Assessment Run 22 2008
CD15

The slide to be stained for CD15 comprised:
1. Tonsil, 2. Kidney, 3. Hodgkin's lymphoma, NS, 4-5. Hodgkin's lymphomas, MC.
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 all three cases of Hodgkin's lymphoma.
- A strong cytoplasmic staining of the neutrophils in all specimens – a nuclear reaction in the neutrophils was accepted, as this is frequently observed (also described in run 10 2004).

112 laboratories submitted stains. At the assessment 45 achieved optimal marks (40 %), 29 good (26 %), 26 borderline (23 %) and 12 poor marks (11 %).

The following Abs were used:
mAb clone **MMA** (Becton Dickinson, n=32; Ventana, n=15; NeoMarkers, n=3; Cell Marque, n=1)
mAb clone **C3D-1** (Dako, n=51)
mAb clone **BY87** (Novocastra, n=3; Ventana, n=1)
mAb clone **Carb-3** (Dako, n=4)
mAb clone **DT07+BC97** (Biocare Medical, n=1)
mAb clone **H198** (BD Pharmingen, n=1)

Optimal staining for CD15 in this assessment was obtained with the mAbs clone **MMA** (31/51)*, clone **C3D-1** (11/51), clone **Carb-3** (2/4) and clone **H198** (1/1).
* (number of optimal results/number of laboratories using this Ab).

MMA: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (20/23)*, Cell Conditioning1 (BenchMark, Ventana) (5/15)**, Cell Conditioning2 (BenchMark, Ventana) (1/1)*, Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems)(3/4)*, EDTA/EGTA pH 8 (1/2)* or Citrate pH 6 (1/2)*. The mAb was typically diluted in the range of 1:10 – 1:200 depending on the total sensitivity of the protocol employed or as a Ready-To-Use antibody. Using these protocol settings 41 out of 48 (85 %) laboratories produced a sufficient staining (optimal or good).
** (number of optimal results/number of laboratories using this buffer).

C3D-1: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (9/31) or Target Retrieval Solution pH 9 (FLEX TRS high pH, Dako) (2/5)*. The mAb was diluted in the range of 1:5 – 1:25 depending on the total sensitivity of the protocol employed. Using these protocol settings 22 out of 24 (92 %) laboratories produced a sufficient staining (optimal or good).

Carb-3: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (1/1) or Target Retrieval Solution pH 9 (FLEX TRS high pH, Dako, (1/1).The Ab was diluted 1:50 using TE pH 9 or as Ready-To-Use using TRS high pH. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining (both optimal).

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

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER – usage of citrate pH 6.0 as heating buffer with the clone C3D-1
- Inappropriate choice of epitope retrieval – proteolysis, irrespective of the clone applied
- Less successful primary antibody.

In this assessment and in concordance with the previous runs, virtually all laboratories were able to detect CD15

in the neutrophil granulocytes, whereas the demonstration of CD15 in the three Hodgkin's lymphomas was much more difficult and only achieved in the correctly calibrated protocols. In the optimal stain 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 quality indicator for CD15.
It was also observed that the follicular dendritic network could be used as a good quality indicator and this might be preferable to laboratories still using a biotin based detection system (as endogenous biotin in the renal tubules mimic the true staining reaction, complicating the interpretation). However the CD15 reaction in dendritic cells has neither been validated by NordiQC nor described in the literature, and the reaction has to be investigated further.

This was the third assessment of CD15 in NordiQC. A constant increase of the proportion of sufficient results has been seen, as shown in table 1:

Table 1	Run 10 2004	Run 14 2005	Run 22 2008
Participants, n=	71	84	112
Sufficient results	50%	61%	66%

Many factors may contribute to this increase of sufficient results, but especially the identification of a reliable quality indicator as kidney facilities a correct calibration of the immunohistochemical protocol for CD15. Also the tailored recommendations giving to the laboratories obtaining an insufficient mark seem to have an impact: From run 10 to run 22, 60 laboratories have been given a recommendation and send in a staining in the following run. 30 laboratories followed the recommendations and 22 (73%) improved to optimal or good. 20 did not change their protocol and only 3 (15%) improved their result. 10 laboratories changed their entire system of which 4 laboratories (40%) improved. The recommendations given were typically: 1. increase the concentration of the primary Ab, 2. use an alkaline buffer for HIER and 3. consider change of the primary Ab.

The choice of clone seems to be an important parameter. The two clones C3D-1 and MMA have been the most widely used markers throughout the three assessments. MMA has shown to be more robust giving a higher proportion of sufficient results compared to C3D-1, as shown in table 2:

Table 2	Run 10 2004		Run 14 2005		Run 22 2008		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone C3D-1	45	17	46	22	51	25	142	45% (64/142)
mAb clone MMA	18	16	29	25	51	43	98	86% (84/98)

The clone C3D-1 can give an optimal result but the protocol has to be based on a high concentration of the primary Ab and efficient HIER in an alkaline buffer, whereas MMA can be used in a wider range of protocols and still give an optimal result. However, it is striking, that the main provider of the clone MMA, BD, does not have CE IVD labelled their product (in contrast to Ventana and LabVision). NordiQC highly recommends that both manufactors and laboratories comply with the EU directive (98/79/EC) on In Vitro Diagnostic medical devices and products and only apply CE labelled products for diagnostic use.

Conclusion
The mAbs clones MMA (LabVision, Ventana), C3D-1 and Carb-3 are all useful antibodies for CD15.
HIER preferable in an alkaline buffer is mandatory to obtain an optimal reaction for CD15. Kidney is recommended as control: A strong predominantly membranous reaction shall be seen in the epithelial cells lining the renal proximal tubules.

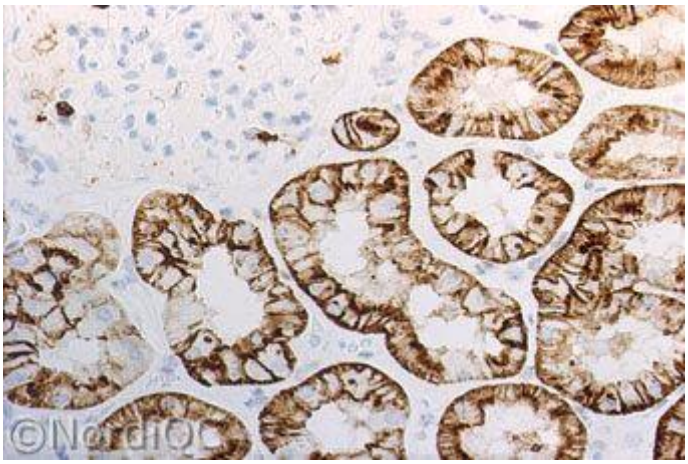

Fig. 1a
Optimal CD15 staining of the kidney using the mAb clone C3D-1. Both the proximal tubules show a strong predominantly membranous but also cytoplasmic staining in all of the cells.

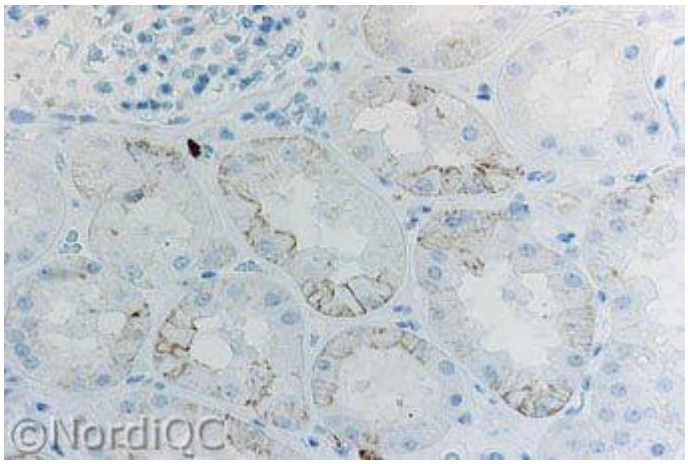

Fig. 1b
Insufficient CD15 staining of the kidney using the mAb clone C3D-1 too diluted. Only scattered cells of the proximal tubules show a weak staining. Also compare with Fig. 2b and 3b – same protocol.

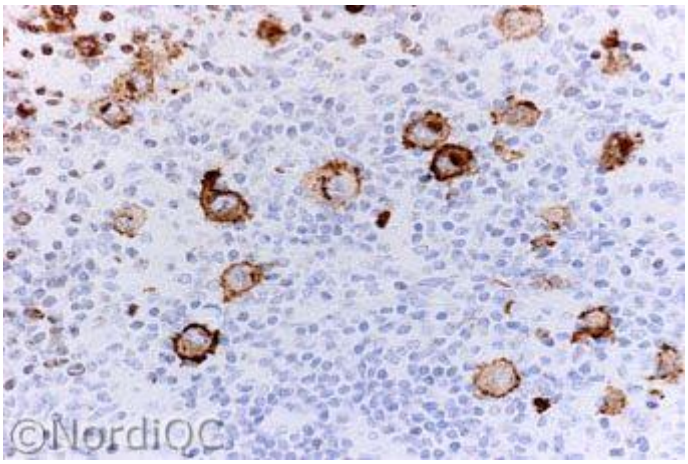

Fig. 2a
Optimal CD15 staining of the Hodgkin's lymphoma no 5 (MC) using same protocol as in Fig. 1a. The Reed-Sternberg and Hodgkin's cells show a strong membranous staining and a dot-like positivity. Also note the focal nuclear reaction of the inflammatory cells in upper right corner.

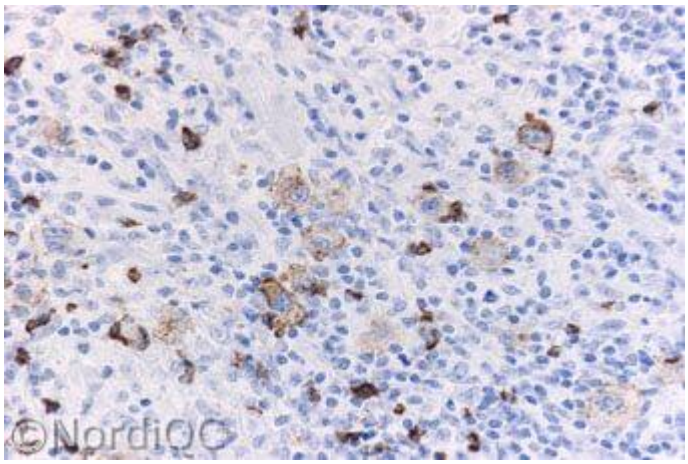

Fig. 2b
CD15 staining of the Hodgkin's lymphoma no 5 (MC) using same protocol as in Fig. 1b. The Reed-Sternberg and Hodgkin's cells are demonstrated, but the reaction is weak and the number reduced compared to Fig. 2a (same field). Also compare with Fig. 3b.

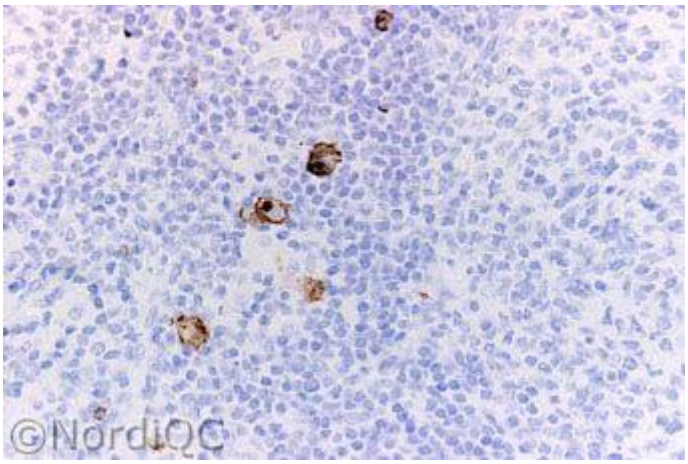

Fig. 3a
Optimal CD15 staining of the Hodgkin's lymphoma no 5 (NS) using same protocol as in Fig. 1a & 2a. The scattered Reed-Sternberg and Hodgkin's cells show a distinct staining.

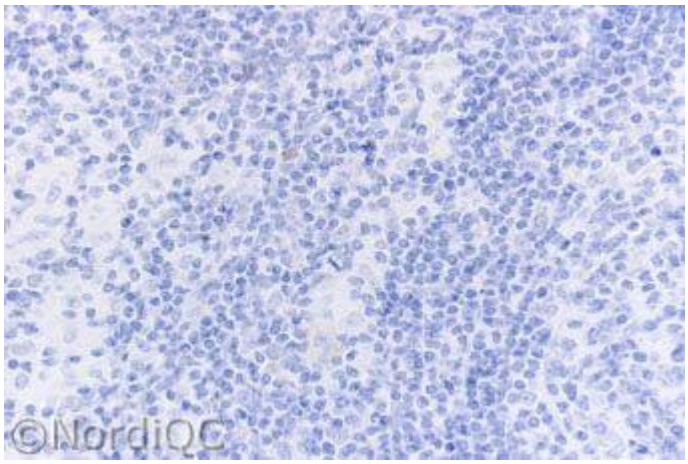

Fig. 3b
Insufficient CD15 staining of the Hodgkin's lymphoma no 5 (NS) using same protocol as in Fig. 1b & 2b. The Reed-Sternberg and Hodgkin's cells are virtually negative – same field as in Fig. 3a.

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