Assessment Run 25 2009
CD15

The slide to be stained for CD15 comprised:
1. Kidney, 2-3. Hodgkin lymphoma classical type, NS.
All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD15 staining as optimal included:

- A strong predominantly membranous reaction of the epithelial cells lining the renal proximal tubules.
- A strong and distinct predominantly membranous staining as well as a dot-like (Golgi) staining of the Hodgkin and Reed-Sternberg cells in the two cases of Hodgkin lymphoma.
- A strong cytoplasmic staining of the neutrophils in all three specimens.
- No or only a minimal background reaction.

121 laboratories participated in this assessment. 76 % achieved a sufficient mark. In Table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and scores for CD15, run 25**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MMA**	29 10 1	Becton Dickinson NeoMarkers Cell Margue	21	8	6	5	73 %	74 %
mAb clone **C3D-1**	43	Dako	9	22	5	7	72 %	84 %
mAb clone **Carb-3**	11	Dako	8	1	1	1	82 %	82 %
mAb clone **BY87**	2	Novocastra	0	2	0	0	-	-
mAb clone **H198**	1	Becton Dickinson	1	0	0	0	-	-
mAb clone **MMA+BY87**	1	Biocare Medical	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **MMA**	18	Ventana	3	11	3	1	78 %	86 %
mAb clone **Carb-3**	5	Dako	5	0	0	0	100 %	100 %
Total	121		48	44	15	14	-	-
Proportion			40 %	36 %	12 %	12 %	76 %	81 %

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

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **MMA**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (15/23)*, Cell Conditioning 1 (BenchMark, Ventana) (2/3), Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/4), or Citrate pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:10 – 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 29 out of 39 (74 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **C3D-1**: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (6/22), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (2/7) or EDTA/EGTA pH 8 (1/2). The mAb was diluted in the range of 1:5 – 1:20 depending on the total sensitivity of the protocol employed. Using these protocol settings 31 out of 37 (84 %) laboratories produced a sufficient staining.

mAb clone **Carb-3**: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (5/7), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (1/1), Cell Conditioning 1 (BenchMark, Ventana) (1/2) or EDTA/EGTA pH 8 (1/1). The mAb was typically diluted in the range of 1:50 – 400 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 11 (82 %) laboratories

produced a sufficient staining.

mAb clone **H198**: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Citrate pH 6. The Ab was diluted 1:20.

Ready-To-Use Abs
mAb clone **MMA** (prod. no 760-2504, Ventana): The protocols giving an optimal result were based on HIER in Cell Conditioning 1, standard, an incubation time of 32 min in the primary Ab and UltraView as the detection system. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining.

mAb clone **Carb-3** (prod. no IR062, Dako): The protocols giving an optimal result were all based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex as the detection system. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER – too short heating time or use of citrate pH 6.0
- Inappropriate choice of epitope retrieval – proteolysis, irrespective of the clone applied
- Use of a biotin based detection system complicating the interpretation of kidney as positive control.

In this assessment and in concordance with the three previous runs for CD15, virtually all laboratories were able to detect CD15 in the neutrophil granulocytes, whereas the demonstration of CD15 in the two Hodgkin lymphomas was more challenging and required a higher sensitivity and a correctly calibrated protocol. In order to validate a correct calibration of the immunohistochemical protocol for CD15, kidney was a suitable control. In the optimally calibrated protocols virtually all the epithelial cells of the proximal tubules showed a strong and distinct predominantly membranous reaction, indicating that these cells can serve as a reliable critical staining quality indicator (CSQI) for CD15. However, kidney is only a reliable positive control for CD15, when a non-biotin based detection system is applied, as the non-specific reaction of endogenous biotin in the renal tubules mimics the specific staining reaction, which complicates the interpretation. 19 of the participants (16 %) used a biotin based detection system out of which 8 (42 %) obtained a sufficient score.

Table 2. **Sufficient results with CD15 in four runs**

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

This was the 4th assessment of CD15 in NordiQC. A constant increase of the proportion of sufficient results has been seen, as shown in table 2. Many factors contribute to this increase of sufficient results, but the identification of kidney as CSQI and the tailored recommendations given to the laboratories obtaining an insufficient mark seem to be central for the improvement. From run 10 to run 25, 91 laboratories have been given a recommendation and submitted a staining in the following run. 44 laboratories followed the recommendations and 33 (75 %) improved to a sufficient mark, while 33 did not change their protocol and only 5 (15 %) improved their mark to sufficient. 14 laboratories changed their entire system and 8 of these (57 %) improved their mark to sufficient. The recommendations given were typically: 1. Increase the concentration of the primary Ab, 2. Use HIER with an alkaline buffer, and 3. consider change of the primary Ab. Consequently, the mean concentration used for, e.g., the clone C3D-1 has changed from 1:40 in run 10 2004 to 1:20 in the current run, and the proportion of laboratories using Citrate pH 6.0 has been reduced from 14 % in run 10 to 6 % in the current run.

The choice of Ab clone still seems to be an important parameter to secure a robust immunohistochemical demonstration of CD15. The two clones C3D-1 and MMA are still the most widely used and gave in this assessment almost the same proportion of sufficient results of 72 % and 73 % respectively. However the proportion of optimal results was markedly higher for the clone MMA than the clone C3D-1: 53 % of the protocols based on the clone MMA resulted in an optimal staining versus 21 % for C3D-1 (also see table 1). Clone MMA in a RTU format gave a lower proportion of optimal results than the concentrated format. The newly introduced clone Carb-3 (Dako) seems to be very robust and provided a high number of optimal results both applied in the concentrated format and as RTU.

The main provider of the clone MMA, Becton Dickinson, has not CE IVD labelled their product and only Ventana and LabVision have CE IVD registered their clone MMA. NordiQC highly recommends to use clone MMA as CE IVD labelled or change to another IVD Labelled clone as Carb-3.

Conclusion
The mAbs clones MMA, C3D-1 and Carb-3 are all useful markers for CD15.
HIER preferable in an alkaline buffer seems to be mandatory to have an optimal reaction for CD15. Kidney is recommended as positive control: The epithelial cells lining the renal proximal tubules shall show a strong predominantly membranous reaction and can thus serve as CSQI for CD15, provided that a non-biotin based detection system is used.

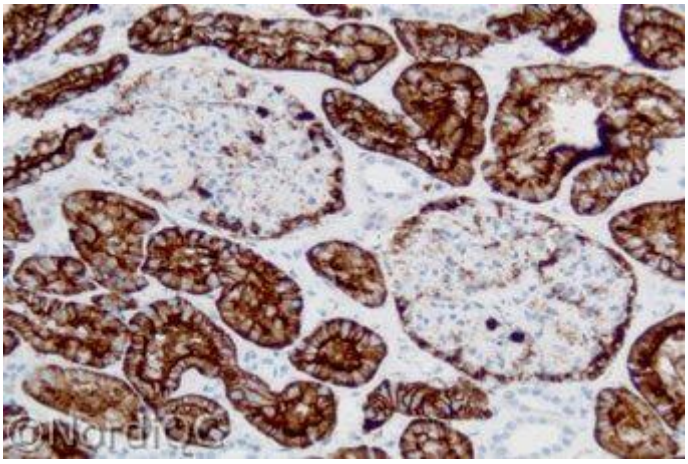

Fig. 1a
Optimal CD15 staining of the kidney using the mAb clone Carb-3. The epithelial cells lining the proximal tubules show a strong predominantly membranous but also cytoplasmic staining in virtually all the cells.

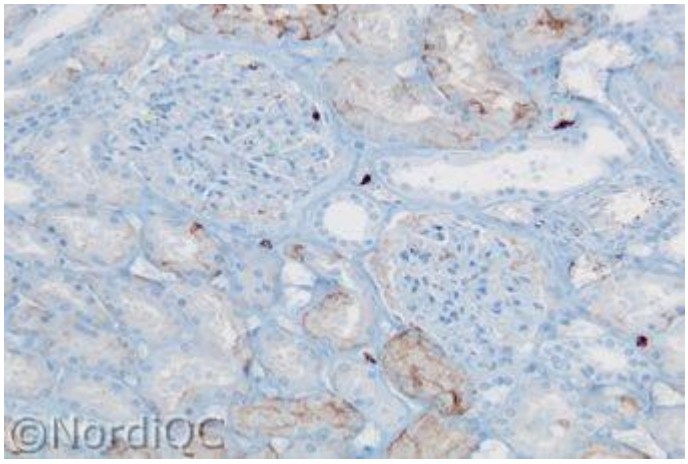

Fig. 1b
Insufficient CD15 staining of the kidney using the mAb too diluted. Only scattered epithelial cells of the proximal tubules show a weak staining. Also compare with Fig. 2b and 3b – same protocol.

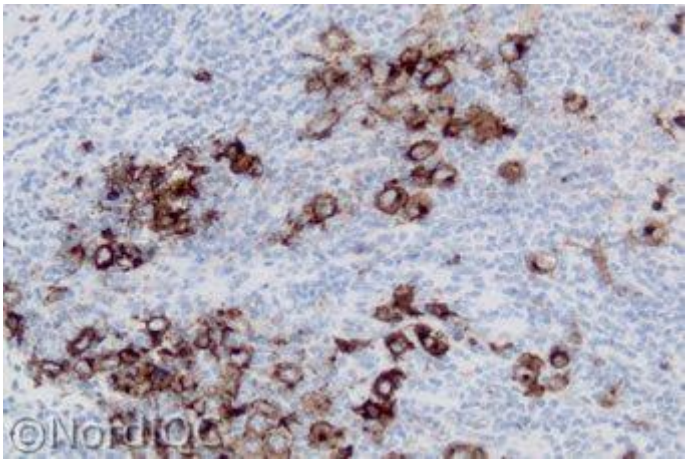

Fig. 2a
Optimal CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin cells show a strong membranous staining and a dot-like positivity.

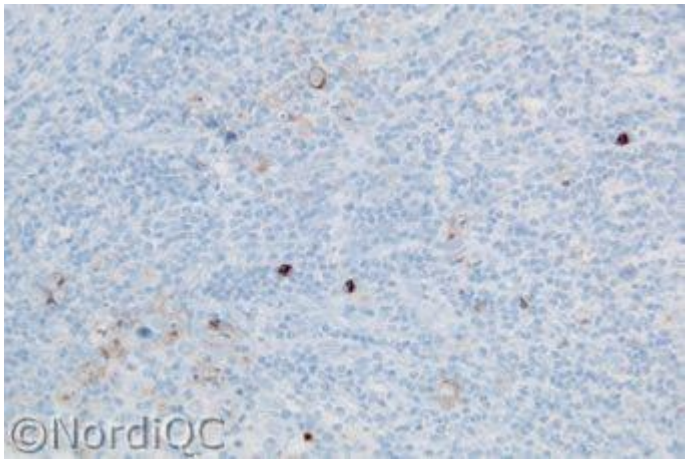

Fig. 2b
CD15 staining of the Hodgkin lymphoma no 2 (NS) using same protocol as in Fig. 1b. Only few Reed-Sternberg and Hodgkin cells show a weak staining - same field as in Fig. 2a.

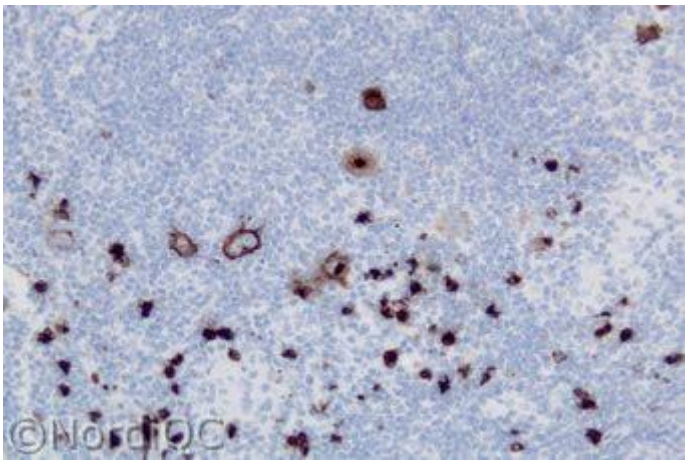

Fig. 3a
Optimal CD15 staining of the Hodgkin lymphoma no 3 (NS) using same protocol as in Fig. 1a & 2a. The scattered Reed-Sternberg and Hodgkin cells show a distinct staining. Also note the strong staining in the granulocytes.

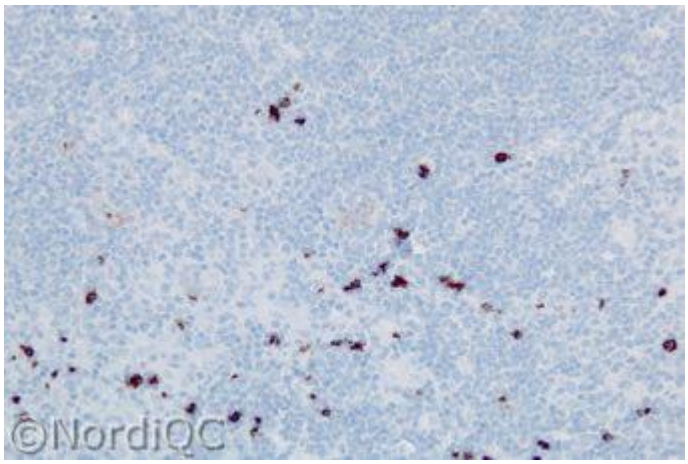

Fig. 3b
Insufficient CD15 staining of the Hodgkin lymphoma no 3 (NS) using same protocol as in Fig. 1b & 2b. The Reed-Sternberg and Hodgkin cells are virtually negative, while the granulocytes show a strong staining – same field as in Fig. 3a.

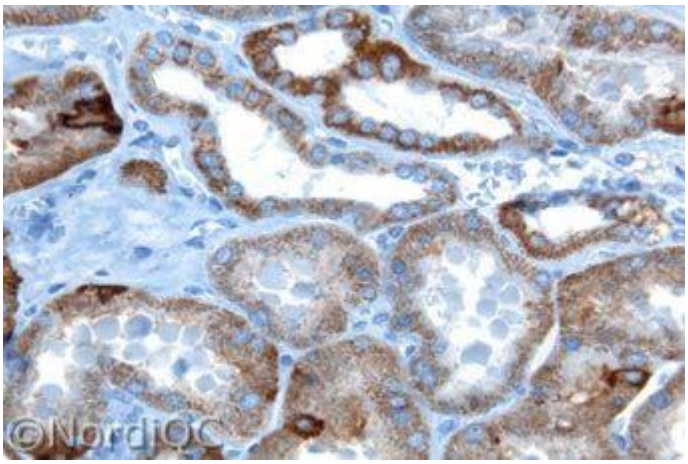

Fig. 4a
Insufficient CD15 staining of the kidney using the mAb clone MMA too diluted, using efficient HIER in an alkaline buffer and a biotin based detection system. The epithelial cells of the proximal tubules are demonstrated but only show a granular cytoplasmic reaction due to endogenous biotin. Also compare with Fig. 4b – same protocol.

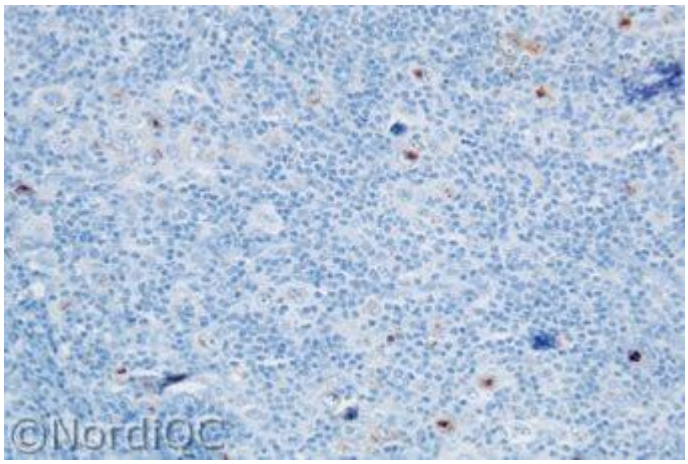

Fig. 4b
CD15 staining of the Hodgkin lymphoma no 2 (NS) using same insufficient protocol as in Fig. 4a. Only few Reed-Sternberg and Hodgkin cells show a weak staining – also compare with Fig. 2a.

SN/MV/LE 6-4-2009