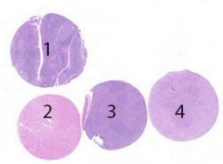
Assessment Run 42 2014
CD15

Material
The slide to be stained for CD15 comprised:

1. Tonsil 2. Kidney 3. Hodgkin lymphoma, classical lymphocyte rich 4. Hodgkin Lymphoma, classical nodular sclerosing (NS).



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CD15 staining as optimal included:

- An at least weak but distinct predominantly membranous staining reaction of follicular dendritic cells in the germinal centres of the tonsil.
- A moderate to strong predominantly membranous staining reaction of the epithelial cells lining the renal proximal tubules.
- 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 four specimens.
- No or only minimal background reaction

Participation

Number of laboratories registered for CD15, run 42	259
Number of laboratories returning slides	239 (92%)

Results
Of the 239 participating laboratories, 1 laboratory used an inappropriate antibody. 79% (n=188) of the remaining participants achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Insufficient HIER (too short heating time or too low temperature)
- Inappropriate primary Ab (mAb clone BY87)
- Use of biotin based detection systems (providing low sensitivity and specificity)

Performance history
This was the fifth NordiQC assessment of CD15. The overall pass rate was improved slightly compared to run 25, 2009 (see table 2).

Table 2. **Proportion of sufficient results for CD15 in the five NordiQC runs performed**

	Run 10 2004	Run 14 2005	Run 22 2008	Run 25 2009	Run 42 2014
Participants, n=	71	84	112	121	238
Sufficient results	50%	61%	66%	76%	79%

Conclusion
The mAbs clones **Carb-3**, **MMA and HI98** could all be used to obtain optimal staining results for CD15. Irrespective of the clone applied, efficient HIER, use of sensitive non-biotin based 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 and Ventana provided the highest proportion of sufficient and optimal results.
Kidney is recommended as positive tissue control: Virtually all epithelial cells lining the renal proximal tubules must show a strong predominantly membranous reaction. Supportive to kidney tonsil can be used as positive and negative tissue control. The majority of follicular dendritic cells in the germinal centres 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 42**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **Carb-3**	53 1 1	Dako Zytomed Systems Nordic Biosite	31	16	6	2	85%	89%
mAb clone **MMA**	24 7 3 2	BD Biosciences Cell Marque Thermo/NeoMarkers Immunologic	12	9	8	7	60%	64%
mAb clone **BY87**	7	Leica/Novocastra	0	0	0	7	-	-
mAb clone **HI98**	2	BD Biosciences	1	1	0	0	-	-
mAb clone **MMA+BY87**	2	Biocare	0	0	2	0	-	-
mAb clone **C3D-1**	1	Dako	0	0	0	1	-	-
mAb **BRA4F1**	1	BioGenex	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **Carb-3 IS/IR062**	49	Dako	38	9	1	1	96%	100%
mAb clone **Carb-3 GA062**	4	Dako	3	1	0	0	-	-
mAb clone **Carb-3 MSG005**	1	Zytomed Systems	0	0	0	1	**-**	**-**
mAb clone **MMA 760-2504**	70	Ventana	46	17	6	1	90%	90 %
mAb **MMA MAD-005151QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **MMA 115M-18**	1	Cell Marque	0	1	0	0	-	-
mAb clone **MMA PDM 127**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone **Carb-1 PA0039**	4	Leica/Novocastra	0	1	1	2	-	-
mAb clone **MMA+BY87 PM073 AA**	2	Biocare	0	1	0	1	-	-
mAb **BRAF4F1 AM302-5M**	1	BioGenex	0	0	0	1		
Total	238		132	56	24	26	-	
Proportion			55%	24%	10%	11%	79%	

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

Detailed analysis of CD15, Run 42

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) (8/10)*, TRS pH 9 (Dako) (4/5), Cell Conditioning 1 (CC1; Ventana) (7/20), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (5/5), Bond Epitope Retrieval Solution1 (BERS1; Leica) (2/2), Diva Decloaker pH 6.2 (Biocare) (2/2) or Tris-EDTA/EGTA pH 9 (3/6) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 40 of 45 (89%) 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 Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/3), TRS pH 9 (Dako) (1/1), CC1 (Ventana) (5/12), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/8) or Tris-EDTA/EGTA pH 9 (3/8) 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 16 of 25 (64%) laboratories produced a sufficient staining result.

mAb clone **HI98**: One protocol with an optimal result was based on HIER using CC1 (Ventana) (1/2) as retrieval buffer. The mAb was diluted 1:20.

Table 3. **Proportion of optimal results for CD15 for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **Carb-3**	11/15** (73%)	0/1	7/20 (35%)	-	5/5 (100%)	2/2
mAb clone **MMA**	2/4**	-	5/12 (42%)	-	2/8 (25%)	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, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 20-30 min. at 97-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 19 of 19 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **Carb-3**, product no. **GA062**, Dako, 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 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) as detection system. Using these protocol settings 4 of 4 (100%) laboratories produced a sufficient staining result.

mAb clone **MMA**, product no. **760-2504**, Ventana, BenchMark XT/Ultra:
Protocols with optimal result were typically based on HIER using Cell Conditioning 1 (efficient heating time 32-64 min.), 16-64 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection systems. Using these protocol settings 45 of 50 (90%) laboratories produced a sufficient staining result.

mAb clone **MMA,** product no. **MAD-005151QD**, LabVision Autostainer 720:
One protocol with an optimal result was based on HIER using EDTA/EGTA pH 8 (efficient heating time 20 min.), 15 min. incubation of the primary Ab and Quanto Detection Kit (MAD-021881QK) as detection system.

Comments
In this fifth NordiQC assessment for CD15, the prevalent features of an insufficient staining result was a generally too weak or completely false negative staining reaction of the cells expected to be demonstrated. This was observed in 98% of the insufficient results (49 of 50).
Virtually all laboratories were able to demonstrate CD15 in the neutrophil granulocytes (high-level antigen expressors) whereas demonstration of CD15 in neoplastic cells of the two Hodgkin lymphomas was more difficult and required optimal calibrated protocols. Insufficient HIER (too short heating time or too low temperature), inappropriate primary Abs and/or usage of detection systems providing low sensitivity were the main parameters causing a too weak staining reaction for CD15. 10 of the participants (4 %) used a biotin based detection system out of which 4 obtained a sufficient score. In 3 of the insufficient cases, false positive staining of the epithelial cells lining the renal tubules (due to endogenous biotin) and a too weak staining reaction of the Reed-Sternberg cells in the Hodgkin lymphomas was observed simultaneously.

The choice of the primary Ab clone was shown to have a high impact on the final result. Demonstration of CD15 using three different mAbs for CD15 (BY87, MMA and Carb-3) has recently been tested thoroughly using in-house optimized protocols (Røge et al. *Appl Immunnohistochem Mol Morphol* 2014;22:449-458), showing that the mAb BY87 was inferior to the concentrated formats of Carb-3 and MMA. This result is in concordance with the results of this run for CD15, where all protocols (n=7) based on the concentrate of mAb BY87 (Leica/Novocastra) were assessed as poor.

The mAb clone Carb-3 gave the highest proportion of sufficient results, and using the Ab as a concentrate within a laboratory developed (LD) assay, optimal staining could be obtained on all 3 main IHC systems (see table 3). Although the number of participants was low, best performance was achieved on the Leica

platforms (Bond III / Max) as 7 out of 7 (100%) were assessed as optimal regardless of HIER buffer (BERS1 or BERS2) applied.
The Dako Ready-To-Use systems **IS/IR062** or **GA062** based on the mAb clone Carb-3 also provided a high number of sufficient and optimal results. The overall pass rate was 96% (50 of 53) and 77% were assessed as optimal. The Ventana RTU system (760-2504) based on the mAb MMA gave a slightly lower pass rate of 90% (63 of 70) and 66% were evaluated as optimal. Both RTU systems provided a higher pass rate compared to the corresponding pass rates using the concentrates within a LD assay. For both the Dako and Ventana RTU systems, optimal results could be obtained using official protocol recommendations provided by 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 total number of participating laboratories in this run, has increased considerably (97%) compared to the latest assessment (Run 25, 2009). Any firm details to the specific causes for the minor improvement of the pass rate obtained in this run (79% versus 76%) is difficult to elucidate as many laboratories participated for the first time and many laboratories have changed their IHC systems since 2009. However, the extended use of high quality and robust RTU systems for CD15 seems to be one of the central elements. In run 25, 19% of the participants (23 of 121) used one of the above mentioned RTU systems. Compared to this run, 52% (123 of 238) of the participants used the same RTU systems giving a total pass rate of 93% when grouped together. Additionally, the concentrated mAb C3D-1 used by 43 laboratories in run 25 (2009) has been discontinued by the vendor (Dako) and for some of those participants, the shift to the more robust concentrated primary mAb Carb-3 (used by 55 laboratories in this run), may also account for the improvement in pass rate. Only one participant was still using the concentrated mAb C3D-1 and the staining was assessed as poor.

As mentioned in the previously run for CD15 (2009), the main provider of the clone MMA, Becton Dickinson, is to date not CE IVD labelled. Therefore, NordiQC recommends to use the CE IVD registered MMA clone from the two vendors; Ventana or Thermo Scientific / LabVision. Alternatively, change to another IVD labelled clone as Carb-3.

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. In tonsil, follicular dendritic cells of the germinal centres 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.

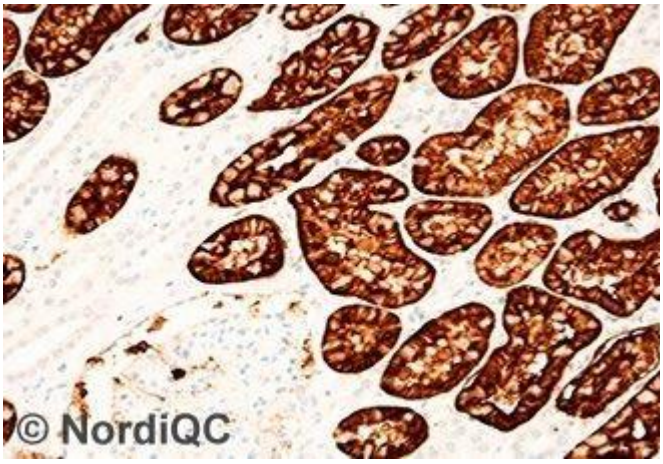


Fig. 1a (x200)
Optimal CD15 staining of the kidney using the mAb clone Carb-3 diluted 1:100, using HIER in an alkaline buffer (CC1 pH 8.5, Ventana) for 48min. at 99°C and a 3-step multimer based detection system (OptiView, Ventana). Virtually all epithelia cells lining the proximal tubules show a strong predominantly membranous but also cytoplasmic staining reaction. Same protocol used in Figs. 2a - 3a.

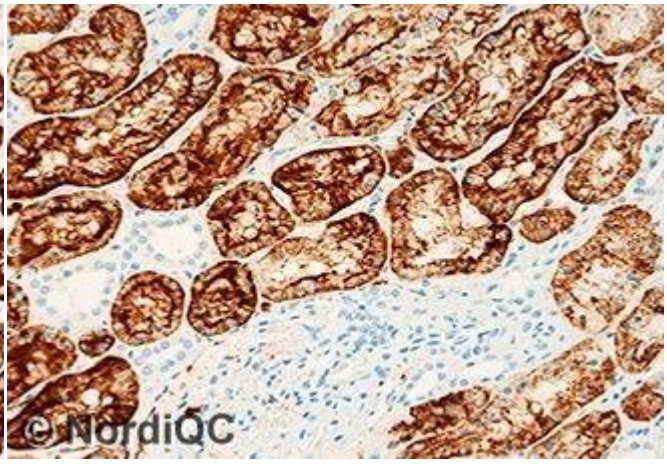


Fig. 1b (x200)
CD15 staining of the kidney using the mAb clone Carb-3 by an insufficient protocol providing a too low sensitivity. Same titer of the primary antibody and detection kit as in Fig. 1a, but a reduced HIER time to 16 min. and temp. to 99°C. The intensity of the staining reaction of the epithelial cells is reduced. Also compare with Figs. 2b – 3b, same protocol.

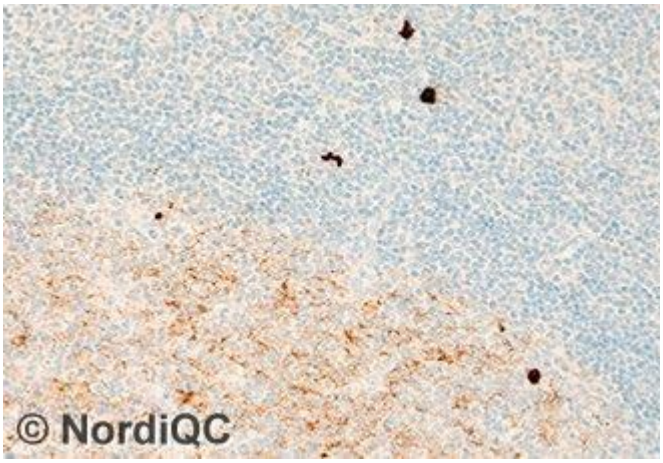


Fig. 2a (x200)
Optimal CD15 staining of the tonsil using same protocol as in Fig. 1a. Neutrophile granulocytes show a strong cytoplasmic reaction whereas the germinal centre dendritic cells show a weak predominately membranous staining reaction - also compare with Fig.3 b.

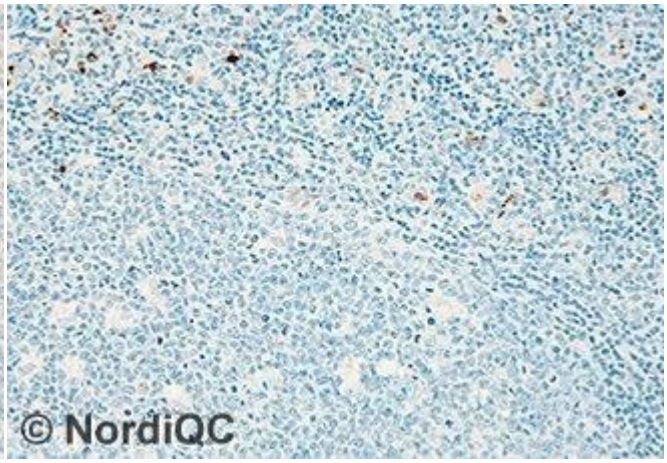


Fig. 2b (x200)
Insufficient CD15 staining of the tonsil using same protocol as in Fig. 1b. The neutrophile granulocytes are demonstrated but the germinal centre dendritic meshwork is barely visible.

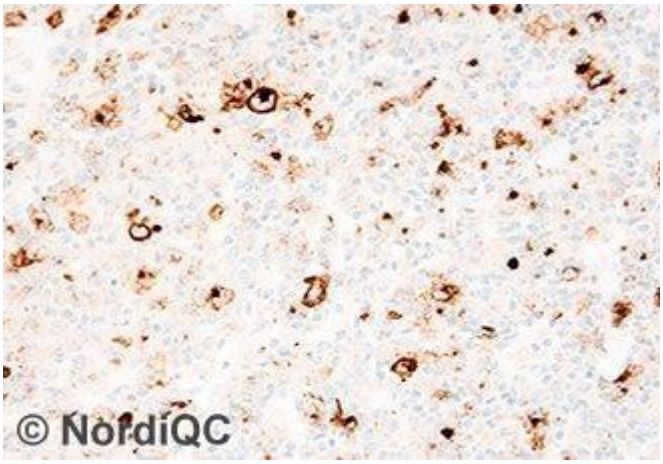


Fig. 3a (x200)
Optimal CD15 staining of the Hodgkin lymphoma, classical type (NS) (core no 4) using same protocol as in Figs. 1a and 2a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

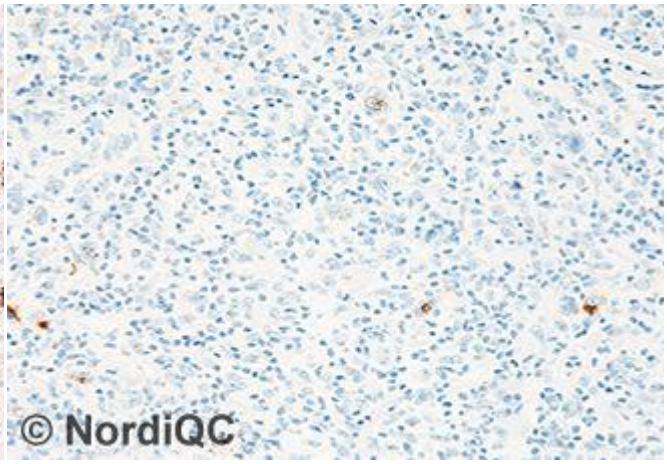
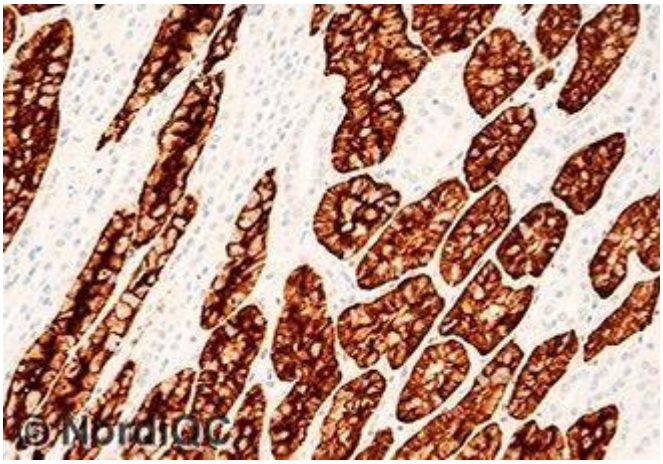


Fig. 2b (x200)
Insuffcient CD15 staining of the Hodgkin lymphoma, classical type (NS) (core no 4) using same protocol as in Figs. 1b and 2b. The vast majority of Reed-Sternberg cells and Hodgkin cells are false negative and only scattered neoplastic cells show a weak staining reaction - compare with Fig. 3a.



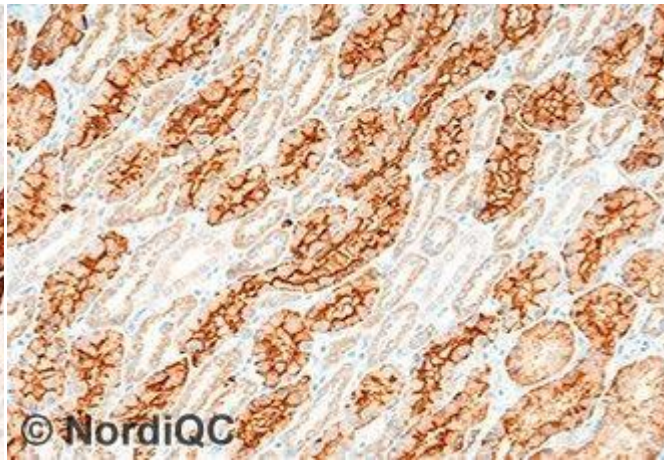


Fig. 4a (x200)
Optimal CD15 staining of the kidney using the mAb clone MMA as RTU format, using HIER in an alkaline buffer (CC1 pH8.5, Ventana) and a 3-step multimer based detection system (OptiView, Ventana). Only the epithelia cells of the proximal tubules are stained whereas the distal and collecting tubules are completely negative – compare with Fig. 4b.

Fig. 4b (x200)
Insufficient CD15 staining of the kidney using the mAb clone MMA as RTU format, using HIER in an alkaline buffer (CC1 pH8.5, Ventana) and a biotin based detection system (i-View, Ventana). The distal and collecting tubules are false positive (a typical granular reaction pattern) due to endogenous biotin. The proximal tubules show a too weak staining intensity – compare with Fig.4a. Kidney cannot be recommended as positive tissue control if a biotin based detection system is used.

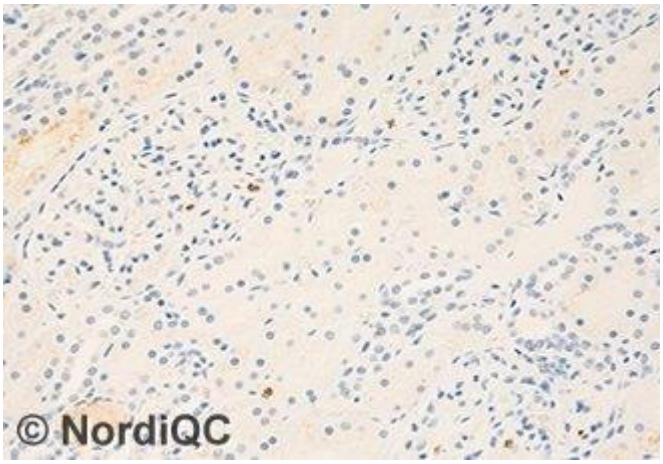


Fig. 5a (x200)
Insufficient CD15 staining of the kidney using the mAb clone BY87, using HIER in an alkaline buffer (BERS2 pH 9, Leica) and a 3-step polymer based detection system (Bond Refine, Leica). The epithelial cells of proximal tubules are false negative. The mAb clone BY87 has consistently provided a too low sensitivity and thus a poor pass rate has been observed in the NordiQC runs for CD15 - also see the staining result of the Hodgkin lymphoma using same protocol in Fig. 5b.

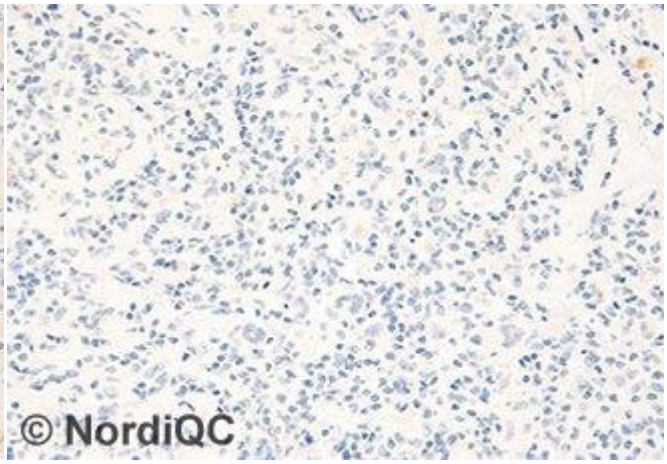


Fig. 5b (x200)
Insufficient CD15 staining of the Hodgkin lymphoma, classical type (NS) (core no 4) using the same protocol settings as in Fig. 5a. The Reed-Sternberg cells and Hodgkin cells are completely negative and only few scattered neutrophile granulocytes show a too weak staining reaction – compare with the optimal result based on the mAb clone Carb-3 in Fig. 3a.

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