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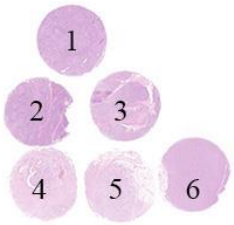
Assessment Run 65 2022
CD30

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD30, typically identifying classical Hodgkin lymphoma, anaplastic large cell lymphoma, anaplastic variant of diffuse large B-cell lymphoma or subtypes of cutaneous lymphoproliferative disorders. CD30 is also useful in identification of embryonal carcinoma and subtypes of mixed germ cell tumours. Relevant clinical tissues, both normal and neoplastic were selected, displaying a broad spectrum of antigen densities for CD30 (see below).

Material
The slide to be stained for CD30 comprised:



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

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD30 staining as optimal included:

- A weak to moderate, distinct membranous staining reaction of activated B-cells primarily located in the rim of the germinal centres and of interfollicular activated lymphocytes in the tonsils.
- A strong, distinct membranous but also dot-like (Golgi zone) staining reaction of virtually all neoplastic cells in the ALCL.
- A weak to moderate, distinct membranous staining reaction of virtually all neoplastic cells in the embryonal carcinoma - a weak background staining due to necrosis was accepted.
- An at least moderate, distinct membranous staining reaction but also Golgi reaction of the majority of Hodgkin`s/Reed-Sternberg cells in the Hodgkin`s lymphoma.
- No staining reaction of other cells (all cores) including the neoplastic cells of the DLBCL.

Cytoplasmic staining of the plasma cells was accepted. As observed in the previous run 51 for CD30, the mAb clone JCM182 from Leica Biosystems gave an unexpected staining reaction of both endothelial cells and subpopulations of macrophages. This aberrant staining pattern was also accepted in this run, providing that interpretation of the specific reaction for CD30 was not compromised. However, in situations causing interpretation difficulties, the assessment score was reduced by one grade e.g., from optimal to good.

Participation

Number of laboratories registered for CD30, run 65	388
Number of laboratories returning slides	366 (94%)

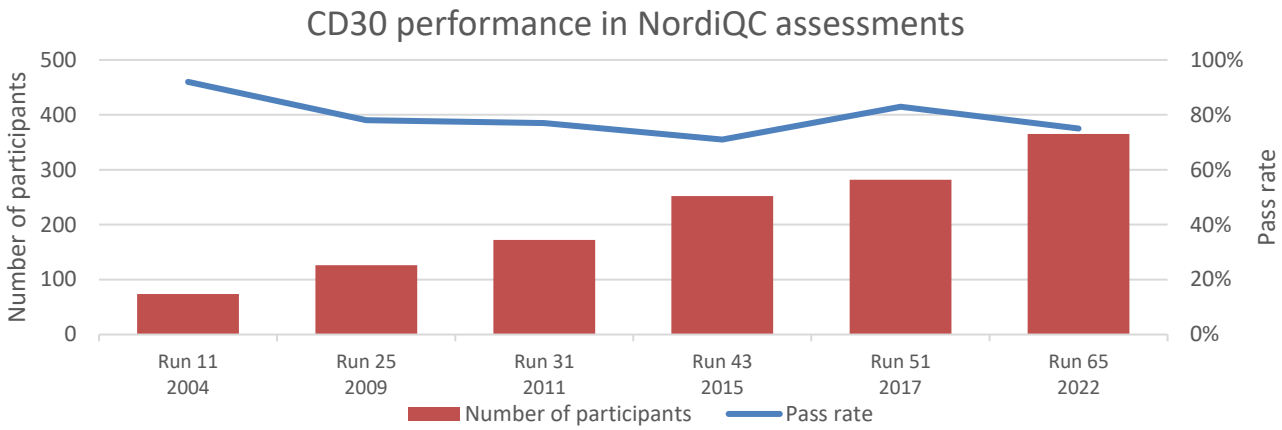
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides for CD30. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but data were not included in this report.

One laboratory stained an incorrect NordiQC slide (BAP1), and thus, was not included in this report. 365 laboratories participated in this assessment and 75% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining were:
- Less successful performance of the Ready-to-use (RTU) format 790-4858 (Ventana/Roche).
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems
- Inefficient Heat Induced Epitope Retrieval (HIER).

Performance history
This was the sixth NordiQC assessment of CD30. The pass rate decreased marginally compared to previous run 51 (2017) and has almost been at the same level since run 25 in 2009 (see Graph 1). A significant increased number of new participants was enrolled in this run (n=83) compared to the latest run.

Graph1. **Proportion of sufficient results for CD30 in the six NordiQC runs performed**



Conclusion
The mAb clones **Ber-H2**, **JCM182**, **CON6D/B5**, **IHC030** and **HRS4** could all be used to obtain an optimal staining result for CD30. For the mAb clone Ber-H2, used by the 89% (325/365) of the participants, efficient HIER either in modified citric based buffer (TRS pH 6.1, Dako/Agilent) or an alkaline buffer, careful calibration of the primary antibody and use of a 3-step multimer/polymer detection system were the most important requirements for an optimal staining result.
In this assessment, using both vendor and laboratory modified protocol settings, the RTU system PA0790 (Leica Biosystems) based on the mAb clone JCM182 provided a high pass rate and all results (25/25) using this system were assessed as sufficient. However, proportion of optimal results were relatively low primarily due to excessive background staining and/or unspecific staining of subpopulations of macrophages/mononuclear cells, compromising interpretation of the specific reaction for CD30 in especially the Hodgkin`s lymphoma. Compared to other RTU systems from the main vendors/manufactures, the RTU system 790-4858 (Ventana/Roche) based on the mAb clone Ber-H2 gave less successful results with an overall pass rate of only 54% (69/127). For this particular RTU system, the choice of detection system was essential and at present, OptiView with tyramide amplification was definitely the most efficient combination for optimal performance.
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 but distinct membranous staining reaction. Virtually all other cells must be negative.

Table 1. **Antibodies and assessment marks for CD30, Run 65**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **Ber-H2**	82 10 2 2 1 1	Dako/Agilent Cell Marque Diagnostic Biosystems Epredia Zytomed Systems Santa Cruz	59	26	9	4	87%	60%
mAb clone **JCM182**	6	Leica Biosystems	1	3	1	1	67%	17%
mAb clone **IHC130**	1	GenomeMe	1	0	0	0	-	-
mAb clone **CON6D/B5**	2	Biocare Medical	2	0	0	0	-	-
mAb clone **HRS4**	1	Thermo Scientific	1	0	0	0	-	-
rmAb clone **ZR248**	1	Zeta Corporation	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **Ber-H2 IR602**[3]	3	Dako/Agilent	1	1	1	0	-	-
mAb clone **Ber-H2 IR602**[4]	44	Dako/Agilent	23	18	3	0	93%	52%
mAb clone **Ber-H2 GA602**[3]	25	Dako/Agilent	13	9	3	0	88%	52%
mAb clone **Ber-H2 GA602**[4]	22	Dako/Agilent	10	7	4	1	77%	45%
mAb clone **Ber-H2 790-4858**[3]	14	Ventana/Roche	0	2	9	3	14%	0%
mAb clone **Ber-H2 790-4858**[4]	113	Ventana/Roche	41	26	38	8	59%	36%
mAb **Ber-H2 MAD-002045QD**	2	Master Diagnostica	0	2	0	0	-	-
mAb **Ber-H2 130M-XX**	1	Cell Marque	0	1	0	0	-	-
mAb clone **Ber-H2 MS-361-R7**	1	Epredia	0	1	0	0	-	-
mAb clone **Ber-H2 AM327**	1	BioGenex	0	0	0	1	-	-
mAb clone **Ber-H2 BSB 5212**	1	Bio SB	0	0	0	1	-	-
mAb clone **Ber-H2 8265-C010**	1	Sakura Finetek	0	0	1	0	-	-
mAb clone **JCM182 PA0790**[3]	13	Leica Biosystems	4	9	0	0	100%	31%
mAb clone **JCM182 PA0790**[4]	12	Leica Biosystems	5	7	0	0	100%	42%
mAb clone **C5E10 CCM-0523**	1	Celnovte Biotechnology	0	1	0	0	-	-
mAb clone **BerH2+ConD6/B5 PM074**	1	Biocare Medical	0	0	0	1	-	-
mAb clone **442F7G3 PA137**	1	Abcarta	0	1	0	0	-	-
Total	365		161	114	69	21	-	
Proportion			44%	31%	19%	6%	75%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols

Detailed analysis of CD30, Run 65
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) (Dako/Agilent) (6/14)*, TRS pH 6.1 (Dako/Agilent) (7/10), Cell Conditioning 1 (CC1, Ventana/Roche) (32/48), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (12/20), Tris-EDTA/EGTA pH 9 (1/2) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/1) 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, 76 of 86 (88%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **JCM182**: One protocol with an optimal result was based on HIER using BERS1 as retrieval buffer. The mAb was diluted 1:50 and Bond Refine (Leica Biosystems) was used as the detection system.

mAb clone **CON6D/B5**: Two protocols with optimal results were both based on HIER using TRS pH 6.1 as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 and Envision FLEX+ (Dako/Agilent) was used as the detection system.

mAb clone **IHC030**: One protocol with an optimal result was based on HIER using CC1 as retrieval buffer. The mAb was diluted 1:120 and OptiView (Ventana/Roche) was used as the detection system.

mAb clone **HRS4**: One protocol with an optimal result was based on HIER using TRS pH 6.1 as retrieval buffer. The mAb was diluted 1:30 and Envision FLEX+ was used as the detection system.

Table 2. **Proportion of optimal results for CD30 for the most used antibody as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III**	
	TRS pH 9.0	TRS pH 6.1	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**	2/5** (40%)	3/5 (60%)	4/8 (50%)	3/3	28/42 (67%)	-	12/20 (60%)	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
mAb clone **Ber-H2**, product no. **IR602**, Dako/Agilent, Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) 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+ (K8002) as detection system. Using these protocol settings, 11 of 11 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Ber-H2**, product no. **GA602**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 6.1 (efficient heating time 30 min. at 97°C), 10-20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (GV800/GV800+821) as detection system. Using these protocol settings, 34 of 38 (89%) laboratories produced a sufficient staining result.

mAb clone **Ber-H2**, product no. **790-4858**, Ventana/Roche, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-72 min. at 95-100°C), 24-44 min. incubation of the primary Ab and OptiView (760-700) with amplification as detection systems. Using these protocol settings, 38 of 39 (97%) laboratories produced a sufficient staining result - 29/39 (74%) being optimal.

mAb clone **JCM182,** product no. **PA0790**, Leica Biosystems, BOND III:
Protocols with optimal results were typically based on HIER using BERS1 (efficient heating time 20 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, 16 of 16 (100%) laboratories produced a sufficient staining result.

Table 3 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 according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **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 **IR602**	2/3	1/3	89% (16/18)	72% (13/18)
Dako Omnis mAb Ber-H2 **GA602**	88% (22/25)	52% (13/25)	85% (17/20)	50% (10/20)
VMS Ultra/XT/GX mAb Ber-H2 **790-4858**	14% (2/14)	0% (0/14)	59% (67/113)	36% (41/113)
Leica BOND MAX/III mAb JCM182 **PA0790**	100% (13/13)	31% (4/13)	100% (12/12)	42% (5/12)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In concordance with the previous NordiQC assessments for CD30, the prevalent features of an insufficient staining result was a generally too weak or false negative staining reaction of the cells expected to be demonstrated. This was observed in 96% of the insufficient results (86/90). The remaining insufficient results were characterized by false positive reactions and/or poor signal-to-noise ratio. Almost all laboratories were able to demonstrate CD30 in the neoplastic cells of the anaplastic large cell lymphoma (high-level antigen expressing cells), whereas detection of CD30 in low-level antigen expressing cells as activated B- and T-cells in the tonsils, neoplastic cells of the embryonal carcinoma and neoplastic cells of the Hodgkin lymphoma was more challenging and required optimally calibrated protocols.
As described in the previous report (Run 51), the mAb clone JCM182 from Leica Biosystems also gave an atypical staining reaction of endothelial cells and subpopulations of macrophages in this run. This aberrant staining pattern was accepted, providing that it did not compromise interpretation of the specific and expected staining reaction for CD30.

The mAb clones Ber-H2 and JCM182 were the most widely used antibodies for demonstration of CD30 and in total applied by 98% (357/365) of the laboratories (see Table 1).
Used as concentrated format within laboratory developed (LD) assays, the mAb clone Ber-H2 provided a pass rate of 87% (85/98) of which 60% (59/98) of the protocols gave an optimal result. This is a minor improvement in performance compared to the latest run 51, giving an overall pass rate of 83% and 47% being optimal. Optimal results could be obtained on all main IHC platforms (see Table 2). The prevalent feature for an optimal result was use of efficient HIER in an alkaline (e.g., TRS pH9, CC1 and BERS2) or modified citric based buffer (TRS pH 6.1), careful calibration of the primary Ab (typically dilution range of 1:20-100) and use of 3-step multimer/polymer detection system (e.g., OptiView, EnVision FLEX+ and Bond Refine). Using these protocol settings 92% (63/68) of the protocols were assessed as sufficient and 72% (49/68) being optimal. Insufficient results were typically characterized by applying protocol settings providing too low analytical sensitivity and often caused by a combination of e.g., too diluted primary Ab and a low sensitive 2-step multimer/polymer detection system.
As observed in the previous assessment for CD30, and for users of the IHC-platforms Autostainer or Omnis (Dako/Agilent), the proportion of sufficient and optimal results were influenced by the choice of HIER buffer. Using the mAb clone Ber-H2 as concentrate within a LD-assay, any dilution range of the primary Ab, EnVision FLEX/FLEX+ as the detection systems and HIER in TRS pH 9, the pass rate was of 71% (10/14) of which only 43% (6/14) were assessed as optimal. If same protocol settings were applied apart from substituting HIER in TRS pH 9 with the modified acidic HIER buffer TRS pH 6.1, the pass rate increased to 90% (9/10) and 70% (7/10) were optimal. Overall, the mAb clone Ber-H2 is a challenging Ab and require that each protocol parameter is optimized to provide the appropriate level of analytical sensitivity and fulfilling the intended use of the assay.

Using the mAb clone JCM182 as concentrate within a LD assay, the overall pass rate was 67% (4/6) of which only 17% (1/6) of the results were assessed as optimal. The mAb was mainly used by laboratories on the BOND III (Leica Biosystems) and all protocols (4/4) were assessed as sufficient. Protocols were

based on HIER in either BERS1 or BERS2, the primary Ab was diluted in the range 1:50-1:400 and BOND refine was applied as the detection system.

Optimal results could also be obtained with the mAb clones COND6/B5, HRS4 and IHC030. For the mAb clones COND6/B5 and HRS4, HIER in modified acidic buffer (e.g., TRS pH 6.1) was mandatory for optimal performance and for mAb clone IHC030, optimal result could be obtained using HIER in an alkaline buffer (CC1). All protocols were based on 3-step multimer/polymer detection systems (OptiView or Envision FLEX+).

In total, 70% (256/365) of the laboratories used a RTU format for demonstration of CD30 which is a significant increase compared to 52% (147/282) in Run 51. For the RTU system IR602 (Dako/Agilent, Autostainer) based on the mAb clone Ber-H2, the number of participants following vendor recommended protocol settings (VRPS) was surprisingly low (see Table 3) and gave a low pass rate of 67% (2/3) – one being optimal. However due to low number of data points, the observation must be taken with caution. The official recommendation for the RTU system IR602 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. In contrast, by using laboratory modified protocol settings (LMPS), the proportion of sufficient and optimal results increased significantly, to 89% (16/18) and 72% (13/18), respectively. The most prevalent feature accounting for this improvement in performance, was related to a substitution of Envision FLEX with Envision FLEX+. The use of EnVision FLEX+ irrespectively of all other protocol settings applied e.g., HIER conditions (buffer and time/temperature) and/or different primary Ab incubation times, provided a pass rate of 100% (12/12) and 92% (11/12) being optimal. Similar, and following the basic VRPS apart from substituting EnVision FLEX with Envision FLEX+, gave 100% optimal results (5/5).
A significant proportion of participants (n=22) used the RTU format IR602 on the Omnis, providing a pass rate of 95% (21/22) of which only 32% (7/22) were assessed as optimal and similar performance patterns were thus seen for the IR602 format being performed on either Autostainer or Omnis. In general, "direct" transfer of a RTU product developed for the Autostainer to the Omnis platform should be avoided, especially when the manufacturer has an alternative product (GA602) based on exactly the same clone and validated for the Omnis platform (see below and Table 3).

The RTU format GA602 (Dako/Agilent, Omnis) also based on the mAb clone Ber-H2, provided a relative high proportion of sufficient results applying both VRPS and LMPS (see Table 3). The VRPS are based on HIER in TRS low pH for 30 min. at 97°C, 10 min. incubation of the primary Ab and EnVision FLEX as the detection system. The most successful modified settings were based on prolonged incubation time of primary Ab and/or use of EnVision FLEX+ as detection system giving an overall pass rate of 93% (13/14) and 64% (9/14) being optimal. Less successful modified settings were mostly associated with usage of TRS high pH as HIER buffer instead of TRS low pH, giving a pass rate of 60% (3/5) and no optimal. Two laboratories used the RTU product GA602 off-label on the Benchmark Ultra platform (Ventana/Roche) and none were able to produce a sufficient result. As mentioned above, this practice with mitigation of a RTU format to another platform than intended should be avoided, or laboratories should at least be committed to initiate a thorough validation process with focus on purpose and intended use.

The RTU system 790-4858 (Ventana/Roche, Benchmark GX/XT/Ultra) based on the mAb clone Ber-H2, provided an overall low pass rate and proportion of optimal results (see Table 3). Using VRPS, only 14% (2/14) obtained a sufficient mark of which none were optimal, although protocol settings in principle were based on parameters providing high analytical sensitivity e.g., the use OptiView or UltraView with amplification as the detection systems. The proportion of optimal results has declined over the three latest assessments and currently, there is no good explanation for this deviation. However, the proportion of sufficient and optimal results was higher using LMPS typically adjusting parameters such as HIER time, incubation time of the primary antibody and choice of detection system. The most important parameter affecting the overall performance of the assays was the choice of detection system. Applying all protocol settings (unsorted), the following pattern was seen: 1; 7% (2/29) of the protocols based on UltraView with or without amplification were assessed as sufficient – 4% (1/29) being optimal, 2; 33% (14/43) of the protocols based on OptiView were assessed as sufficient – 9% (4/43) being optimal, and 3; 96% (53/55) of the protocols based on OptiView with amplification were assessed as sufficient – 65% (36/55) being optimal. This evidently demonstrates that optimal performance for this RTU product is difficult to achieve, at least if protocols are based on the traditional 3-step detection systems as UltraView with amplification or OptiView (both VRPS and LMPS), despite these detection systems normally provide an appropriate and high-level analytical sensitivity for most markers. Therefore, the RTU system seems to require the sensitive detection system OptiView with tyramide amplification for detection of CD30, and thereby, fulfilling intended diagnostic use of the assay. In this context, it is highly encouraged that the performance of the RTU is re-evaluated by the provider to either adjust the recommended protocol or to adjust the titer

of the Ab in the RTU format to match the level of sensitivity for the presently recommended detection systems.
The RTU system PA0790 (Leica Biosystems, BOND III) based on the mAb clone JCM182 provided among all RTU systems the highest proportion of sufficient results and using both VRPS and LMPS, the pass rate was 100% (25/25) – see Table 3. 89% (8/9) of the optimal results were based on the vendor recommended HIER buffer BERS1. In general, the overall proportion of optimal results was relative low (36%, 9/25) compared to 70% (7/10) in the previous run 51. The decline in proportion of optimal results in this assessment was caused by a significantly high proportion of protocols (16/25) giving a too excessive background staining and/or aberrant staining of macrophages/mononuclear cells interfering with the identification of specific and expected reaction of CD30, especially in the Hodgkin lymphoma. Hence, the extend of the problem might be depending on individual biological level in different samples and explain the difference in proportion of optimal results between the two latest runs. No technical parameters (e.g., lot numbers) could with certainty be identified to differentiate optimal from "downgraded good results" – see Figs. 6a–6b.

This was the sixth assessment of CD30 in NordiQC (see Graph 1). The pass rate declined marginally compared to run 51, 2017. The primary parameters influencing the assessment result in negative direction were 1; challenging material circulated to the participants e.g., the embrynonal carcinoma and the Hodgkin lymphoma 2; less sensitive detection systems and 3; less successful performance of the RTU system 790-4858 (Ventana/Roche) used by 35% (127/365) of the participants, providing an overall low pass rate of 54% (69/127). This was a significant decline in pass rate compared to the previous Run 51, in which 81% (61/75) of the laboratories could produce a sufficient result. Importantly, protocols must stain according to the expected antigen levels, and tonsil is recommendable as critical assay performance control assisting to monitor the required level of the analytical sensitivity and specificity of the assay (see below).

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).

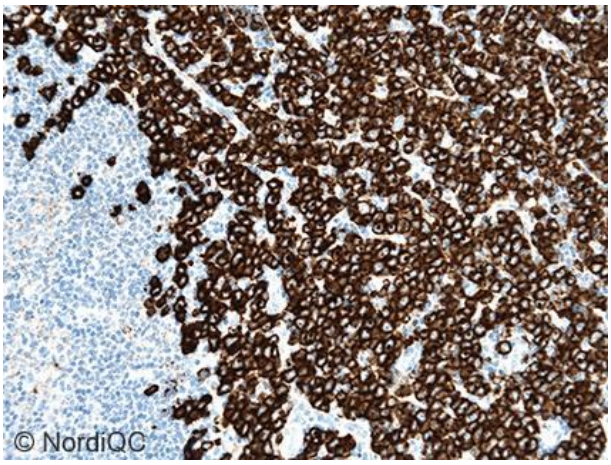


Fig. 1a (x200)
Optimal staining for CD30 of the ALCL using the mAb clone Ber-H2 as RTU format (Ventana/Roche, 790-4858), incubation time in primary Ab for 32 min., efficient HIER in CC1 for 64 min. (100°C) and OptiView with amplification as the detection system. Staining was performed on the Benchmark Ultra platform (Ventana/Roche) - same protocol used in Figs. 2a - 5a. All neoplastic cells display a strong, distinct membranous staining reaction and a dot-like reaction of the Golgi zone. No background staining is seen.

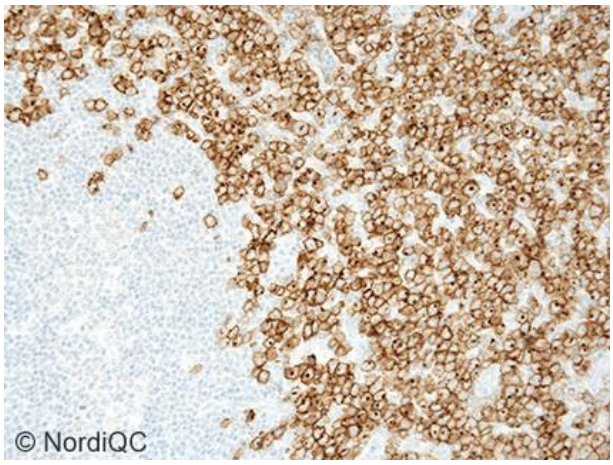


Fig. 1b (x200)
CD30 staining of the ALCL using exactly the same RTU product and protocol settings as in Fig. 1a (same field), except for excluding the amplification step of the detection process. In general and using this RTU system without the tyramide amplification step, protocols provided too low analytical sensitivity (see description in the comments). Although the neoplastic cells of the ALCL are stained, the protocols based exclusively on OptiView (or UltraView with amplification) as detection system, was challenged in tissue structures having significantly lower antigen densities of CD30 compared to the ALCL. - see Figs. 1a - 5b.

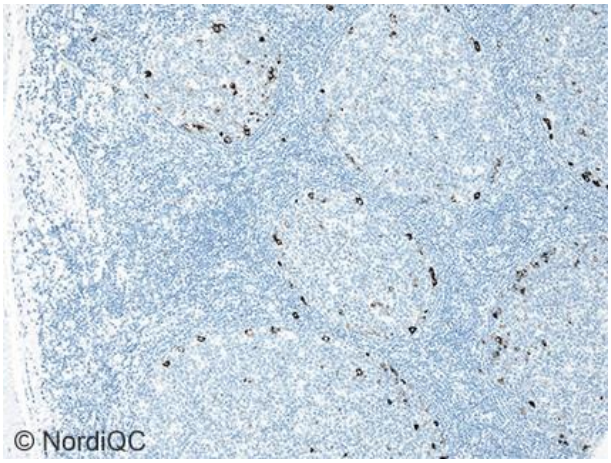


Fig. 2a (x100)
Optimal CD30 staining of the tonsil using same protocol as in Fig. 1a. The activated B- and T-cells, particularly B-cells located at the rim of the germinal centres, show a moderate to strong predominantly membranous staining reaction.

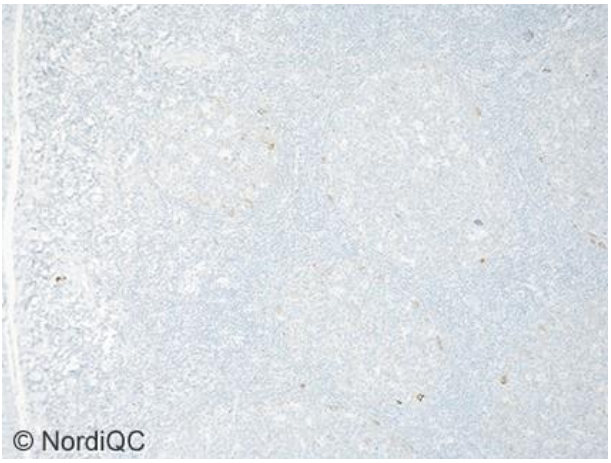


Fig. 2b (x100)
Insufficient CD30 staining of the tonsil using same protocol as in Fig. 1b. Both the proportion and intensity of activated B-cells located at the rim of the germinal centres is significantly reduced to an unacceptable level - compare with Fig. 2a (same field). These activated B-cells are important and verify low level of detection of the assay, and thus, the RTU protocol must be adjusted (either by vendor or laboratory) to fulfil intended use and provide the expected reaction pattern.

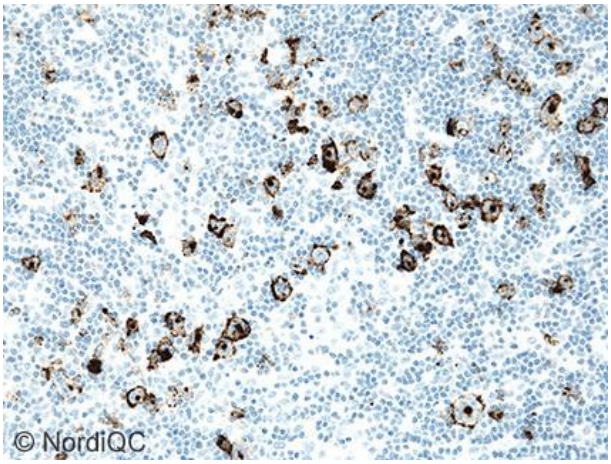


Fig. 3a (x200)
Optimal CD30 staining of the Hodgkin`s lymphoma using same protocol as in Figs. 1a and 2a. All Hodgkin/Reed-Sternberg cells show a moderate to strong, distinct membranous and cytoplasmic dot-like staining pattern.
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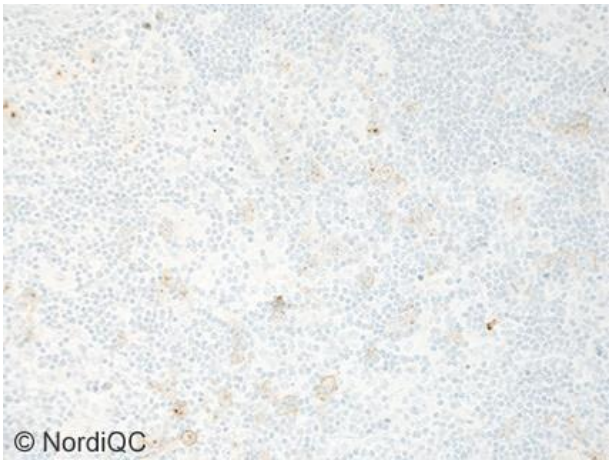

Fig. 3b (x200)
Insufficient CD30 staining of the Hodgkin`s lymphoma, using the same protocol as in Figs. 1b and 2b. A significant proportion of the neoplastic cells are false negative or only faintly demonstrated - compare with Fig. 3a (same field).

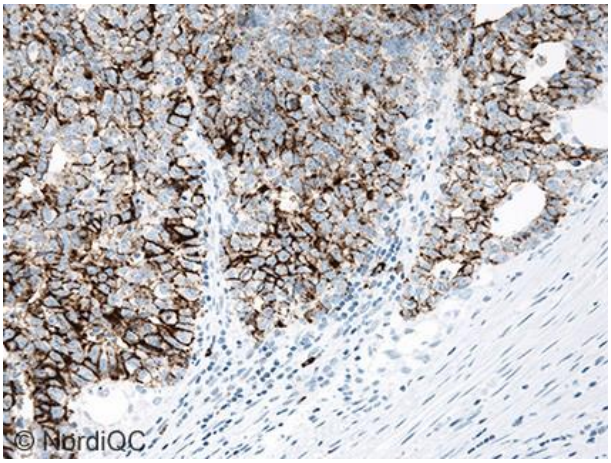

Fig. 4a (x200)
Optimal CD30 staining of the embryonal carcinoma using same protocol as in Figs. 1a - 3a. Virtually all neoplastic cells show a moderate to strong and distinct membranous staining reaction.

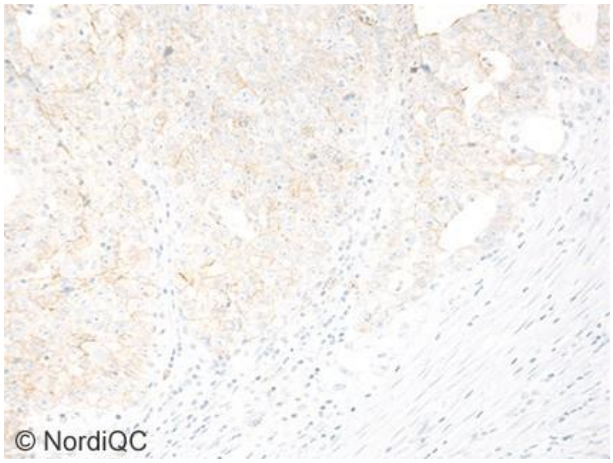

Fig. 4b (x200)
Insufficient CD30 staining of the embryonal carcinoma using the same protocol as in Figs. 1b - 3b. The staining intensity and proportion of positive cells is reduced, and a significant proportion of the neoplastic cells are false negative or display too weak staining reaction - compare with Fig. 4a (same field).

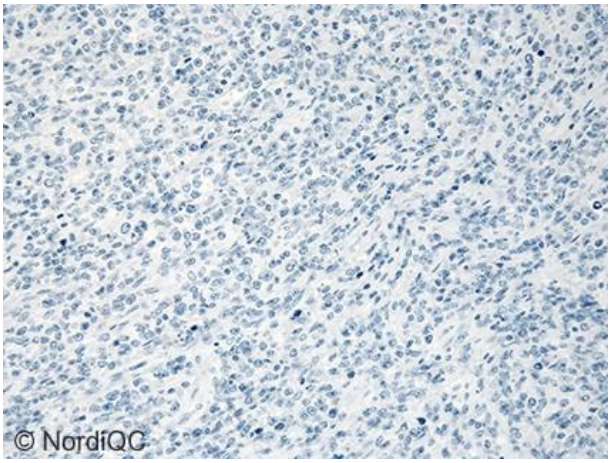

Fig. 5a (x200)
Optimal CD30 staining of the DLBCL using same protocol as in Figs. 1a - 4a. The neoplastic cells are negative as expected.

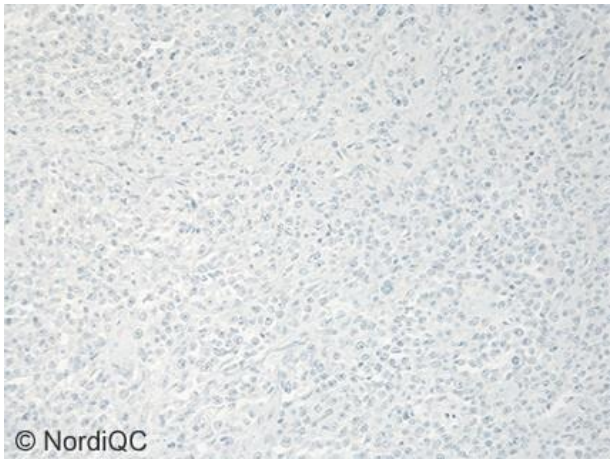

Fig. 5b (x200)
CD30 staining of the DLBCL using same protocol as in Fig. 1b - 4b. Although the protocol provided the expected reaction pattern, the assay is unreliable for demonstration of CD30 due to too low analytical sensitivity, risking misclassification of CD30 positive tumours - compare with Figs. 1a-4b.

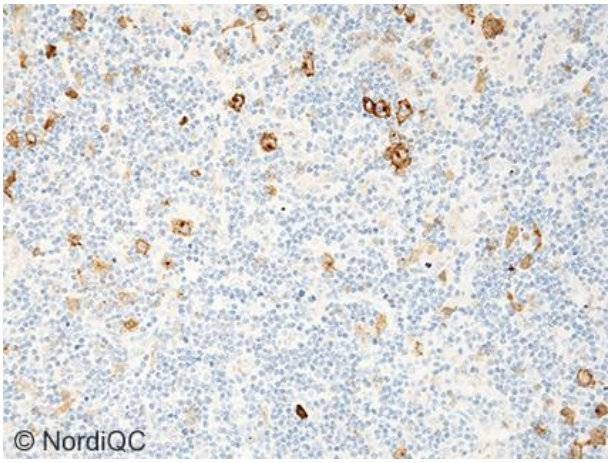

Fig. 6a (x200)
Optimal CD30 staining of the Hodgkin`s lymphoma using the mAb clone JCM182 as RTU format (PA0790, Leica Biosystems), efficient HIER in BERS1 (10 min./100ºC) and BOND Refine as detection system. The neoplastic cells show the characteristically, distinct reaction pattern and are as expected highlighted against a background providing good signal-to-noise ratio. In this assessment, and for protocols based on this particular clone, the aberrant staining of subtypes of macrophages and endothelial cells could cause interpretation problems, and thus, were downgraded by one assessment score e.g., from optimal to good – compare with Fig. 6b.

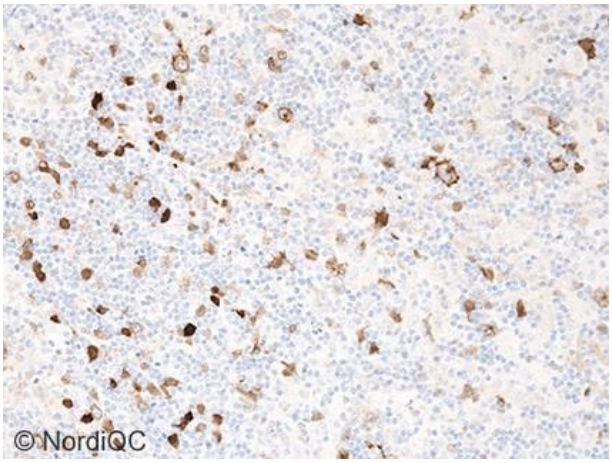

Fig. 6b (x200)
CD30 staining of the Hodgkin lymphoma using the mAb clone JCM182 as RTU format (PA0790, Leica Biosystems) with similar protocol settings as in Fig. 6a, except for extending HIER time in BERS1 to 20 min. The protocol was "only" assessed as good due to the staining of macrophages/mononuclear cells, intermingling and compromising interpretation of the specific signal in the Hodgkin/Reed-Sternberg cells. No technical parameters could be identified, explaining for this unwanted aberrant staining pattern– compare with Fig. 6a.

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