fig. 2a-5b.

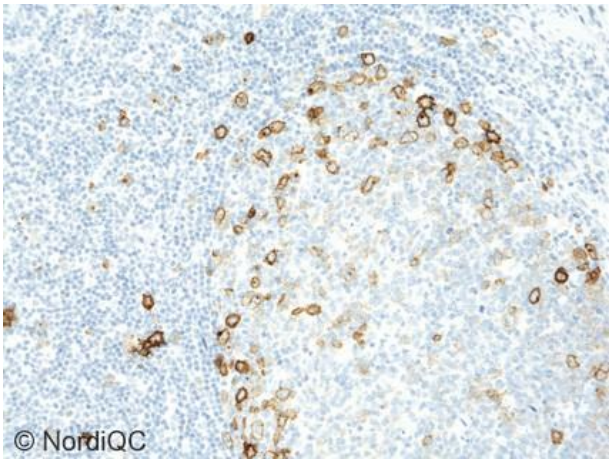


Fig. 2a (x200)
Optimal staining for CD30 in the tonsil, tissue core no 2, using same protocol as in Fig. 1a. The activated B- and T-cells, particularly B-cells located at the rim of the germinal centres, show a moderate to strong predominantly membranous staining reaction.

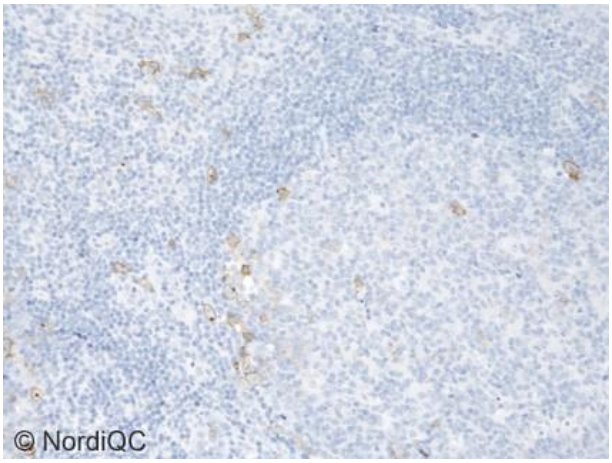


Fig. 2b (x200)
Insufficient staining for CD30 in the tonsil, tissue core no 2, using same protocol as in Fig 1b. The proportion of activated B- and T-cells is significantly reduced and staining intensity is too weak - compare with Fig. 2a (same field).

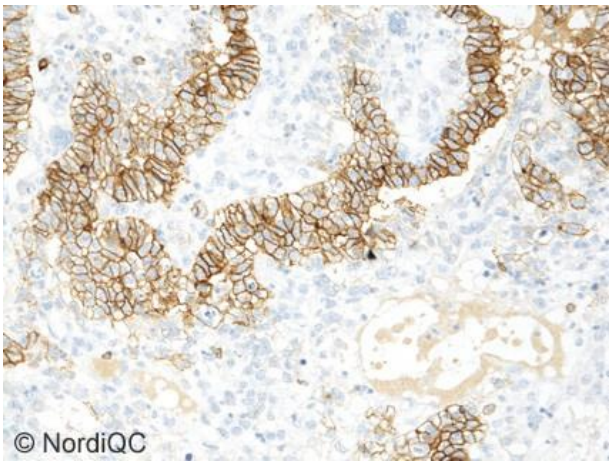


Fig. 3a (x200)
Optimal staining for CD30 in the embryonal carcinoma using same protocol as in Fig. 1a. All the neoplastic cells displays a strong continuous membranous staining reaction.

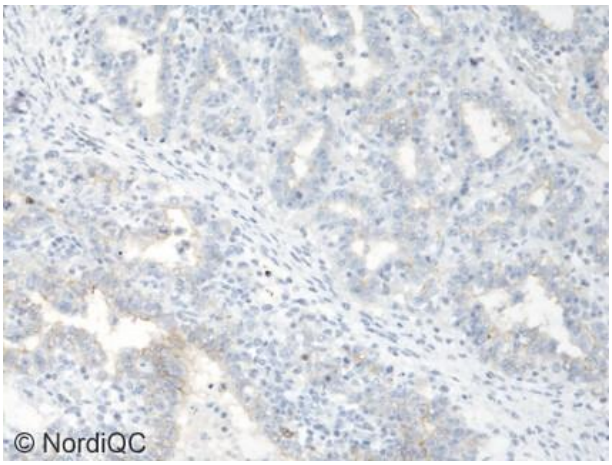


Fig. 3b (x200)
Insufficient staining for CD30 in the embryonal carcinoma using same protocol as in Fig. 1b. The neoplastic cells are false negative or only display a faint inconsistent membranous staining reaction - compare with Fig. 3a (same field).

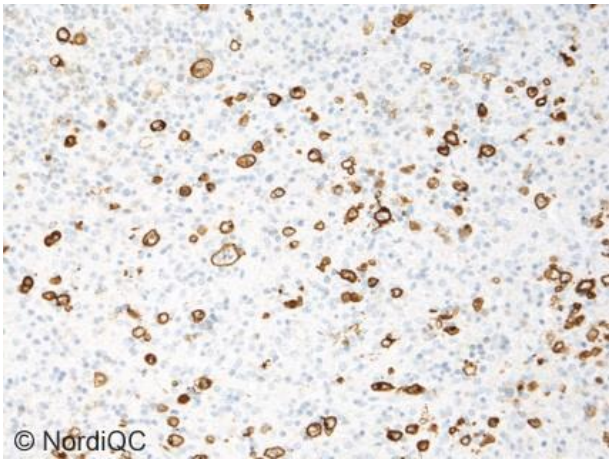


Fig. 4a (x200)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 5, using same protocol as in Fig. 1a - 3a. Virtually all the neoplastic cells show a strong predominately membranous staining reaction.

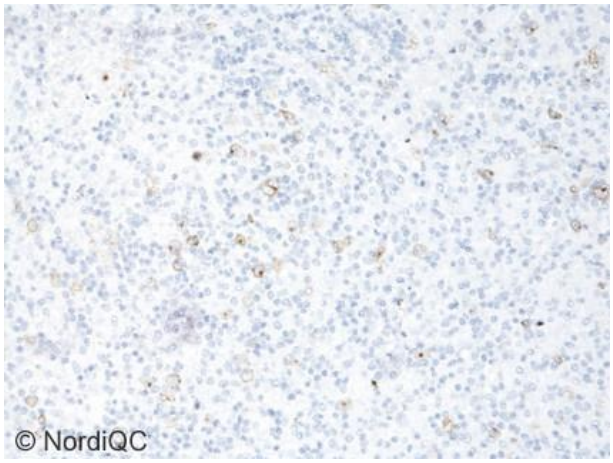


Fig. 4b (x200)
Insufficient staining for CD30 of the Hodgkin lymphoma, tissue core no 5, using same protocol as in Fig. 1b -3b. Staining intensity of the neoplastic cells is too weak or false negative - compare with Fig. 4a (same field).

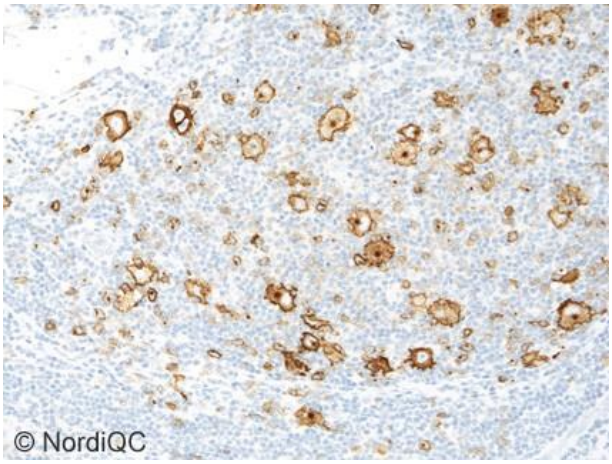


Fig. 5a (x200)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using same protocol as in Fig. 1a - 4a. Virtually all Reed-Sternberg and Hodgkin cells show a moderate to strong, distinct membranous and cytoplasmic dot-like staining pattern.

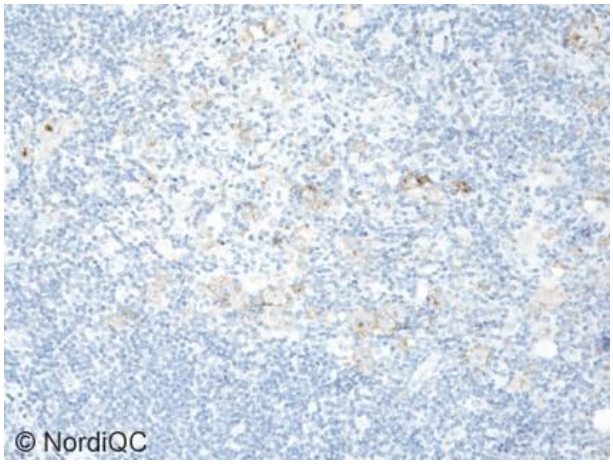


Fig. 5b (x200)
Insufficient staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using same protocol as in Fig. 1b - 4b. The Reed-Sternberg and Hodgkin cells only display weak, inconsistent membranous staining reaction. In addition, the cytoplasmic dot-like staining reaction of the Reed-Sternberg and Hodgkin cells is weak and proportion of positive cells is significantly reduced - compare with Fig. 5a (same field).

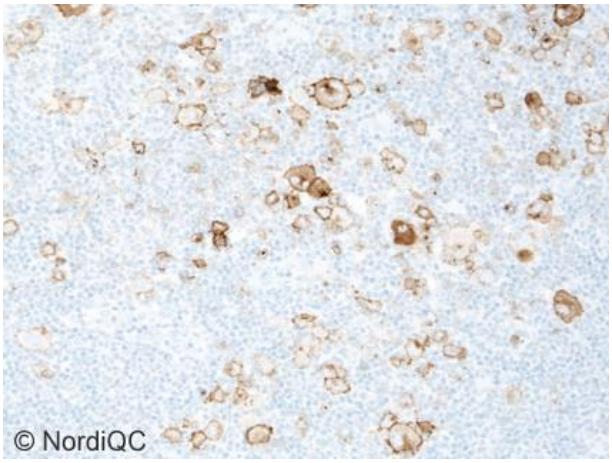


Fig. 6a (x100)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using the RTU system 790-4858 (Ventana) based on the mAb Ber-H2 following the protocol settings given by the vendor (efficient HIER in CC1 for 64 min. and Optiview as the detection system). Staining intensity and proportion of stained cells are as in Fig. 5a – also, compare with Fig. 6b-6c.

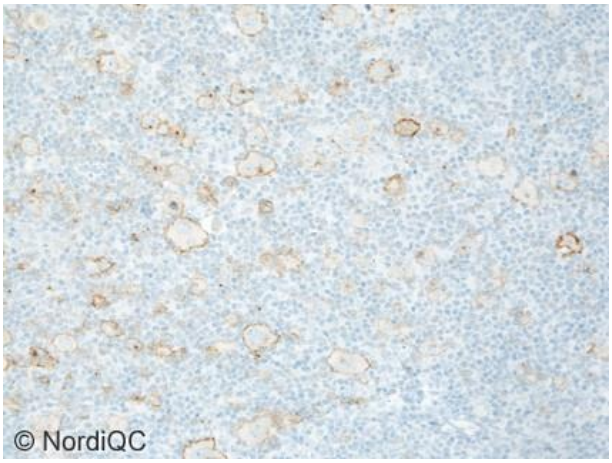


Fig. 6b (x100)
Sufficient staining (good) for CD30 of the Hodgkin lymphoma, tissue core no 6. Although using exactly same protocol settings as in Fig. 6a, staining intensity of the Reed-Sternberg and Hodgkin cells is weaker. This deviant reaction pattern was frequently seen using the RTU system 790-4858 (Ventana) and no specific causes could be identified, explaining for the unexpected difference in performance between procedures with completely identical protocol settings - compare with Fig. 6a (same field).

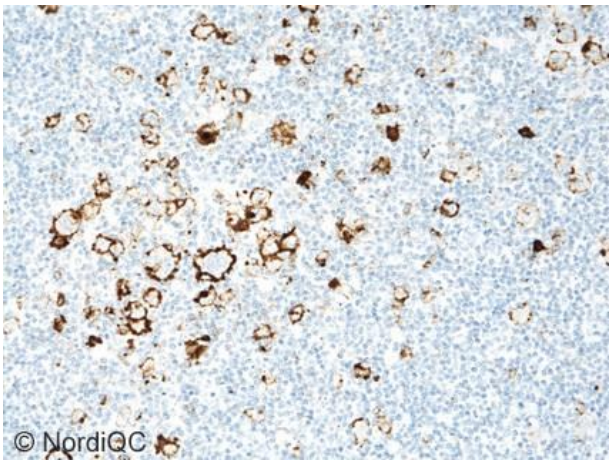


Fig. 6c (x200)
Optimal staining for CD30 of the Hodgkin lymphoma, tissue core no 6, using using exactly same protocol settings as in Fig. 6a., except for use of Optiview with amplification as the detection system. Staining intensity is strong and proportion of stained cells are as in Fig. 5a. All laboratories using OptiView with amplification produced a sufficient result and 89% were giving an optimal score – also, compare with Fig. 6a-6b.

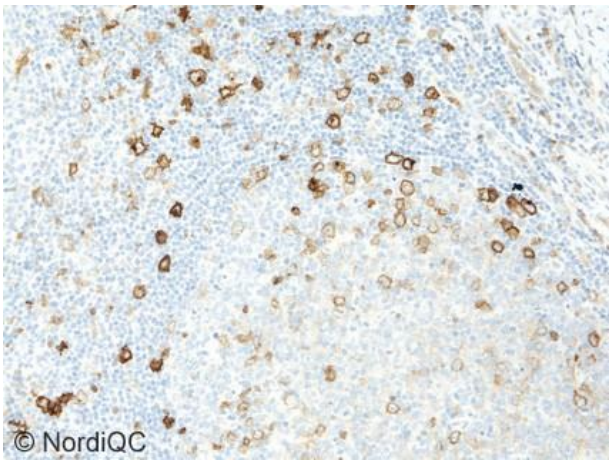


Fig. 7 (x200)
Optimal CD30 staining of the tonsil, tissue core no 2, using the mAb clone JCM182 as concentrate, HIER in BERS1 buffer (Leica) and a 3-step polymer based detection system (Bond Refine, Leica). Note, endothelial cells (upper right corner) and macrophages (dendritic morphology), intermingling with activated B- and T-cells in the interfollicular zone, are weakly stained – compare with Fig. 2 a. This aberrant reaction pattern was accepted, as it did not compromise interpretation of the neoplasias in this assessment.

MB/LE/MV/RR 27.11.2017