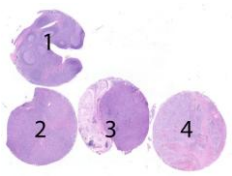
Assessment Run 43 2015
CD30

Material
The slide to be stained for **CD30** comprised



1. Tonsil, 2-3. Hodgkin's lymphoma, classical type. 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 reaction of interfollicular activated B- and T-cells and perifollicular germinal centre B-cells in the tonsil.
- A moderate to strong, predominantly membranous and dot-like (Golgi zone) cytoplasmic staining reaction of Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma (tissue core 2).
- An at least weak to moderate, predominantly membranous and dot-like (Golgi zone) cytoplasmic staining reaction of the majority of Hodgkin and Reed-Sternberg cells in the Hodgkin lymphoma (tissue core 3).
- A moderate to strong, predominantly membranous staining reaction of virtually all neoplastic cells in the embryonal carcinoma - a weak background staining due to necrosis was accepted.
- No or only a weak background staining. Cytoplasmic staining of the plasma cells was accepted.

Participation

Number of laboratories registered for CD30, run 43	273
Number of laboratories returning slides	253 (93%)

Results
Of the 253 participating laboratories, 1 laboratory used an inappropriate primary antibody. 179 laboratories (71%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and 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

Performance history
This was the 4th NordiQC assessment of CD30. The overall pass rate decreased slightly compared to run 31, 2011 (see table 2).

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

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

Conclusion
The mAbs clones **Ber-H2**, **CON6D/5, 1G12, JCM182 and the rmAb EP154** could all be used to obtain optimal staining results for CD30. Irrespective of the antibodies applied, efficient HIER in an alkaline buffer or a modified low pH buffer (Target Retrieval Solutions pH 6.1, Dako or Diva Decloaker pH 6.2, BioCare), 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.
Tonsil is recommended as positive tissue control. Interfollicular activated B- and T-cells and perifollicular germinal centre B-cells must at least display a weak to moderate but distinct membranous staining reaction.

Table 1. **Antibodies and assessment marks for CD30, run 43**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Ber-H2**	102 9 2 1 1 1 1	Dako Cell Marque Thermo/Neomarkers Biosystems GeneMed Immunologic Zytomed Systems	38	46	27	6	72%	77%
mAb clone **1G12**	9	Leica/Novocastra	4	3	2	0	78%	100%
mAb clone **JCM182**	5	Leica/Novocastra	4	1	0	0	100%	100%
mAb clone **CON6D/5**	3	Biocare	3	0	0	0	-	-
mAb clone **15B3**	2	Leica/Novocastra	0	2	0	0	-	-
mAb clone **HRS4**	1	Thermo/Neomarkers	0	0	1	0	-	-
rmAb **EP154**	1	Beijing Zhongsan	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **Ber-H2 IS/IR602**	47	Dako	17	21	8	1	81%	74%
mAb clone **Ber-H2 790-2926**	25	Roche/Ventana	6	11	7	1	68%	88%
mAb clone **Ber-H2 790-4858**	25	Roche/Ventana	6	3	8	8	36%	86%
mAb **Ber-H2 MAD-002045QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **Ber-H2 MAB-0023**	1	Maxin	1	0	0	0	-	-
mAb clone **Ber-H2 MS-361-R7**	1	Thermo/Neomarkers	0	1	0	0	-	-
mAb clone **Ber-H2 AM327-5M**	1	BioGenex	0	0	1	0	-	-
mAb clone **Ber-H2 130M**	1	Cell Marque	0	0	0	1	-	-
mAb clone **JCM182 PA0790**	5	Leica/Novocastra	4	0	1	0	80%	80%
mAb clone **1G12 PA0153**	3	Leica/Novocastra	1	2	0	0	-	-
mAb clone **1G12 CD30-R-7-CE**	2	Leica/Novocastra	0	2	0	0	-	
mAb clone **CON6D/5 PM346**	1	Biocare	0	0	1	0	-	
Total	252		86	93	56	17	-	
Proportion			34%	37%	22%	7%	71%	

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

Detailed analysis of CD30, Run 43

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **Ber-H2**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (4/14)*, TRS pH 9 (Dako) (3/13), TRS pH 6.1 (Dako) (6/8) CC1 (Ventana) (18/53), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (5/8), Diva Decloaker pH 6.2 (Biocare) (1/2) or Tris-EDTA/EGTA pH 9 (1/11) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:75 depending on the total sensitivity of the protocol employed. Using these protocol settings 60 of 78 (77%) laboratories produced a sufficient staining result (optimal or good).

* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **1G12**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (3/7) or CC1 (Ventana) (1/2) as retrieval buffer. The mAb was typically diluted

in the range of 1:10-1:25 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 of 6 (100%) laboratories produced a sufficient staining result.

mAb clone **JCM182**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (3/3) or Bond Epitope Retrieval Solution 1 (BERS1; Leica) (1/1) 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 a sufficient staining result.

mAb clone **CON6D/5**: Protocols with optimal results were all based on HIER using TRS pH 6.1 (Dako) (3/3) as retrieval buffer. The mAb was diluted 1:50 in all three protocols.

rmAb clone **EP154**: One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (1/1) as retrieval buffer. The rmAb was diluted 1:200.

Table 3. **Proportion of optimal results for CD30 with the most commonly used antibody as concentrate (mAb Ber-H2) on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**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 **Ber-H2**	5/19** (26%)	2/2	13/35 (37%)	0/1	4/4	0/1

** 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
mAb clone **Ber-H2**, product no. **IS602/IR602**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were all based on HIER in PT-Link using TRS pH 9 (3-in-1), TRS pH 9 or TRS pH 6.1 (efficient heating time 10-30 min. at 95-98°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings 26 of 35 (74%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Ber-H2**, product no. **790-2926**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using Cell Conditioning 1 (efficient heating time 30-76 min. at 95-100°C), 28-120min. incubation of the primary Ab. and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection systems. Using these protocol settings 15 of 17 (88%) laboratories produced a sufficient staining result.

mAb clone **Ber-H2**, product no. **790-4858**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using Cell Conditioning 1 (efficient heating time 40-76 min. at 94-100°C), 28-120 min. incubation of the primary Ab. and UltraView (760-500) with amplification or OptiView (760-700) as detection systems. Using these protocol settings 6 of 7 (86%) laboratories produced a sufficient staining result.

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 min. at 100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 4 of 5 (80%) laboratories produced a sufficient staining result.

mAb clone **1G12,** product no. **PA0153**, Leica, BOND-max/BOND-III:
One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (BERS2) (efficient heating time 20 min. at 100°C) and 20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 2 of 2 laboratories produced a sufficient staining result.

Comments
In this fourth NordiQC assessment for CD30 and in concordance to the previous runs 25 and 31, 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 95% of the insufficient results (69 of 73). 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 Hodgkin lymphoma (tissue core 2) and neoplastic cells of the embryonal carcinoma

(tissue core 4). 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 3) was more challenging and required optimally calibrated protocols.
Optimal staining results could be obtained with the mAb clones Ber-H2, 1G12, JCM182, CON6D/5 and the rmAb EP154 (see Table 1). It was observed that the mAb clones 1G12 and JCM182 from Leica/Novocastra gave an unexpected staining reaction of endothelial cells in all tissue cores included in this run. It is uncertain if this aberrant staining reaction of endothelial cells is a true positive reaction, but since this staining pattern did not interfere with the specific reaction in cells expected to be positive for CD30 it was fully accepted.

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 Dako, Leica and Ventana, respectively (see Table 3). Used as a concentrate within a laboratory developed (LD) assay, mAb clone Ber-H2 gave an overall pass rate of 72% (84 of 117) out of which 32% were optimal.
Within a LD assay, the mAb clone JCM182 was most successful giving an overall pass rate of 100% (5 of 5) out of which 80% were assessed as optimal. Also the mAb clones 1G12 and CON6D/5 provided a high proportion of optimal results of 44% (4 of 9) and 100% (3 of 3), respectively.
Irrespective of the antibodies applied, efficient HIER in an alkaline buffer or a modified low pH buffer (Target Retrieval Solutions pH 6.1, Dako or Diva Decloaker pH 6.2, BioCare), 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. In particular the use of modified low pH buffers provided an improved result for the mAb clone Ber-H2. If the LD assay for the mAb clone BER-H2 was performed on the Dako Autostainer platform, any dilution range, using EnVision Flex/Flex+ (Dako) as detection system and HIER with an alkaline buffer as Target Retrieval Solution pH 9 (Dako), a pass rate of 79% (22 of 28 protocols) was seen (25% optimal). If same protocol settings were applied using HIER in Target Retrieval Solution pH 6.1 (Dako), a pass rate of 80% (7 of 8 protocols) was seen out of which 75% were optimal.
In this context it was also observed, that the mAb clone CON6D/5 required HIER in a modified low pH buffer for optimal performance.

In this assessment optimal results could be obtained by the RTU systems from Dako, Leica and Ventana. In general the pass rates and proportion of optimal results were comparable to LD assays using same clones.
Optimal results for the Dako and Leica RTU systems could be obtained by both the official recommendations and by modified protocol settings as change of HIER buffer, HIER time, incubation time of the primary antibody and choice of the detection system.
There are two RTU formats available for the Ventana BenchMark platform. For the RTU format 790-2926 of the mAb clone Ber-H2 (developed for UltraView or iView) optimal results could only be obtained by a laboratory modified protocol prolonging the time in the primary Ab and/or using amplification kit.
The RTU format 790-4858 developed for OptiView provided an optimal result using the recommended protocol settings based on HIER in CC1 64 min., 32 min. incubation of the primary Ab and OptiView or UltraView + amplification as detection kit. Using these settings 6 of 7 protocols (86%) were assessed as optimal. If the same protocol settings were applied with the detection system UltraView, only 25% (2 of 8) produced a sufficient result and none (0%) was assessed as optimal (see Fig. 5a & 5b).

A slight decrease in the pass rate was seen compared to the previous run 31, 2011. As many laboratories participated for the first time it is difficult to identify the specific causes for the minor decrease obtained in this run (71% versus 77% in run 31). Virtually same composition of the material has been used in the assessments for CD30, but slight different expression levels can be expected especially in the Hodgkin lymphomas.

Controls
Tonsil is recommended as positive 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 perifollicular germinal centre B-cells. Virtually all other cells must be negative. Plasma cells, macrophages and endothelial cells may be positive depending on the primary antibody clone. E.g plasma cells can be stained by Ber-H2, endothelial cells by JCM182 and 1G12 and macrophages by 1G12.
The choice of correct control material is of immense importance. Several laboratories used a Hodgkin lymphoma as positive control. This cannot be recommended since some lymphomas have high-level CD30 expression while others have a low-level expression. Lymphomas with high-level expression will not provide information on the limit of detection level and consequently impair the ability to demonstrate CD30 in neoplasias with low-level expression - see Fig. 6a and Fig. 6b.

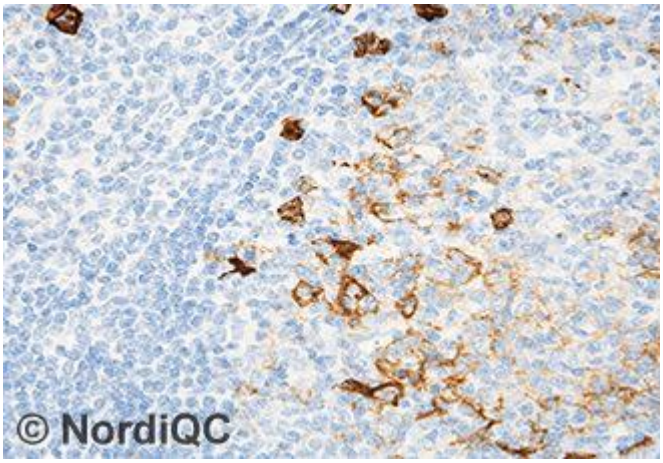


Fig. 1a (x400).
Optimal CD30 staining of the tonsil using the mAb clone CON6D/5 as a concentrate, HIER in an modified low pH buffer (TRS pH 6.1, Dako) and a 3-step polymer based detection system (Flex+, Dako). The activated B- and T-cells, particularly located at the rim of the germinal centers, show a moderate to strong predominantly membranous staining reaction - compare with Fig.1b.
Same protocol used in Figs. 2a - 4a.

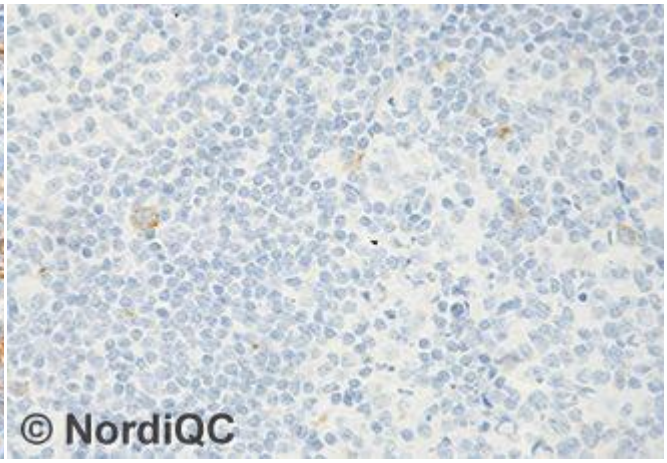


Fig. 1b (x400).
Insufficient CD30 staining of the tonsil using the mAb clone Ber-H2 as a concentrate and use of a 2-step polymer based detection system (GTVision, Gene Tech). The protocol provided an overall too low sensitivity most likely due to a combination of insufficient HIER in Tris-EGTA/EDTA pH 9 (too short time), low sensitivity of the detection system and a too low concentration of the primary Ab - compare with Fig. 1a (same field).
Also compare with Figs. 2b - 4b – same protocol.

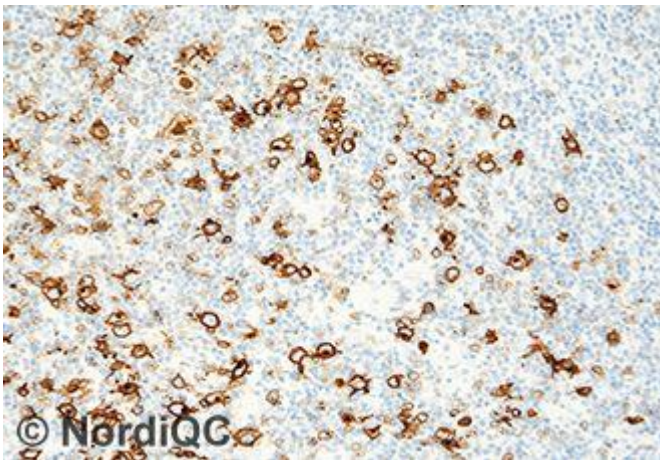


Fig. 2a (x200).
Optimal CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 2) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a moderate to strong membranous staining reaction and a dot-like positivity.

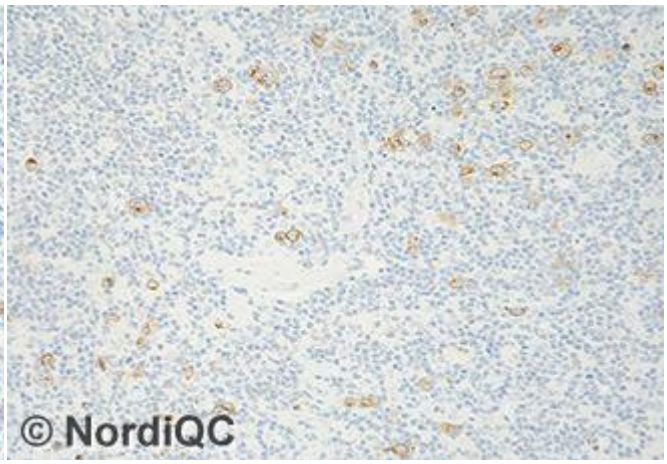


Fig. 2b (x200).
Insufficient CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 2) using same protocol as in Fig. 1b.
The vast majority of Reed-Sternberg cells and Hodgkin cells are only weakly stained or completely false negative - compare with Fig. 2a (same field).
Also compare with Figs. 3b and 4b – same protocol.

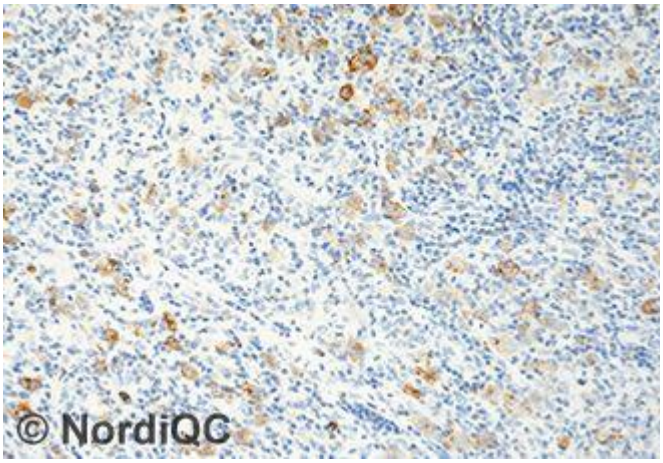


Fig. 3a (x200).
Optimal CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 3) using same protocol as in Figs. 1a & 2a. The Reed-Sternberg and Hodgkin cells show a weak to moderate membranous staining reaction and a dot-like positivity.

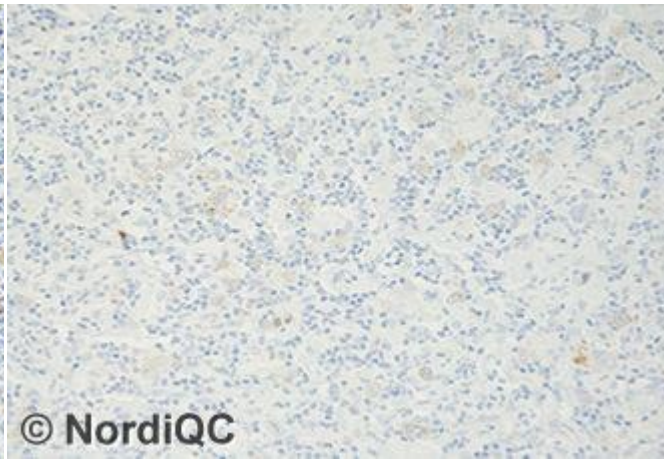


Fig. 3b (x200).
Insufficient CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 3) using same protocol as in Figs. 1b & 2b. The vast majority of Reed-Sternberg cells and Hodgkin cells are false negative - compare with Fig. 3a (same field).

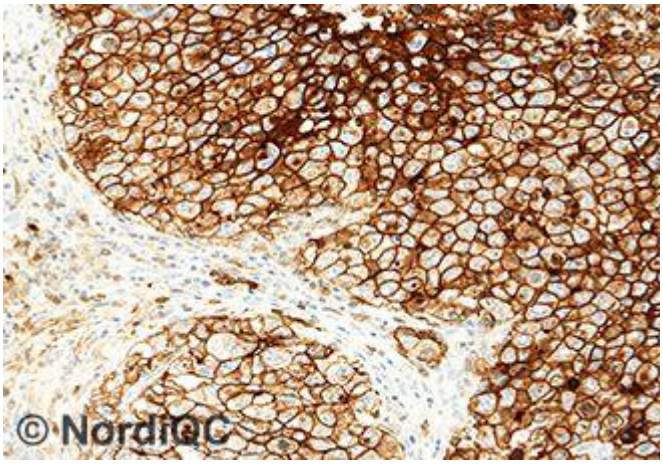


Fig. 4a (x200).
Optimal CD30 staining of the embryonal carcinoma using same protocol as in Figs. 1a - 3a. All the neoplastic cells show a strong membranous staining intensity for CD30.

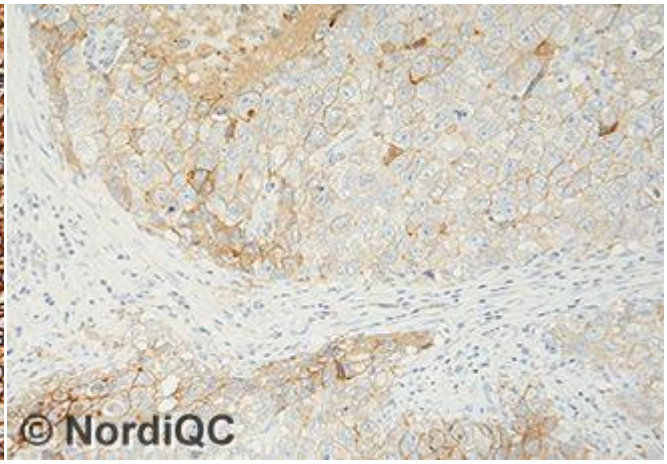


Fig. 4b (x200).
Insufficient CD30 staining of the embryonal carcinoma using same protocol as in Figs. 1b - 3b. The neoplastic cells show a too weak and faint staining intensity for CD30 - compare with Fig.4a (same field).

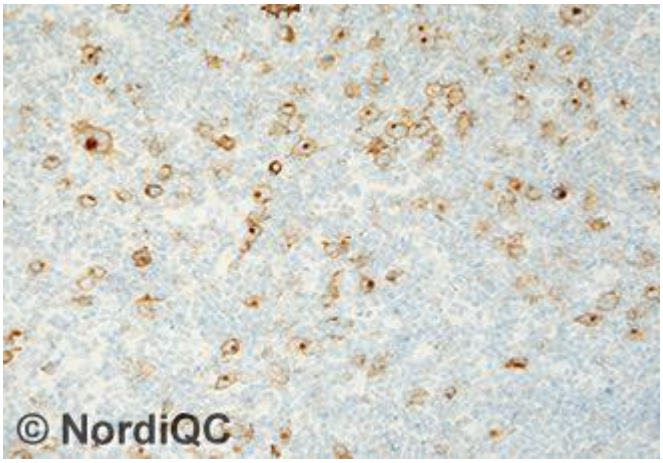


Fig. 5a (x200).
Optimal CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 2) based on the RTU format 790-4878 (Ventana), HIER in an alkaline buffer (CC1 pH 8.5) and a 3-step multimer based detection system (OptiView, Ventana) as recommended in the package insert for the RTU format. Virtually all the Reed-Sternberg and Hodgkin cells show a moderate to strong membranous staining reaction with a dot-like positivity - compare the result with an insufficient laboratory modified protocol based on the use of the 2-step multimer based detection system UltraView (Ventana) - Fig. 5b.

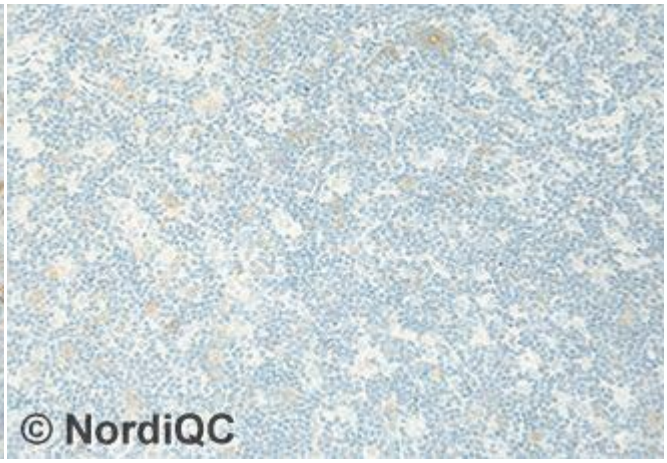


Fig. 5b (x200).
Insufficient CD30 staining of the Hodgkin lymphoma, classical type (tissue core no. 2) based on the RTU format 790-4878 (Ventana) using similar protocol settings as applied for the result shown in Fig. 5a except for the application of a 2-step multimer based detection system (UltraView, Ventana). The majority of Reed-Sternberg cells and Hodgkin cells are too weakly stained or completely false negative - compare with the optimal result obtained by the officially recommended protocol based on the detection system OptiView (Ventana) - Fig. 5a (same field).

MB/SN/RR/LE/MV 20-3-2015