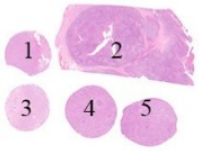
Assessment Run 74 2025
CD30

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD30, used for subclassification of lymphoproliferative disorders typically identifying classical Hodgkin lymphoma, anaplastic large cell lymphoma, variants of diffuse large B-cell lymphoma and subtypes of cutaneous lymphomas. 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 comprised:

1. Diffuse large B-cell lymphoma (DLBCL), NOS 2. Tonsil, 3. Anaplastic Large Cell Lymphoma (ALCL), 4-5. Classic Hodgkin Lymphomas (CHL)



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 in the tonsil but also in activated interfollicular B- and T-cells.
- A strong, distinct membranous and dot-like (Golgi zone) staining reaction of virtually all neoplastic cells in the ALCL.
- An at least weak to moderate, distinct membranous and dot-like Golgi staining reaction of the majority of Hodgkin /Reed-Sternberg cells in the CHL (tissue core no. 5).
- A moderate to strong, distinct membranous and dot-like Golgi staining reaction of virtually all Hodgkin/Reed-Sternberg cells in the CHL (tissue core no. 4).
- No staining reaction of other cells (all cores) including the neoplastic cells of the DLBCL.

Cytoplasmic staining of the plasma cells was accepted. As noted in the previous runs 51 and 65 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, providing that interpretation of the specific reaction for CD30 was not compromised.

KEY POINTS FOR CD30 IMMUNOASSAYS
- The mAb clones **Ber-H2** and **JCM182** were used by 98% of all participants, providing a cumulated pass rate of 74%.
- mAb clone **Ber-H2** was less successful on the Bond platforms (Leica Biosystems)
- The RTU systems **IR/IS/GA602** (Dako/Agilent) and **790-4858** (Ventana/Roche) both based on mAb clone Ber-H2 provided superior performance when applied by laboratory modified protocol setting compared to vendor recommended settings

Participation

Number of laboratories registered for CD30, run 74	470
Number of laboratories returning slides	423 (90%)

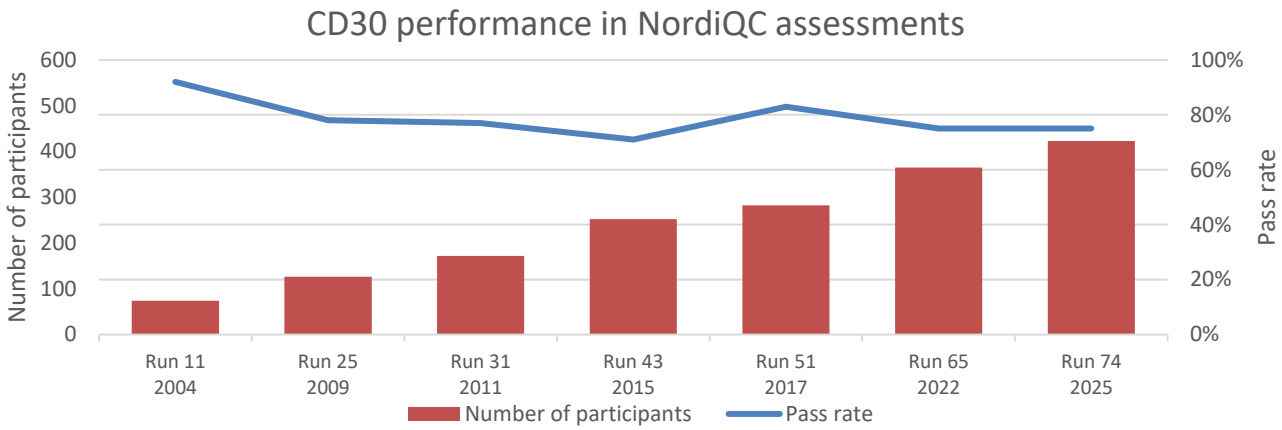
All slides returned after the assessment were assessed and participants received advice if the result was insufficient - data from these outcomes were not included in this report.

Results
423 laboratories participated in this assessment and 315 (75%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of mAb clone **Ber-H2** on the Bond platforms (Leica Biosystems)
- Less successful performance of the RTU format **790-4858** (Ventana/Roche) when using vendor recommended protocol settings.
- Too low concentration of the primary antibody.
- Application of less sensitive detection systems.
- Inefficient HIER.

Performance history
This was the seventh NordiQC assessment of CD30. The pass rate was at the same level as in the previous run 65, 2012 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD30 in the seven NordiQC runs performed**



Controls
Normal tonsil is recommended as positive and negative tissue control for demonstration of CD30. Activated B-cells primarily located in the rim of the germinal centers must show an at least weak but distinct membranous staining reaction (low-level of CD30 expression). In addition, a subpopulation of activated interfollicular lymphocytes should also be demonstrated and typically display a weak to strong membranous staining reaction. Virtually all other lymphocytes must be negative. In this context, it must be mentioned that the mAb clone JCM182 labels subpopulations of macrophages and occasionally also endothelial cells being negative by mAb clone Ber-H2. The number of CD30 positive activated B-cells may vary from tonsil to tonsil.

Conclusion
The widely used mAb clones **Ber-H2** and **JCM182** could both be used to obtain an optimal staining result for CD30. The IHC assays for CD30 should be carefully calibrated and based on protocol settings providing high analytical sensitivity including efficient HIER and at least a 3-step multimer/polymer detection system. mAb clone Ber-H2 has a more selective reaction pattern compared to clone JCM182, as the latter also labels subpopulations of macrophages in addition to e.g. activated lymphocytes. Hodgkin cells were equally recognized by both clones.
mAb clone Ber-H2 showed an inferior performance on BOND IHC platforms (Leica Biosystems), whereas clone JCM182 was more successful on this platform series, stressing that the selection of antibody clone both must be based on the purpose of the IHC assay but also on the IHC platform available in the laboratory.
Similar to the observations in run 65, the extensively used RTU format 790-4858 (Ventana/Roche) based on the mAb clone Ber-H2 gave less successful results with an overall pass rate of only 26% (26/35) when used by vendor recommended protocol settings. For this RTU system, the application of OptiView with Amplification kit was found essential for optimal performance.

Table 1a. **Overall results for CD30, run 74**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	89	39	26	21	3	73%	44%
Ready-To-Use antibodies	334	126	124	77	7	75%	38%
Total	423	165	150	98	10	-	
Proportion		39%	36%	23%	2%	75%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CD30, run 74**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **Ber-H2**	61 12 2 1 1	Dako/Agilent Cell Marque Zytomed Systems Zeta Corporation Quartett	35	22	18	2	74%	46%
mAb clone **JCM182**	9	Leica Biosystems	3	2	3	1	56%	33%
mAb clone **CON6D/B5**	1	Biocare Medical	1	0	0	0	-	-
rmAb clone **ZR248**	1	Zeta Corporation	0	1	0	0	-	-
rmAb clone **HGL-CD30**	1	Bio-Highgrade	0	1	0	0	-	-
Total	89		39	26	21	3	-	
Proportion			44%	29%	24%	3%	73%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).

Table 1c. **Ready-To-Use antibodies and assessment marks for CD30, run 74**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **Ber-H2 IR602 (VRPS)**[3]	4	Dako/Agilent	1	2	1	0	-	-
mAb clone **Ber-H2 IR602 (LMPS)**[4]	46	Dako/Agilent	18	21	7	0	85%	39%
mAb clone **Ber-H2 GA602 (VRPS)**[3]	26	Dako/Agilent	5	15	6	0	77%	19%
mAb clone **Ber-H2 GA602 (LMPS)**[4]	38	Dako/Agilent	16	19	3	0	92%	42%
mAb clone **Ber-H2 790-4858 (VRPS)**[3]	35	Ventana/Roche	1	8	25	1	26%	3%
mAb clone **Ber-H2 790-4858 (LMPS)**[4]	130	Ventana/Roche	62	37	26	5	76%	48%
mAb **Ber-H2 MAD-002045QD**	1	Master Diagnostica	0	0	1	0	-	-
mAb **Ber-H2 130M-XX**	2	Cell Marque	1	0	1	0	-	-
mAb clone **Ber-H2 8265-C010**	2	Sakura FineTek	0	0	2	0	-	-
mAb clone **Ber-H2 AM327**	1	BioGenex	0	0	1	0	-	-
mAb clone **Ber-H2 PDM018**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone **JCM182 PA0790 (VRPS)**[3]	28	Leica Biosystems	14	11	3	0	89%	50%
mAb clone **JCM182 PA0790 (LMPS)**[4]	15	Leica Biosystems	7	7	1	0	93%	47%
mAb clone **C5E10 CCM-0523**	1	Celnovte Biotechnology	1	0	0	0	-	-
mAb clone **ConD6/B5 PM346**	1	Biocare Medical	0	1	0	0	-	-
mAb clone **442F7G3 PA137**	1	Abcarta	0	1	0	0	-	-
mAb clone **MX080 MAB-0868**	1	Fuzhou Maixin	0	1	0	0	-	-
mAb clone **BY018 BFM-0182**	1	Bioin Biotechnology	0	1	0	0	-	-
Total	334		126	124	77	7	-	
Proportion			38%	37%	23%	2%	75%	

Ready-To-Use antibodies and assessment marks for CD30, run 74
1) Proportion of sufficient results (optimal or good). (≥5 assessed 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 assessed 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 assessed protocols)

Detailed analysis of CD30, Run 74

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **Ber-H2**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (4/7)*, TRS pH 6.1 (3-in-1) (Dako/Agilent) (6/8), Cell Conditioning 1 (CC1, Ventana/Roche) (24/40) or PRIME Epitope Retrieval Solution 1 (PERS1, Leica Biosystems) (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, 46 of 55 (84%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **JCM182**: Protocols with optimal results were based based on HIER using Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/5) or TRIS-EDTA/EGTA pH9 (1/1) as retrieval buffer. The mAb was diluted in the range of 1:80-100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 laboratories were giving an optimal mark.

mAb clone **CON6D/B5**: The protocol with an optimal result was based on HIER using TRS pH 6.1 (3-in-1) (Dako/Agilent) as retrieval buffer. The mAb was diluted 1:25 and Envision FLEX+ (Dako/Agilent) was used as the detection system.

Table 2. **Proportion of optimal results for CD30 for the most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **Ber-H2**	2/2**	0/1	2/5 (40%)	6/6 (100%)	22/35 (63%)	-	1/19 (5%)	-
mAb clone **JCM182**	-	-	-	-	-	-	2/2	0/2

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, Ultra, Ultra Plus
3) Bond III/PRIME

Ready-To-Use antibodies and corresponding systems (≥5 protocols).
mAb clone **Ber-H2**, product no. **IR/IS602**, Dako/Agilent, Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS pH 6.1 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8002) as detection system. Using these protocol settings, 6 of 6 (100%) laboratories produced an optimal result.

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, 42 of 50 (84%) laboratories produced a sufficient staining result. Protocols based on the detection system FLEX+ provided 100% (13/13) sufficient results – 69% (9/13) being optimal.

mAb clone **Ber-H2**, product no. **790-4858**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 24-60 min. incubation of the primary Ab and OptiView (760-700) with amplification as detection system. Using these protocol settings, 52 of 56 (93%) laboratories produced a sufficient staining result - 42/56 (75%) being optimal.

mAb clone **JCM182,** product no. **PA0790**, Leica Biosystems, BOND III/MAX/PRIME:
Protocols with optimal results were typically based on HIER using PERS1 or BERS1 (efficient heating time 20 min. at 95-104°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 28 of 31 (90%) laboratories produced a sufficient staining result – 16/31 (52%) being optimal.

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 (in Table 1 LMPS also includes off label use on deviant IHC stainers).

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 **IR/IS602**	75% (3/4)	25% (1/4)	73% (8/11)	64% (7/11)
Dako Omnis mAb Ber-H2 **GA602**	77% (20/26)	19% (5/26)	92% (33/36)	44% (16/36)
VMS Ultra/Ultra PLUS/XT/GX mAb Ber-H2 **790-4858**	26% (9/35)	3% (1/35)	76% (98/129)	47% (61/129)
Leica BOND III/MAX/PRIME mAb JCM182 **PA0790**	89% (25/28)	50% (14/28)	93% (13/14)	43% (6/14)

* 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 are integrated.

Comments

In concordance with the previous NordiQC assessments for CD30, the prevalent feature of an insufficient staining result was related to a generally too weak or false negative staining reaction of the cells expected to be demonstrated accounting for 92% (99/108) of the insufficient results. The remaining insufficient results were typically characterized by false positive reactions and/or poor signal-to-noise ratio, compromising interpretation of the specific CD30 expression. 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-cells of the tonsil (situated at the rim of germinal centres) and neoplastic cells of the two Hodgkin lymphomas and in particular the Hodgkin lymphoma tissue core no. 5 was more challenging and required optimally calibrated protocols.

The mAb clones **Ber-H2** and **JCM182** were the most widely used antibodies for demonstration of CD30 and applied by 98% (415/423) of the participating laboratories (see Tables 1b and 1c).

Used as concentrated format within laboratory developed (LD) assays, the mAb clone **Ber-H2** provided a pass rate of 74% (57/77) - 46% (35/77) being optimal. This is a relatively significant decrease in the performance compared to the latest run 65 with an overall pass rate of 87% (85/98) - 60% (59/98) being optimal. The performance characteristics for mAb clone Ber-H2 on the respective automatic platforms from the major vendors are outlined in Table 2, and as shown optimal results could be obtained on all main platforms, although challenged on the Leica Bond platforms giving only 5% (1/19) optimal results. All protocols giving an optimal staining result were based on efficient HIER in an alkaline buffer (e.g. CC1 from Ventana/Roche) or use of the modified citric based buffer TRS pH 6.1 (3-in-1) (Dako/Agilent) in combination with a 3-layer multimer/polymer-based detection system. Also, the primary antibody was typically applied at a relative high concentration within a dilution range of 1:20-50.
The most prevalent feature impacting the overall pass rate in a negative direction in this assessment was related to the platform used for the CD30 IHC assay. In this assessment it was observed that the mAb clone Ber-H2 revealed an inferior performance on the Leica Biosystems Bond IHC platforms despite being applied by protocol settings providing high analytical sensitivity e.g. efficient HIER in PERS2/BERS2 in combination with the 3-layer detection system Bond Refine (Leica Biosystems). In total, the proportion of sufficient results for the mAb clone Ber-H2 on the Bond platforms was 47% (9/19) and only one result was assessed as optimal, although applying otherwise "optimal protocol settings" (titer of Ab, HIER and detection systems) as seen for other fully automated IHC platforms.
Fon non-Bond IHC platforms insufficient results were caused by a too diluted primary antibody, too short HIER time and/or use of a less sensitive 2-step detection system.

Nine laboratories used the mAb clone **JCM182** within a LD assay and the overall pass rate was 56% (5/9) - 33% being optimal. The mAb was primarily used by laboratories on the BOND III/PRIME (Leica Biosystems) giving 57% (4/7) sufficient results and 29% (2/7) being optimal. The main challenge for this clone was related to an aberrant staining reaction of endothelial cells/subpopulation of macrophages and in combination with a general excessive background staining, which overall accounted for a low pass rate and proportion of optimal results as the final interpretation of the specific CD30 expression was significantly compromised.

78% (334/423) of all participating laboratories used a RTU format for the demonstration of CD30. This is an increase compared to the former run 65, 2022 in which 70% (256/365) of the participants applied a RTU format.

As observed in the previous assessment for CD30, the number of laboratories using the RTU format **IR/IS602** (Dako/Agilent) based on the mAb clone **Ber-H2** with intended use on Dako Autostainer) was quite high (n=50), but only 4 protocols were based on vendor recommended protocol settings (VRPS) providing a pass rate of 75% (3/4) – one being optimal. The official recommendation for the 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. In this assessment, and applying laboratory modified protocol settings (LMPS) substituting Envision FLEX with Envision FLEX+ as detection system, the proportion of optimal results increased to 85% (6/7). One protocol not assessed as optimal (insufficient) was downgraded due to technical issues and aberrant nuclear staining - most likely due to "drying out" during the staining process on the Autostainer. Surprisingly, the overall proportion of sufficient results was nearly identical between laboratories applying either VRPS or LMPS (see Table 3). This observation is difficult to elucidate upon as it was expected that extended use of the 3-layer detection system EnVision FLEX+ would increase the pass rate considerably and as seen in the previous run 65. However, caution must be taken into account due to few data points.
Eight laboratories used the RTU format **IR/IS602** on the Leica Bond (Leica Biosystems) or Ventana BenchMark (Ventana/Roche) platforms giving 100% (8/8) sufficient results - 25% (2/8) being optimal. In addition, a significant proportion of participants (n=27) used the same format on the Omnis, providing a pass rate of 85% (23/27) of which only 33% (9/27) were assessed as optimal. This "direct" transfer of a RTU product, developed for a specific platform e.g. Autostainer, to the Omnis instrument should be avoided, especially when the manufacturer has a validated product (**GA602**) for intended use on 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 relatively low proportion of sufficient and especially optimal results applying VRPS based on HIER in TRS low pH (3-in-1) for 30 min. at 97˚C, 10 min. incubation of the primary Ab and EnVision FLEX as the detection system (see Table 3). In contrast LMPS, typically prolonging incubation time of primary Ab and/or use of EnVision FLEX+ as detection system, was found more successful and increased the proportion of sufficient and optimal results significantly. These modifications seemed advantageous for this "RTU system" and when applied, increased the overall pass rate to 93% (28/30) of which 47% of the results (14/30) were assessed as optimal. Irrespectively of protocol settings applied e.g., HIER conditions (buffer and time/temperature) and/or use of different incubation times in primary Ab the substitution of the 2-step EnVision FLEX detection system with the 3-step EnVision FLEX+ system was observed to be the single most effective positive protocol modification giving a pass rate of 100% (13/13) – 69% (9/13) being optimal. Based on these observations and also being noted in run 65, the vendor is encouraged to change protocol settings and revise package insert for the RTU product GA602.

The RTU system **790-4858** (Ventana/Roche, Benchmark GX/XT/Ultra) based on the mAb clone **Ber-H2**, and using VRPS, provided the lowest proportion of sufficient and optimal results among RTU systems from the major vendors, 26% (9/35) and 3% (1/35), respectively (see Table 3). This observation is in line with the inferior results obtained in previous assessments for CD30, although recommended protocol settings to this RTU system are based on parameters giving a high analytical sensitivity e.g., using effective HIER in CC1 combined with the use of OptiView or UltraView with amplification as the detection systems. From the data analyzed so far in the latest two NordiQC CD30 assessments, the RTU system have provided an overall accumulated pass rate of 22% (11/49 protocols) when used by VRPS and indicate a "miscalibration" of the antibody concentration selected for the RTU format.
This statement of a "miscalibration" is supported by the significant proportion of laboratory developed protocols based on the corresponding concentrated formats (see above) providing sufficient (83%, 33/40) and optimal results (60%, 24/40) with similar protocols settings as recommended by Ventana/Roche for the RTU system. It was as such observed that 96% (23/24) of the LD protocols with an optimal result were based on "standard protocol settings" using HIER in CC1 (average HIER time 56 minutes at 95-100°C), average incubation time in the primary Ab for 32 min. (range 16-60 minutes), average titer of 1:60 of the primary Ab (range 30-150) and use of Ultraview with amplification or OptiView as the detection system.
In contrast to VRPS for the RTU system **790-4858**, LPMS gave a significant improved performance and the most effective modification with positive impact was related to the application of OptiView with Amplification kit as detection system (see Table 1c and Table 3). For this modification a pass rate of 90% (71/79 protocols) was obtained, 71% being optimal results.
The same topic has been addressed in the previous run 65, and the observations clearly indicate, 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 of analytical sensitivity for most markers. Conclusive, the present format of Ventana/Roche RTU system seems to require the sensitive detection system OptiView with tyramide amplification for an optimal detection of CD30 in the full dynamic and diagnostic relevant range of CD30 expression levels. 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, PRIME) based on the mAb clone **JCM182** provided among all RTU systems the highest proportion of sufficient results using VRPS (HIER in BERS1 for 20 min., 15 min. in primary Ab and Refine as detection system) as the pass rate was 89% (25/28) – 50% (14/28) being optimal (see Table 3). LPMS were in general also successful giving an overall pass rate of 93%, 47% optimal. HIER in BERS2 was found less adequate giving an enhanced background reaction in most results based on this modification. Similar to the corresponding concentrated format of the mAb clone JCM182, an extended staining reaction is seen in a subpopulation of macrophages e.g. in tonsils/lymph nodes compared to the pattern seen for the mAb clone Ber-H2.

This was the seventh assessment of CD30 in NordiQC (see Graph 1). The pass rate of 75% was fully on par with the level seen in the previous run 65, 2022. The insufficient results were mainly caused by application of protocols providing a too low analytical sensitivity compromising the demonstration of CD30 in cells, both normal and neoplastic, with low-level expression. Concordant to the analysis in run 65, the inferior performance of the widely used RTU system from Ventana/Roche when applied accordingly to VRPS or by comparable protocol settings contributed quite significant to the overall relatively low pass rate. In this assessment, the clone Ber-H2 was also challenged when used on the Leica Biosystems BOND IHC platforms.
This supports the hypothesis, that IHC assays always must be calibrated for the purpose of the assay addressing the relevant dynamic and diagnostic relevant range of expression of the target analyte and the selection of antibody clone must be anchored with focus on the intended platform – not all clones perform equally on all IHC platforms.

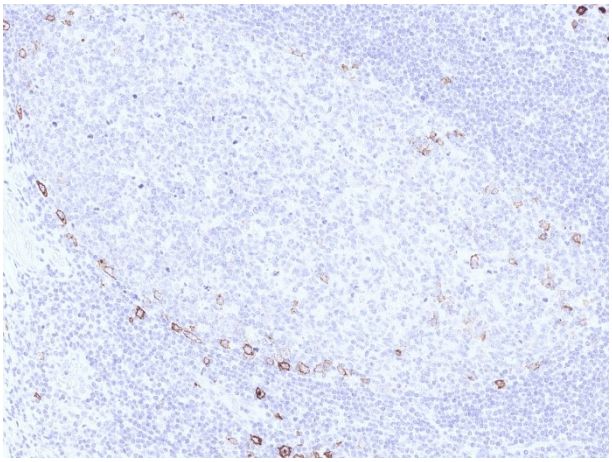


Fig. 1a (x100)
Optimal CD30 staining reaction of the tonsil using the RTU format GA602 (Dako/Agilent) based on mAb clone Ber-H2 on Dako Omnis, following the protocol recommendations given by the vendor except for substituting EnVision FLEX with EnVision FLEX+ (with mouse linker) – same protocol used in Figs. 1a – 5a.

The activated B- and T-cells, particularly B-cells located at the rim of the germinal centers, show a weak to moderate predominantly membranous staining reaction.

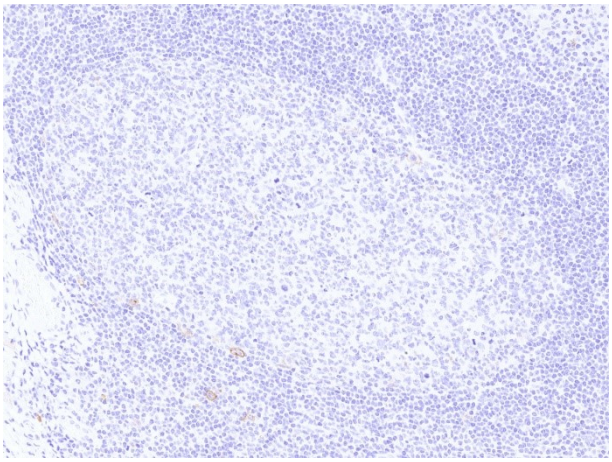


Fig. 1b (x100)
Insufficient staining reaction of the tonsil using the mAb clone Ber-H2 as concentrate (1:50) on Leica BOND III (Leica Biosystems) with HIER in BERS2 and Refine as detection system – same protocol used in Figs. 1b – 5b. Despite selecting protocol settings expected to provide a high analytical sensitivity, the result was not as expected. The activated B-cells within the rim of the germinal center are only faintly positive and the total number of cells is reduced compared to level seen in Fig. 1a.
mAb clone Ber-H2 gave an overall inferior performance on the BOND IHC platform in this assessment.
Also see Fig. 2b, higher magnification.

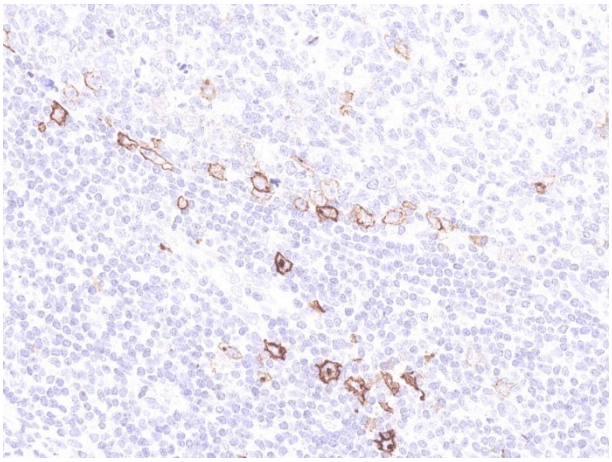


Fig. 2a (x200)
Same field and protocol as in Fig. 1a.
The activated B-cells located at the rim of the germinal centers, show a weak to moderate and distinct membranous staining reaction, while interfollicular lymphocytes show a slightly stronger intensity.

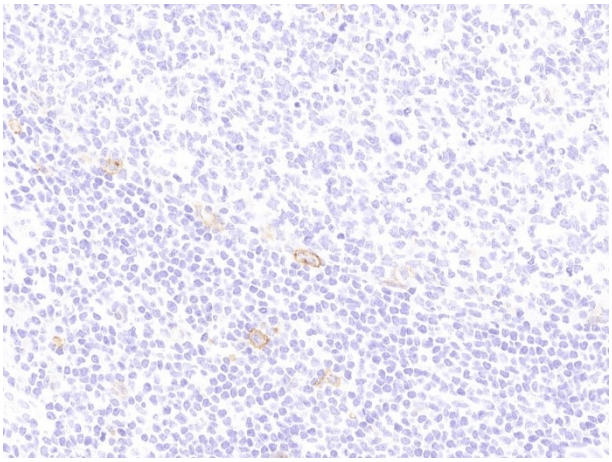


Fig. 2b (x200)
Same field and protocol as in Fig. 1b.
The activated B-cells located at the rim of the germinal centers are hardly visible and only display an equivocal membranous staining reaction.
These activated B-cells are important to verify low-level demonstration of the assay and its ability to identify CD30 in the full dynamic and diagnostic range of expression levels as seen in Figs. 3b – 5b.

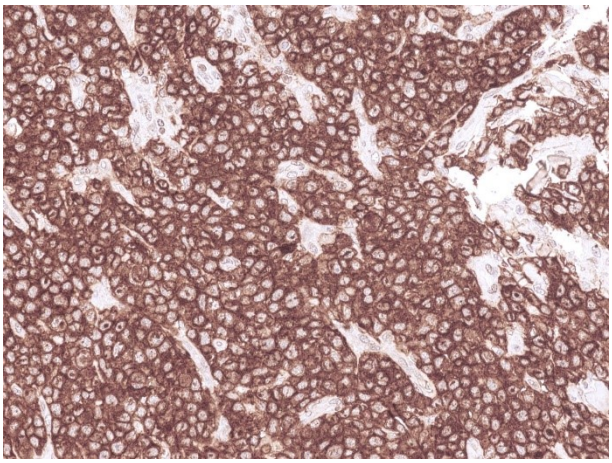


Fig. 3a (x200)
Optimal staining reaction for CD30 of the ALCL using the same protocol used in Figs. 1a - 2a.
All neoplastic cells display a strong, distinct membranous staining reaction and a dot-like reaction of the Golgi zone.

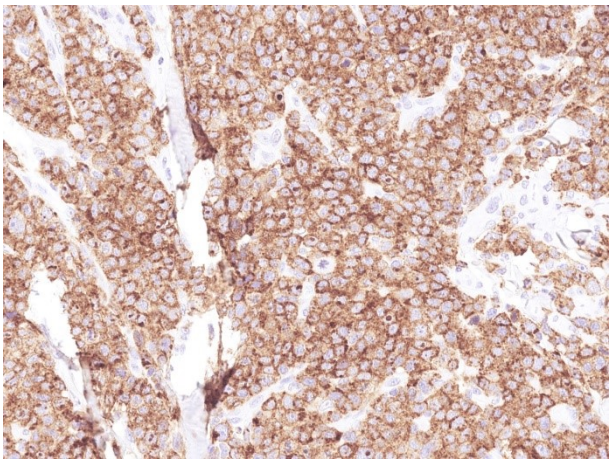


Fig. 3b (x200)
CD30 staining reaction of the ALCL using same insufficient protocol as in Figs. 1b and 2b.
Virtually all the neoplastic cells of the ALCL are demonstrated as expected, as these cells have a high-level CD30 expression. Compare with the result obtained in Figs. 4b and 5b with focus on tissues with lower antigen levels of CD30 compared to the ALCL.

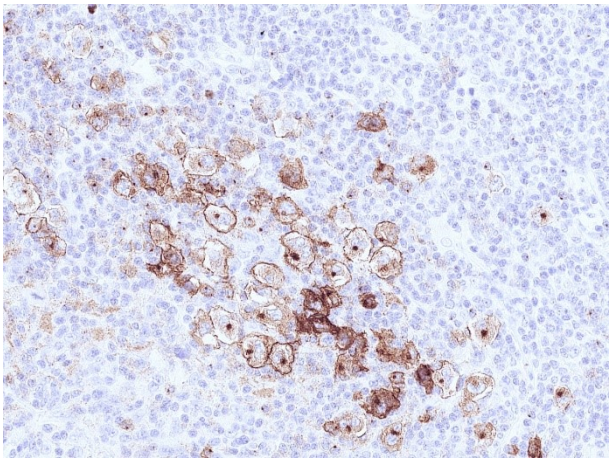


Fig. 4a (x100)
Optimal CD30 staining reaction of the Hodgkin lymphoma, tissue core no. 5, using same protocol as in Figs. 1a - 3a. All Hodgkin/Reed-Sternberg cells show a weak to strong, distinct membranous and cytoplasmic dot-like staining pattern.

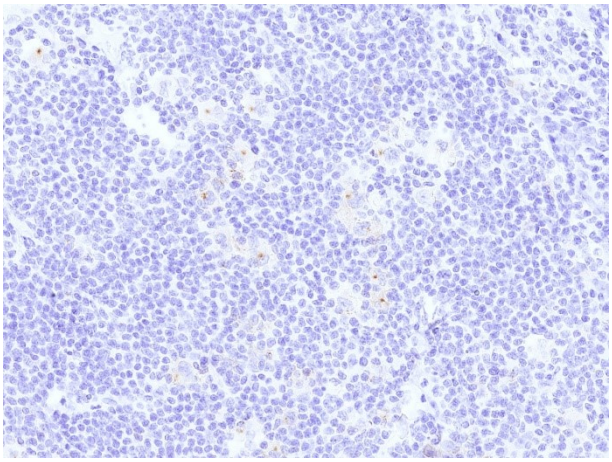


Fig. 4b (x100)
Insufficient CD30 staining reaction of the Hodgkin lymphoma, tissue core no. 5, using the same protocol as in Figs. 1b - 3b. Virtually all the neoplastic cells are false negative or only faintly demonstrated - compare with Fig. 4a (same field).

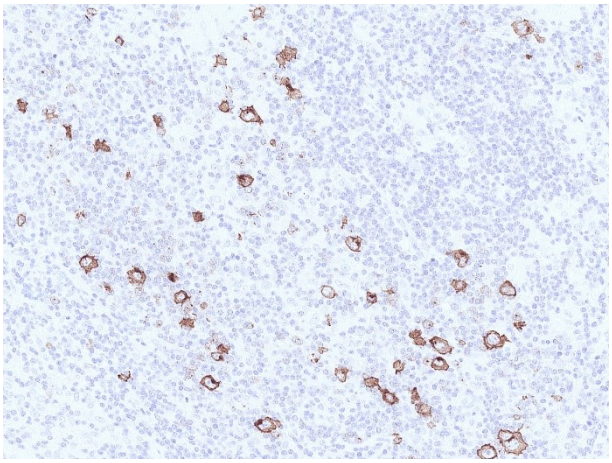


Fig. 5a (x400)
Optimal CD30 staining reaction of the Hodgkin lymphoma, tissue core no. 4, using same protocol as in Figs. 1a - 4a. All Hodgkin/Reed-Sternberg cells show a moderate to strong, distinct membranous and cytoplasmic dot-like staining pattern.

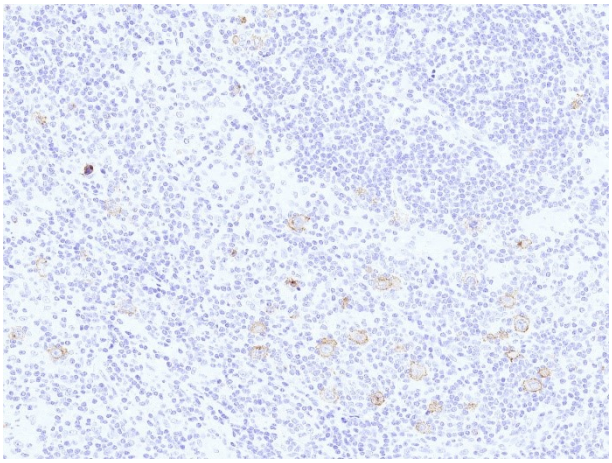


Fig. 5b (x400)
Insufficient CD30 staining reaction of the Hodgkin lymphoma, tissue core no. 4, using the same protocol as in Figs. 1b - 4b. A significant proportion of the neoplastic cells are false negative or only faintly demonstrated - compare with Fig. 5a (same field).

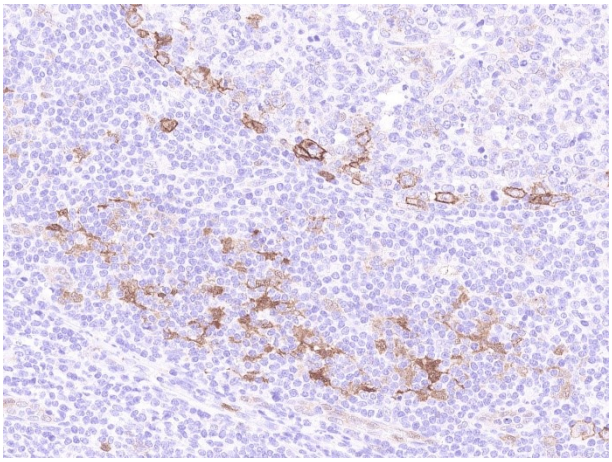


Fig. 6a (x200)
Optimal CD30 staining reaction of the tonsil using the RTU format PA0790 (Leica Biosystems) based on mAb clone JCM182 on BOND III, following the protocol recommendations given by the vendor. The activated B-cells located at the rim of the germinal centers, show a weak to strong and distinct membranous staining reaction. In addition, a subpopulation of macrophages in the interfollicular area is demonstrated. However, the protocol overall provided the results expected and of central importance gave the required level of analytical sensitivity in tissues with low-level CD30 expression as seen in the two Hodgkin lymphomas – see Fig. 6b, same protocol.

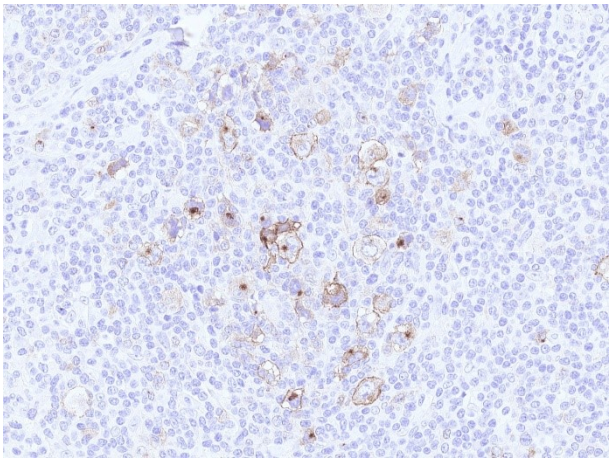


Fig. 6a (x200)
Optimal CD30 staining reaction of the Hodgkin lymphoma, tissue core no. 5, using same protocol as in Fig. 6a.
The Hodgkin/Reed-Sternberg cells with low-level CD30 expression show a weak to moderate distinct membranous and cytoplasmic dot-like staining pattern.

MB/LE/RR/SN 02.07.2025