Assessment Run 15 2005

Immunoglobulin Lambda (IgL)

The slide to be stained for Immunoglobulin Lambda (IgL) comprised:
1. Chronic lymphatic lymphoma (CLL), IgK positive, 2. Mantle cell lymphoma (MCL), IgL positive, 3. Tonsil fixed 4 h, 4. Tonsil fixed 24 h, 5. Tonsil fixed 72 h. All specimens were fixed in 10 % NBF.

Criteria for assessing an IgL staining as optimal included:

- A strong and distinct membranous reaction of the neoplastic cells in the MCL.
- A strong and distinct membranous staining of approximately half of the normal B-cells in the mantle zone in the tonsils.
- A strong cytoplasmic reaction of plasmacells.
- No staining of the neoplastic cells of the CLL.
- A weak general background staining only.

79 laboratories submitted stains including one in situ hybridization (ISH) stain for IgL mRNA, which was accepted as equivalent to the immunohistochemical demonstration of IgL.

At the assessment 12 achieved an optimal marks (15 %), 15 good (19 %), 9 borderline (11 %) and 43 (55 %) poor marks.

The following Abs were used:
mAb clone **N10/2** (Dako, n=12)
mAb clone **HP6054** (BioGenex, n=1; Zymed, n=1)
mAb clone **LcN-2** (BioCare, n=1)
mAb clone **MH29-2** (CLB, n=1)
pAb **760-2515** (Ventana, n=2)
pAb **A0193** (Dako, n=55)
pAb **A0194** (Dako, n=5)
ISH **780-2844** (Ventana, n=1)

Optimal staining for IgL in this assessment was obtained with the following Abs: pAb **A0193** (11 out of 55) and pAb **A0194** (1 out of 5).

All optimal protocols were based on HIER.

Using the pAb **A0193** both Citrate pH 6.0 and Target Retrieval Solution S1699 (TRS; Dako) (4 out of 6) could be used as HIER buffer: 7 out of 23 and 4 out of 6 gave optimal results, respectively. In the optimal protocols the pAb was typically used in the range of 1:2.000 – 8.000 depending on the sensitivity of the applied protocol for IHC.
Using the pAb **A0194** with citrate pH 6.0 as the HIER buffer gave an optimal result with an Ab dilution of 1:5.000.
The combination of pAb **A0193** in a proper dilution (1:2.000 - 8.000) and HIER in Citrate pH 6.0 or TRS, gave an optimal staining in 11 out of 22 laboratories (50 %).

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Inappropriate epitope retrieval (proteolysis or HIER in an alkaline buffer)
- Less succesful primary Ab.

The prevalent feature of an insufficient staining was a too weak or negative staining of the normal mantle zone B-cells and the MCL. The membranes of the normal IgL positive B-cells should be distinctively demonstrated with only a minimal background reaction in the mantle zone. In almost all slides in which the normal IgL positive B-cells were selectively demonstrated, the protocol could be used for the demonstration of the IgL in the MCL (with

no simultaneous positivity of the CLL).

Virtually all protocols demonstrated the plasma cells. However, the cytoplasmic IgL expression in plasma cells is much stronger than that of normal and neoplastic B-cells. Thus, plasma cells can not be used as control for the demonstration of membranous IgL in lymphomas.

Another feature of the insufficient staining was over staining decorating all mantle zone B-cells. This was most frequently due a too high concentration of the primary Ab, which made it impossible to differentiate between the membranous IgL reaction and an intercellular background reaction of immunoglobulin. In these protocols it was also impossible to demonstrate any certain difference between the CLL and MCL.

Proteolytic pre-treatment could not be used to obtain an optimal staining. Typically the membranes of normal B-cells as well as neoplastic cells were digested causing a false negative reaction for IgL while at the same time enhancing the intercellular background reaction. In general the staining result using proteolytic digestion is very dependent on the fixation time in NBF and might be optimised to the individual specimen, but in a diagnostic setting the fixation conditions are very comparable to the range of the applied fixation times (4 – 72 hours) of the tonsils used in this assessment and the procedure should optimally be applicable to various fixation times.

Using HIER in Citrate pH 6 or TRS an optimal reaction could be obtained in all of the specimens in the multitissue block, indicating that HIER is the preferable pre-treatment for IgL.

Conclusion
In this assessment, pAb **A0193** (Dako) was the most useful Ab for IgL. HIER in Citrate pH 6.0 or TRS was the most appropriate pre-treatment.
The concentration of the primary Ab should be carefully calibrated. Normal tonsil is appropriate control tissue: approximately 50% of the mantle zone B-cells should show a distinct membrane staining reaction, while the rest should be unstained.

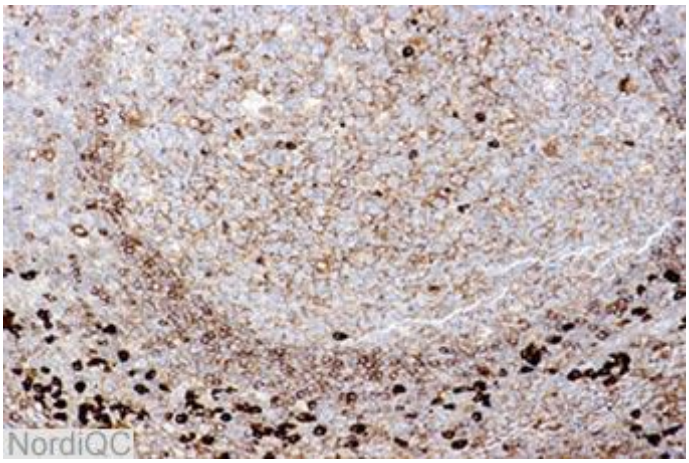

Fig. 1a
Optimal staining for IgL of the tonsil. Low magnification view shows cytoplasmic staining of the plasma cells and - more important - a membrane staining of the mantle zone B-cells.

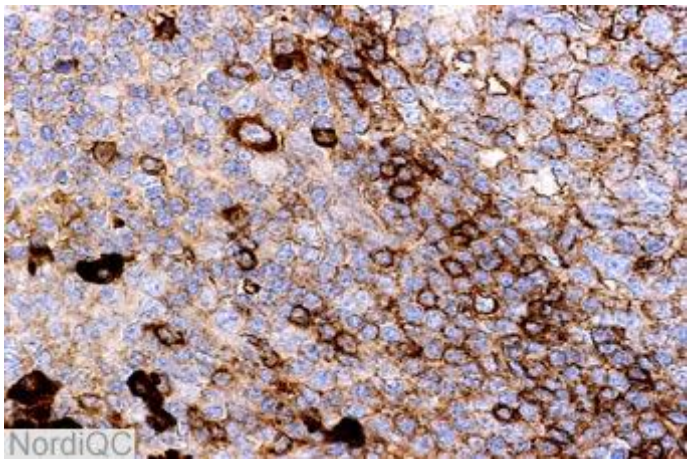

Fig. 1b
Optimal staining for IgL of the tonsil. High magnification view shows a distinct membranous staining reaction of approximately 50 % of the mantle zone B-cells with a minimal background reaction.

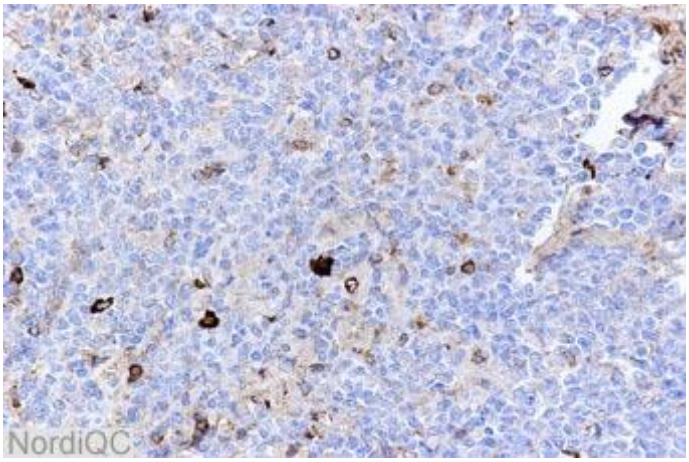

Fig. 1c
Optimal staining for IgL of the CLL. The neoplastic cells are virtually negative. Strong staining of scattered plasma cells, and a weak focal reaction of the macrophages. Same protocol as in Fig. 1a-b.

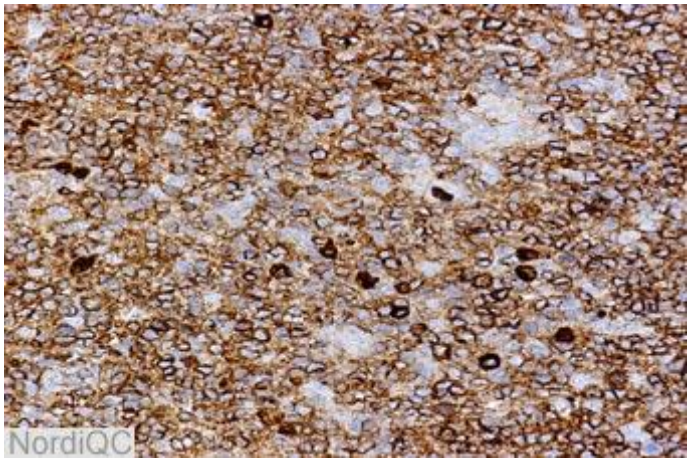

Fig. 1d
Optimal staining for IgL of the MCL. Almost all of the neoplastic cells show a distinct membranous reaction. Same protocol as in Figs. 1a-c.

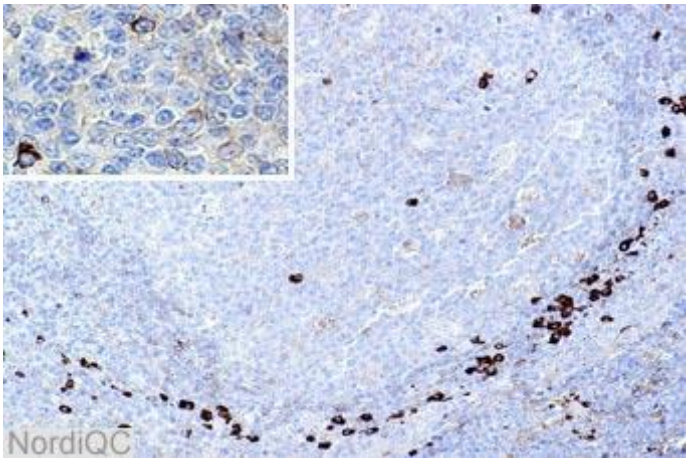

Fig. 2a
Insufficient staining for IgL of the tonsil. Low magnification shows rather strong staining of the plasma cells but only a weak staining of the mantle zone B-cells. Insert: high magnification of the mantle zone.

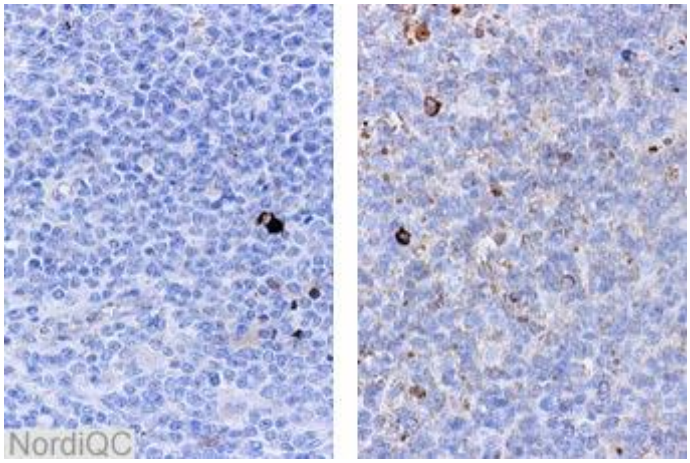

Fig. 2b
Insufficient staining for IgL of the two lymphomas using same protocol as in fig. 2a. Both the CLL (left) and the MCL (right) are negative. The plasma cells are strongly stained.

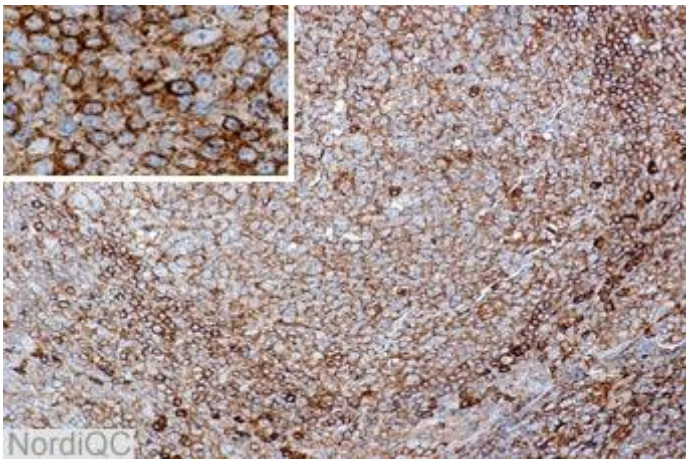

Fig. 3a
Insufficient staining for IgL in the tonsil. Low magnification shows positive reaction of almost all cells. Insert: high magnification of the mantle zone in which all B-cells are stained.

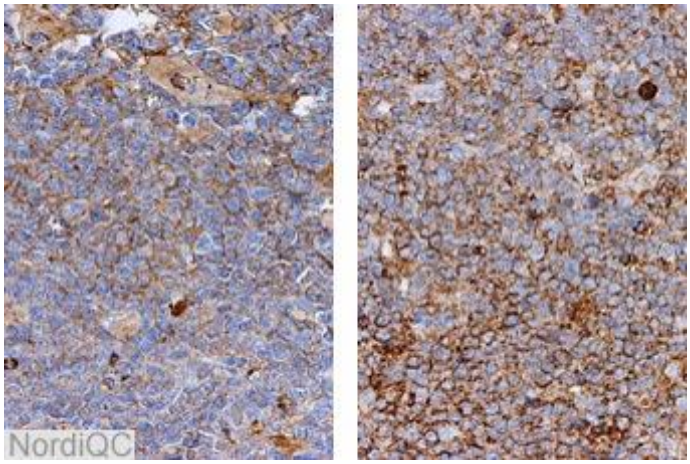

Fig. 3b
Insufficient staining for IgL of the two lymphomas using the same protocol as in fig. 3a. Both the CLL (left) and the MCL (right) appear positive.

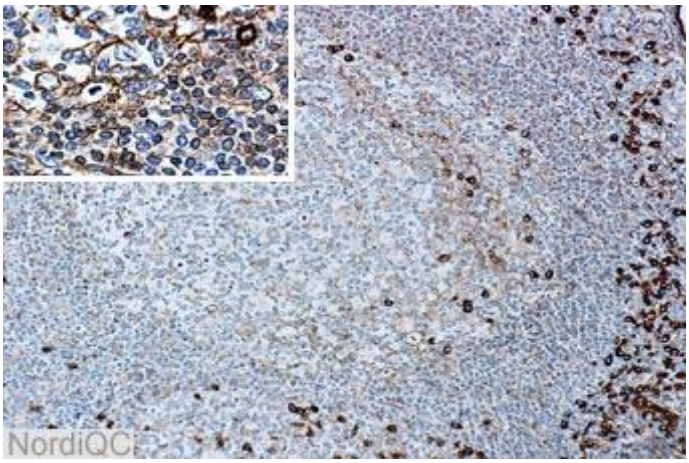

Fig. 4a
Insufficient staining for IgL of the tonsil using proteolytic pre-treatment. The plasma cells are positive but the fragile cytoplasm of the B-cells is over digested causing a too weak staining of the mantle zone B-cells.

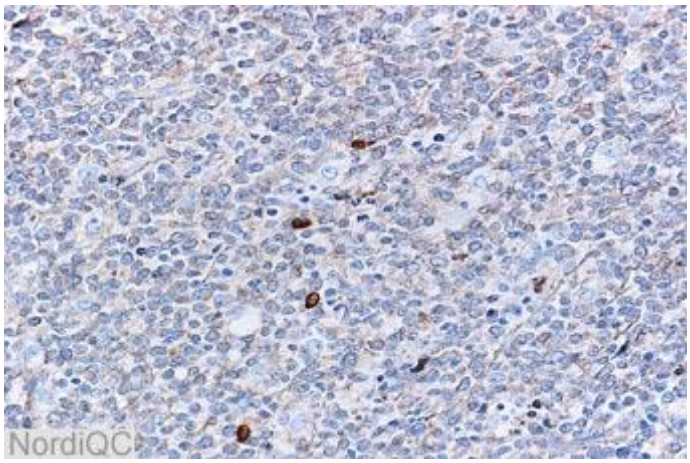

Fig. 4b
False negative staining for IgL of the MCL using the same protocol as in fig. 4a. The cell membranes are over digested giving an "empty" looking slide.

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