Assessment Run 14 2005
CD15

The slide to be stained for CD15 comprised:
1: Breast, 2: Kidney, 3. Spleen with acute myeloid leukaemia, 4. Lymph node with Hodgkin's lymphoma mixed cellularity (MC) type, 5. Lymph node with Hodgkin's lymphoma nodular sclerosing (NS) type.

Criteria for assessing a CD15 staining as optimal included:

- A strong and distinct predominantly membranous staining as well as dot-like (Golgi) staining of Hodgkin and Reed-Sternberg cells in both cases of Hodgkin's lymphoma
- A strong membranous and cytoplasmic staining of the acute myeloid leukaemia
- A strong predominantly membranous reaction of the breast duct epithelium and renal proximal tubules

84 laboratories submitted stained slides. At the assessment 25 achieved optimal staining (30 %), 26 good (31 %), 15 borderline (18 %) and 18 (21 %) poor staining.

The following Abs were used:
mAb clone **C3D-1** (DakoCytomation, n=46)
mAb clone **MMA** (Becton Dickinson, n=23; Ventana, n=4; NeoMarkers, n=3)
mAb clone **BY87** (Novocastra, n=4; Ventana, n=2)
mAb clone **H198** (Becton Dickinson, n=1)
mAb clone **Tu9** (Quartett Immunodiagnostika, n=1)

Optimal stains in this assessment were obtained only with the clones **MMA** (16 out of 29 were optimal), and **C3D-1** (9 out of 46 were optimal).

With clone **MMA** all optimal protocols was based on HIER using either Tris-EDTA/EGTA pH 9 (15 out of 21 were optimal) or Citrate pH 6 (1 out of 4 was optimal).
With clone **3D-1** all optimal protocols were based on HIER using Tris-EDTA/EGTA pH 9 (9 out of 25 were optimal).
MMA was typically used in the range of 1:10 – 1:50 and C3D-1 in the range of 1:5 – 1:25 depending on the total sensitivity of the protocol used.
The combination of clone **MMA**, diluted in the range of 1:10 – 1:50 and HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining in 15 out of 19 laboratories (79 %), while the combination of clone **C3D-1**, diluted 1:5 – 1:25 and HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining in 9 out of 19 laboratories (47 %).

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Insufficient HIER - too short heating time
- Inappropriate choice of HIER buffer – citrate pH 6 combined with a low total sensitivity of the protocol
- Apparently inappropriate choice of primary Ab

The majority of laboratories were able to detect CD15 in AML and in the neutrophil granulocytes, whereas the demonstration of CD15 in the two Hodgkin's lymphomas was much more difficult and only achieved in the correctly calibrated protocol. In the optimal stain the epithelial cells of the proximal tubules typically showed a strong and distinct predominantly membranous reaction, indicating that these cells may serve as a reliable control – using a biotin based detection system care should be taken in the interpretation as cytoplasmic endogenous biotin mimics the true staining reaction.

CD15 was also assessed in run 10. In that run 71 laboratories participated out of which 50 % (35 laboratories) had an insufficient staining. Each laboratory was given a specific recommendation to improve their protocol. 33 laboratories, which obtained an insufficient result in run 10 submitted a new CD15 stain in run 14. 17 out of the 33 laboratories followed the recommendations, and 12 of these (71 %) improved the assessment from insufficient to either good or optimal. 16 laboratories did not follow the recommendation and only 2 (12 %)

improved.

The overall proportion of insufficient staining was in this run reduced from 50 % in run 10 to 40 %, but as the proportion of insufficient staining still is relative high, CD15 will be repeated in 2006.

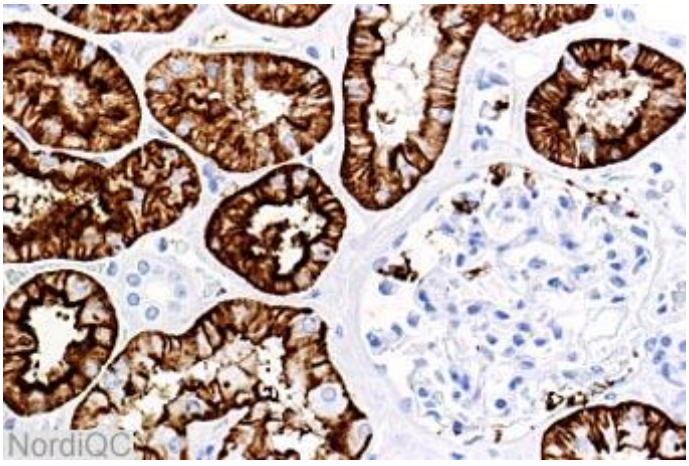

Fig. 1a
Optimal staining for CD15 in the kidney. Both proximal and distal tubules show a strong predominantly membranous but also cytoplasmic staining in all of the cells.

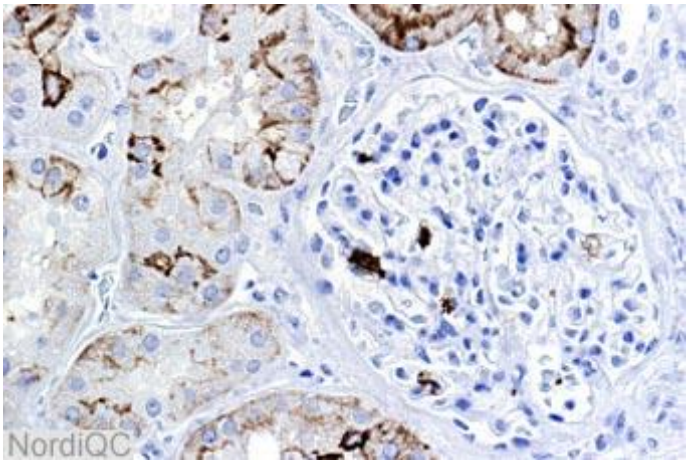

Fig. 1b
Insufficient staining for CD15 in the kidney. The proximal and distal tubules are only focally stained.

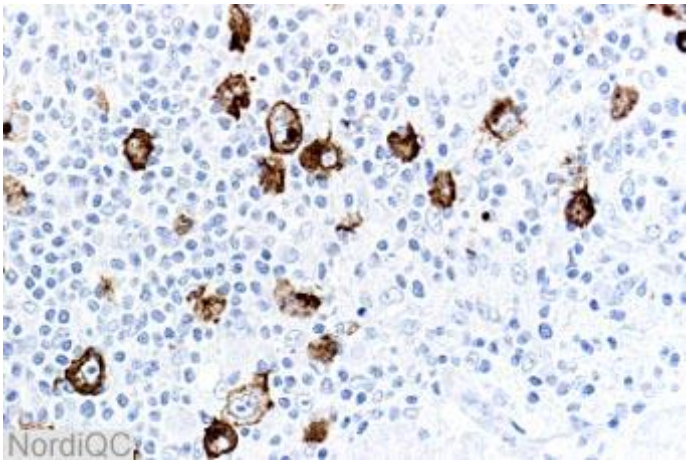

Fig. 2a
Optimal staining for CD15 in the lymph node with Hodgkin's lymphoma (NS). The Reed-Sternberg and Hodgkin's cells show a strong membranous staining and a dot-like positivity.

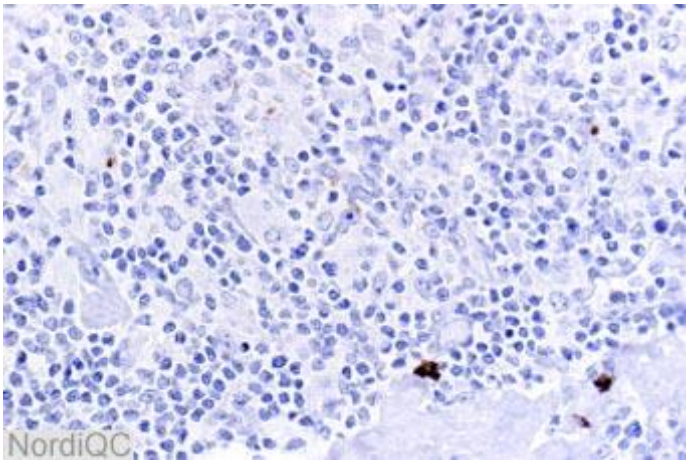

Fig. 2b
Insufficient staining for CD15 in the lymph node with Hodgkin's lymphoma (NS). The Reed-Sternberg and Hodgkin's cells only focally show a weak dot-like positivity.

SN/MV/LE 27-6-2005