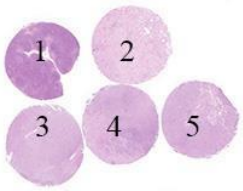
Assessment Run 61 2021
CD15

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD15, identifying classical Hodgkin lymphoma (CHL) and aid in the differential diagnosis of CHL (75-90% pos.) from e.g. diffuse large B-cell lymphoma (DLBCL). Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD15 (see below). This was the sixth NordiQC assessment of CD15.

Material
The slide to be stained for CD15 comprised:



1. Tonsil, 2. Kidney, 3. DLBCL, 4-5. Classical Hodgkin lymphoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD15 staining as optimal included:

- An at least weak but distinct predominantly membranous staining reaction of most follicular dendritic cells in the germinal centers of the tonsil.
- A moderate to strong predominantly membranous staining reaction of virtually all epithelial cells lining the renal proximal tubules.
- A moderate to strong predominantly membranous staining reaction of most parietal epithelial cells of Bowman's capsule in the kidney.
- A moderate to strong and distinct predominantly membranous staining reaction as well as dot-like (Golgi) staining reaction of the vast majority of Hodgkin and Reed-Sternberg cells in the two Hodgkin lymphomas.
- A strong cytoplasmic staining reaction of neutrophil granulocytes in all five specimens.
- No staining reaction of the neoplastic cells of the DLBCL.
- No or only minimal background reaction.

Participation

Number of laboratories registered for CD15, run 61	354
Number of laboratories returning slides	305 (87%)

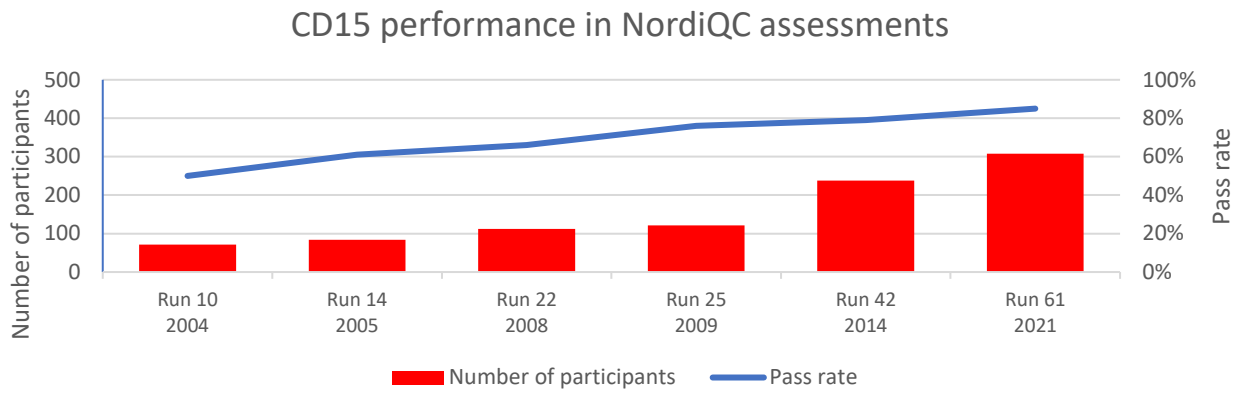
The number of laboratories returning slides has decreased in this run 61 compared to previous assessments, due to the COVID-19 pandemic and associated postal delays. All slides returned after the assessment were assessed and received advice if the result being insufficient but were not be included in this report.

Results
305 laboratories participated in this assessment. 262 (86%) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in Table 1 (see page 2)

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient Heat Induced Epitope Retrieval (HIER)
- Detection systems with low sensitivity

Performance history
This was the sixth NordiQC assessment of CD15. The overall pass rate was improved compared to run 42, 2014 and have in general increased steadily in all runs performed (see Graph 1).

Graph 1. **Proportion of sufficient results for CD15 in the six NordiQC runs performed**



Conclusion

The monoclonal antibodies (mAbs) clones **Carb-3**, **MMA** and **IHC527** could all be used to obtain optimal staining results for CD15. Irrespective of the clone applied, efficient HIER, use of sensitive 3-step detection systems and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Compared to the concentrates, the Ready-To-Use systems for CD15 from Dako/Agilent, Ventana/Roche and Leica Biosystems provided the highest proportion of sufficient and optimal results.

Kidney is recommended as positive tissue control: a strong predominantly membranous staining reaction of virtually all epithelial cells lining the renal proximal tubules and moderate to strong staining of most parietal epithelial cells of Bowman's capsule must be seen. Supportive to kidney, tonsil can be used as positive and negative tissue control. The majority of follicular dendritic cells in the germinal centers must show an at least weak but distinct predominantly membranous staining reaction for CD15. No staining reaction must be seen in other cell types except for a strong cytoplasmic staining reaction in neutrophil granulocytes.

Table 1. **Antibodies and assessment marks for CD15, Run 61**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **Carb-3**	57	Dako/Agilent	24	19	12	2	75%	42%
mAb clone **MMA**	11 5 1 1 1 3 1	Cell Marque Becton Dickinson Biocare medical DBS Leica Biosystems Thermo/NeoMarkers Zeta Corporation	7	8	5	2	68%	32%
mAb clone **MMA+BY87**	1 1	Biocare Zytomed Systems	-	1	1	-	-	-
mAb clone **BRA4F1**	1	BioGenex	-	-	-	1	-	-
Other	1	Other	-	1	-	-	-	-
Ready-To-Use antibodies								
mAb clone **Carb-3 IS/IR062**[3]	15	Dako/Agilent	8	5	1	1	87%	53%
mAb clone **Carb-3 IS/IR062**[4]	18	Dako/Agilent	10	2	6	-	67%	56%
mAb clone **Carb-3 GA062**[3]	29	Dako/Agilent	15	12	1	1	93%	52%
mAb clone **Carb-3 GA062**[4]	20	Dako/Agilent	10	9	1	-	95%	50%
mAb clone **MMA 760-2504**[3]	14	Ventana/Roche	6	7	1	-	93%	43%
mAb clone **MMA***	98	Ventana/Roche	64	28	5	1	94%	64%

760-2504[4]								
mAb clone **Carb-1 PA0039[3]**	2	Leica Biosystems	1	-	-	1	-	-
mAb clone **Carb-1 PA0039[4]**	1	Leica Biosystems	1	-	-	-	-	-
mAb clone **MMA PA0473[3]**	3	Leica Biosystems	3	-	-	-	-	-
mAb clone **MMA PA0473[4]**	10	Leica Biosystems	6	4	-	-	100%	60%
mAb clone **MMA 8256-C010**	4	Sakura Finetek	1	3	-	-	-	-
mAb clone **MMA+BY87 PM073 AA**	2	Biocare	-	2	-	-	-	-
mAb **MMA MAD-005151QD**	1	Vitro SA	1	-	-	-	-	-
mAb clone **MMA 115M-18**	1	Cell Marque	1	-	-	-	-	-
mAb clone **MMA CCM-0431**	1	Celnovte	-	-	1	-	-	-
mAb clone **MMA MAB-0779**	2	Maixin	2	-	-	-	-	-
mAb clone **IHC527 IHC527**	1	GenomeMe	1	-	-	-	-	-
Total	305		161	101	34	9		
Proportion			53%	33%	11%	3%	86%	

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 CD15, Run 61

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **Carb-3**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (4/4)*, TRS pH 9 (Dako/Agilent) (6/9), Cell Conditioning 1 (CC1,Ventana/Roche) (8/24) or Bond Epitope Retrieval Solution 2 (BERS2; Leica) (6/12) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 36 of 44 (82%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **MMA**: Protocols with optimal results were all based on HIER using CC1 (Ventana/Roche) (4/8) or BERS2 (Leica) (3/7) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 of 14 (79%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CD15 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX / XT / Ultra**			**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **Carb-3**	4/4**	0/1	6/9 (66%)	-	8/24 (31%)	0/2	-	6/12 (50%)	0/4
mAb clone **MMA**	0/3	0/1	-	-	4/8 (50%)	-	-	3/7 (43%)	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 **Carb-3**, product no. **IS062/IR062**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 97-99°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 22 of 26 (85%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Carb-3**, product no. **GA062**, Dako/Agilent, Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 30 min. at 97°C), 12-20 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) as detection system. Using these protocol settings 20 of 20 (100%) laboratories produced an optimal staining result.

mAb clone **MMA**, product no. **760-2504**, Ventana/Roche, BenchMark XT/Ultra:
Protocols with optimal result were typically based on HIER using CC1 (efficient heating time 32-64 min.), 16-32 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit (760-080) or OptiView (760-700) as detection systems. Using these protocol settings 84 of 88 (96%) laboratories produced a sufficient staining result.

mAb clone **MMA** product no. **8256-C010,** Sakura, Tissue-Tek Genie:
One protocol with optimal result was based on HIER using Tissue-Tek Genie High pH sol. (efficient heating time 45 min.), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit (8826-K250) as detection systems. Using these protocol settings 4 of 4 laboratories produced a sufficient staining result.

mAb clone **Carb-1** product no. **PA0039**, Leica Biosystems, Leica Bond:
Protocols with optimal results were based on HIER using BERS2 or BERS1 (efficient heating time 20 min. at 98-100°C), 15 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 2 of 3 laboratories produced a sufficient result.

mAb clone **MMA** product no. **PA0473**, Leica Biosystems, Leica Bond:
Protocols with optimal results were typically based on HIER using BERS2 or BERS1 (efficient heating time 10-20 min. at 98-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 11 of 11 (100%) laboratories produced a sufficient result.

Table 3. **Proportion of sufficient and optimal results for CD15 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb Carb-3 **IR062**	87% (13/15)	53% (8/15)	82% (9/11)	73% (8/11)
Dako Omnis mAb Carb-3 **GA062**	93% (27/29)	52% (15/29)	95% (17/18)	50% (9/18)
VMS Ultra/XT mAb MMA **760-2504**	93% (13/14)	43% (6/14)	94% (92/98)	64% (64/98)
Leica BOND MAX/III mAb Carb-1 **PA0039**	1/1	1/1	1/2	1/2
Leica BOND MAX/III mAb MMA **PA0473**	(3/3)	(3/3)	100% (8/8)	63% (5/8)

* 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 this sixth NordiQC assessment for CD15, 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 100% of the insufficient results (43 of 43). Virtually all laboratories were able to

demonstrate CD15 in the neutrophil granulocytes (high-level antigen expressors) (Figs. 4a-4b) whereas demonstration of CD15 in neoplastic cells of the two Hodgkin lymphomas, the parietal epithelial cells of Bowman's capsule in the kidney and the follicular dendritic cells of the germinal centres in the tonsil were more difficult and required optimally calibrated protocols. Insufficient HIER (too short heating time or too low temperature, too diluted primary Abs and/or usage of detection systems providing low technical and analytical sensitivity were the main parameters causing a too weak staining reaction for CD15.

27% (83 of 305) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for CD15 which is a decrease from the latest run 42, 2014 where 47% were using concentrated formats.

Carb-3 was the most popular clone for the concentrated antibodies in this assessment and used by 64% (57 out of 83) of the laboratories. One submitted protocol was not included in this assessment due to technical problems. This clone was used on all four main IHC instruments, and an optimal result could be obtained on all four. For optimal performance HIER at high pH was found superior to low pH, as no optimal results were generated by use of HIER at low pH at any platform/system as shown in Table 2.

21 laboratories used a 2-layer detection system with a pass rate of 62% (13 out of 21) while 36 used a 3-layer detection system obtaining a pass rate of 89% (32 out of 36) indicating more sensitive detection system was superior for this clone (Figs. 5a-5b). The Average Dilution Value (ADV) for sufficient results was 1:89 (range 1:20-400, whereas an ADV of 1:298 (range 1:20-2.000) was seen in protocols with insufficient results. Consequently, the titer must be carefully calibrated to provide an IHC protocol that is able to demonstrate CD15 in structures with both low- and high-level CD15 expression.

The conc. mAb MMA clone was used by 22 laboratories and optimal results were obtained on both the Ventana/Roche and Leica platforms (see Table 2). The required protocol settings for optimal performance were similar as observed for Carb-3 with an efficient HIER at high pH and preferable in combination with a 3-layer detection system. For optimal results, the mAb clone MMA typically was diluted in the range of 1:10-50.

74% of the laboratories (225 of 305) used RTU products, two submitted protocols were not included in the assessment due to technical problems. For the Ventana/Roche, Dako/Agilent and Leica RTU systems, optimal results could be obtained using official protocol recommendations from the respective companies and by laboratory modified protocol settings (typically adjusting HIER time, incubation time of the primary Ab and/or choice of detection system).

The Ventana/Roche RTU format (**760-2504**) based on mAb clone MMA was the most frequently applied assay used by a total of 112 laboratories. The total pass rate of this assay was 92% with 62% optimal. The official RTU protocol provided by the vendor is based on HIER in CC1 for 64 min., a primary antibody incubation time at 16 min. with UltraView as detection system. Out of 112 participants only 14 used these protocol settings with a pass rate of 93%, 43% optimal.
98 laboratories modified the protocol gaining a pass rate of 94%, 64% optimal, which was the highest proportion of optimal results in this assessment. The most efficient modification was related to an exchange of the 2-layer detection system with a 3-layer system. As such, 49 of the laboratories using OptiView as detection system obtained a pass rate of 96%, 80% optimal. 63 used UltraView with a pass rate of 92%, but the level of optimal was only 50% and inferior when compared to OptiView protocols (Figs. 4-5).

The Dako/Agilent RTU format **IS/IR062** based on the mAb clone Carb-3 also provided a high number of sufficient and optimal results. The overall pass rate was 75% (25 of 33) and 55% were assessed as optimal. The official Dako/Agilent RTU protocol for Autostainer is based on HIER in TRS High pH for 20 min. and an Ab incubation of 20 min. using EnVision Flex as detection system. In total 15 laboratories used the vendor recommended protocol with a pass rate of 87%, 53% optimal. The laboratory modified protocols providing optimal results were typically prolonging the Ab incubation time to 25-30 min. One laboratory diluted the IR062 1:4 with a borderline result.

In the latest assessment of CD15 only 4 laboratories used the **GA062** RTU format for Dako Omnis with a success rate of 100%. In this assessment a total of 49 laboratories were using the Omnis format. The recommended protocol from the vendor is based on HIER in TRS high for 30 min., 12½ min. Ab incubation time and EnVision Flex as detection system. 29 used the vendor recommended protocol with a pass rate of 93%, 52% optimal. Laboratory modified protocol settings provided an almost identical pass rate and proportion of optimal results as shown in Table 3. Successful modifications were based on prolonged incubation time in primary Ab and less successful modifications related to HIER at Low pH.

In general protocols assessed "only" as good and not optimal on the Omnis was related to a reduced intensity and less distinct reaction in the follicular dendritic cells of the geminal centres in the tonsil and the parietal epithelial cells of Bowman's capsule in the kidney. In the tonsil the follicular dendritic meshwork (Figs. 1-3) seemed diffuse and weak and was observed in several cases with the vendor recommended protocol. Protocols based on increased primary Ab incubation were in particular successful to improve the performance on Omnis

Leica offers two different RTU formats based on clones mAb Carb-1 (**PA0039**) and mAb MMA (**PA0473**). The PA0039 is in the future replaced by the PA0473. 13 laboratories used the PA0473 format of clone MMA with a 100% sufficient pass rate. The vendor recommended protocol is based on HIER in BERS1 for 20 min. and 15 min. Ab incubation using the Bond Refine detection system. Only 3 laboratories used the vendor recommended protocol and all 3 obtaining optimal results (100%). Laboratory modified protocols also provided a pass rate of 100%, but reduced proportion of optimal results to 63% (see Table 3).

The total number of participating laboratories in this run, has increased (30%) compared to the latest assessment (Run 42, 2014). Any firm details to the specific causes for the minor improvement of the pass rate obtained in this run (86% versus 79% in run 42) is difficult to elucidate on, as many laboratories participated for the first time and many laboratories have changed their IHC systems since 2014. However, the extended use of high quality and robust RTU systems for CD15 seems to be one of the central elements. The most used concentrated antibodies had a pass-rate of 75% (Carb-3) and 68% (MMA) compared to the most commonly used RTU systems based on same clones as displayed in Table 3 all showing higher pass-rates ranging from 87-100%. In run 42, 56% of the participants (134 of 238) used one of the above mentioned RTU systems compared to 73% (222 of 305) in this run.

Controls
Kidney and tonsil are recommended as positive and negative tissue controls for CD15. In the kidney the protocol must be calibrated to provide a distinct and strong predominantly membranous staining reaction in virtually all the epithelial cells of the proximal tubules and most parietal epithelial cells of Bowman's capsule. In tonsil, follicular dendritic cells of the germinal centers must show an at least weak but distinct predominantly membranous staining reaction (the proportion of follicular dendritic cells can vary from tonsil to tonsil). The neutrophil granulocytes will show a strong cytoplasmic staining reaction. All other cell types including B- and T cells must be negative. As a supplement to kidney and tonsil, especially in the technical calibration phase of the CD15 assay, it is recommended to verify the protocol on Hodgkin lymphomas, classical subtype.

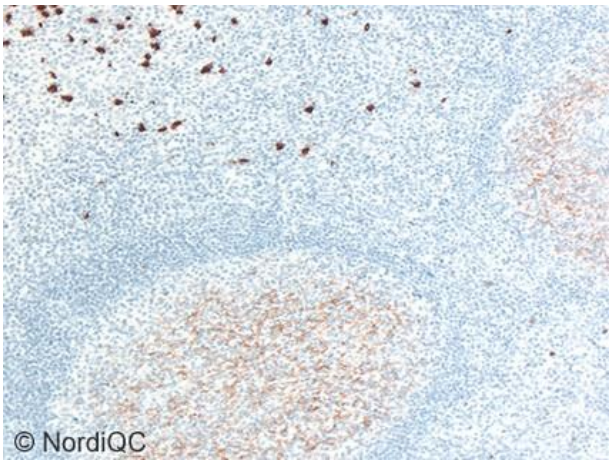


Fig. 1a (x100)
Optimal CD15 staining of the tonsil using the mAb clone Carb-3 (Dako/Agilent RTU GA062) with HIER in an alkaline buffer (TRS pH 9.0, Dako/Agilent) for 30 min., primary Ab for 20 min., Envision FLEX as detection system and performed on the Dako Omnis. Neutrophil granulocytes show a strong cytoplasmic reaction whereas the germinal center follicular dendritic cells show a weak to moderate predominately membranous staining reaction (same protocol used in Figs. 2a - 3a)

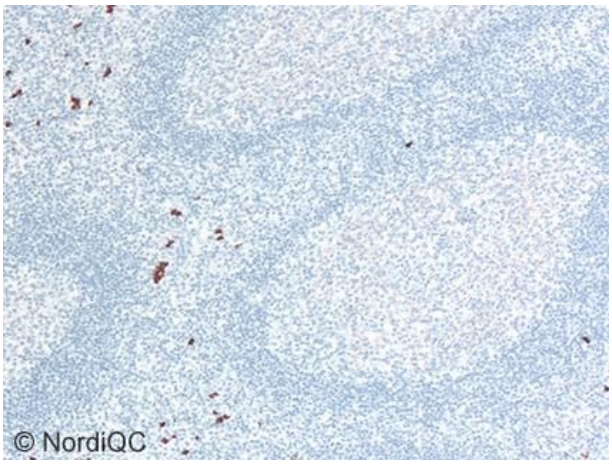


Fig. 1b (x100)
Insufficient CD15 staining of the tonsil using the mAb clone Carb-3 (with a less successful protocol on the Dako Omnis. The neutrophil granulocytes are demonstrated but the germinal center follicular dendritic meshwork is barely visible. Compare with Fig. 1a, also, compare with Figs. 2b and 3b – same protocol.

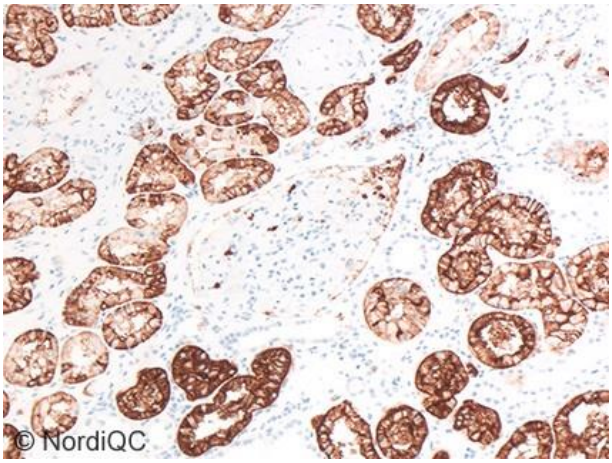


Fig. 2a (x100)
Optimal CD15 staining of the kidney using the same protocol as in Fig. 1a. Virtually all epithelial cells lining the proximal tubules show a strong predominantly membranous but also cytoplasmic staining reaction. The parietal epithelial cells of Bowman's capsule show a moderate staining reaction.

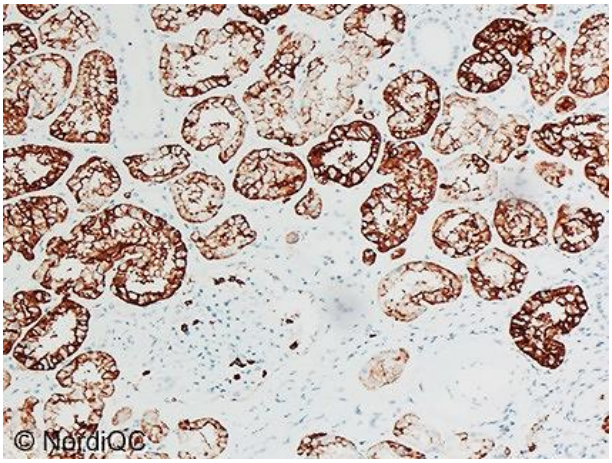


Fig. 2b (x100)
CD15 staining of the kidney using the same protocol as in Figs. 1b and 3b. The intensity of the staining reaction of the epithelial cells is slightly reduced whereas of more critical importance the parietal cells of the Bowman's capsule are barely visible. Compare with Fig. 2a. and most critically Fig. 3b. – The protocol provided an overall insufficient result.

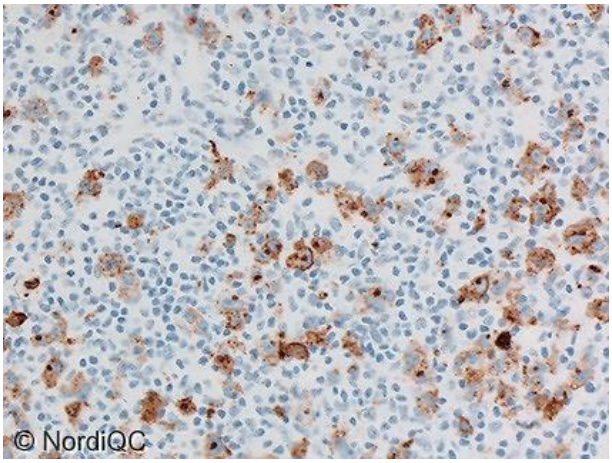


Fig. 3a (x200)
Optimal CD15 staining of the Hodgkin lymphoma, tissue core no 4, using same protocol as in Figs. 1a and 2a. The Reed-Sternberg and Hodgkin cells show a moderate membranous staining reaction and a strong dot-like positivity (same protocol used in Figs. 1a - 2a).

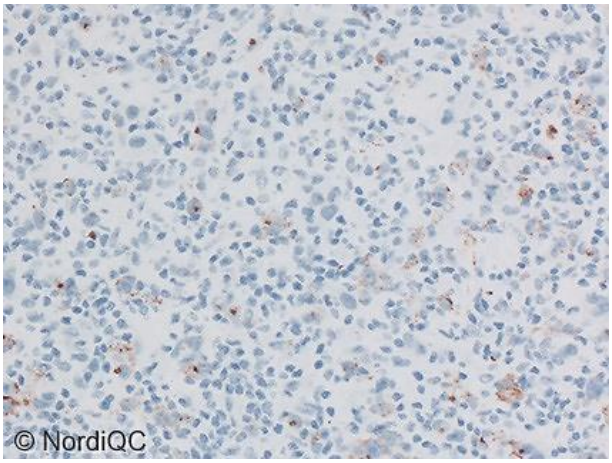


Fig. 3b (x200)
Insufficient CD15 staining of the Hodgkin lymphoma, tissue core 4, using same protocol as in Figs. 1b and 2b. The vast majority of Reed-Sternberg cells and Hodgkin cells are false negative. Compare with Fig. 3a

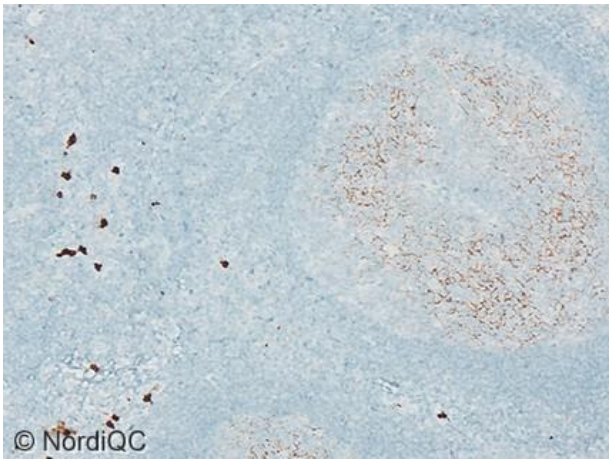


Fig. 4a (x100)
Optimal CD15 staining of the tonsil using mAb clone MMA (Ventana/Roche RTU 760-2504) with HIER in an alkaline buffer (CC1, Ventana/Roche) for 64 min., primary Ab 32 min., OptiView as detection system and performed on the Ventana Benchmark.
Neutrophil granulocytes show a strong cytoplasmic reaction whereas the germinal centre follicular dendritic cells show a weak to moderate predominately membranous staining reaction. Compare with Fig 5a – same protocol.

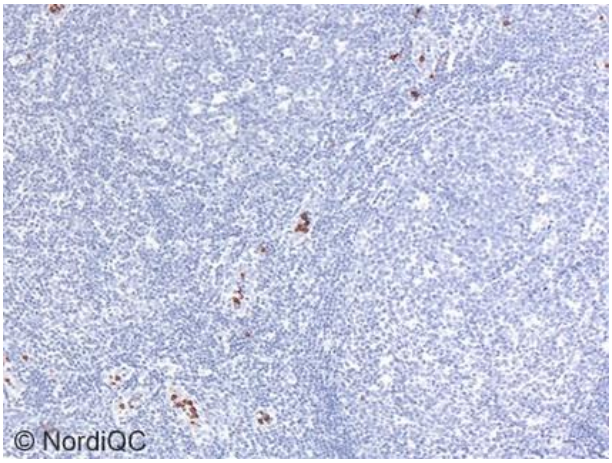


Fig. 4b (x100)
Insufficient CD15 staining of the tonsil using mAb clone MMA (Ventana/Roche RTU 760-2504) with HIER in an alkaline buffer (CC1, Ventana/Roche) for 60 min., primary Ab 32 min. UltraView as detection system and performed on the Ventana Benchmark.
The reduced sensitivity of the protocol employed causes a false negative result in the dendritic meshwork of the germinal centres and only neutrophil granulocytes with high CD15 expression level are demonstrated. Same protocol used in Fig. 5b.

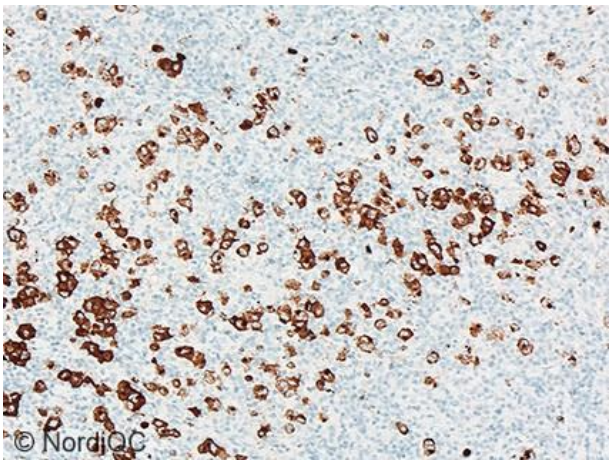


Fig. 5a (x100)
Optimal CD15 staining of the Hodgkin lymphoma using same protocol as in Fig 4a. The Reed-Sternberg and Hodgkin cells show a moderate membranous staining reaction and a strong dot-like positivity. Also, compare with Fig. 5b.

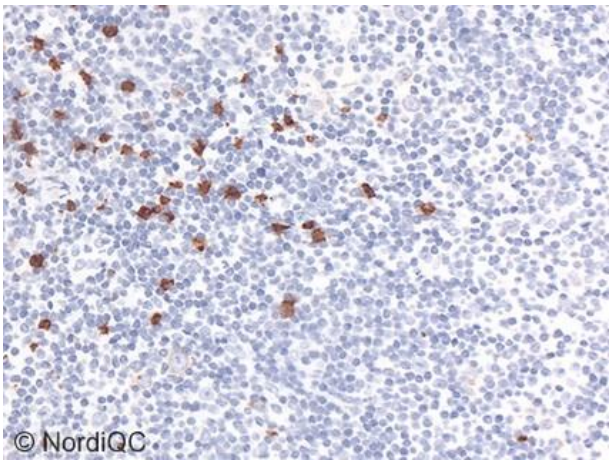


Fig. 5b (x200)
Insufficient CD15 staining of the Hodgkin lymphoma using same protocol as in Fig 4b. The vast majority of Reed-Sternberg cells and Hodgkin cells are false negative and only the neutrophil granulocytes are demonstrated. Also, compare with Fig. 5a.

TJ/LE/SN 13.04.21