Assessment Run 15 2005

Immunoglobulin kappa (IgK)

The slide to be stained for Immunoglobulin Kappa (IgK) 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 IgK staining as optimal included:

- 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 approximately half of the plasma cells.
- A strong and distinct membranous reaction of the majority of the neoplastic cells in the CLL.
- No staining of the neoplastic cells of the MCL.
- A weak general background staining only.

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

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

The following Abs were used:
mAb clone **6E1** (Immunotech, n=1)
mAb clone **A8B5** (Dako, n=5)
mAb clone **HP6053** (Zymed, n=1)
mAb clone **KDB-1** (BioCare, n=1)
mAb clone **MH19-1** (CLB, n=1)
mAb clone **R10-21-F3** (Dako, n=5)
pAb **760-2514** (Ventana, n=2)
pAb **A0191** (Dako, n=55)
pAb **A0192** (Dako, n=2)
ISH **780-2843** (Ventana, n=1)

Optimal staining for IgK in this assessment was obtained with the following Abs: pAb **A0191** (8 out of 55) and pAb **A0192** (1 out of 2).

All optimal protocols were based on HIER.

Using the pAb **A0191** both Citrate pH 6.0 and Target Retrieval Solution S1699 (TRS; Dako) could be used as HIER buffer: 6 out of 19 and 2 out of 8 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 protocol applied.
Using the pAb **A0192** (now discontinued from the vendor) with citrate pH 6.0 as the HIER buffer gave an optimal result with an Ab dilution of 1:10.000.
The combination of pAb **A0191** in a proper dilution (1:2.000 - 8.000) and HIER in Citrate pH 6.0 or TRS gave an optimal staining in 9 out of 21 laboratories (43 %).

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 CLL. The membranes of the normal IgK 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 IgK positive B-

cells were selectively demonstrated, the protocol could be used for the demonstration of the IgK in the CLL (with no simultaneous positivity in the mantle zone lymphoma).

Virtually all protocols demonstrated the plasma cells. However, the cytoplasmic IgK 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 IgK 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 IgK 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 IgK 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 IgK.

Conclusion
In this assessment, pAb **A0191** (Dako) was the most useful Ab for IgK. 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 an appropriate control tissue: approximately 50% of the mantle zone B-cells should show a distinct membrane staining reaction, while the rest should be unstained.

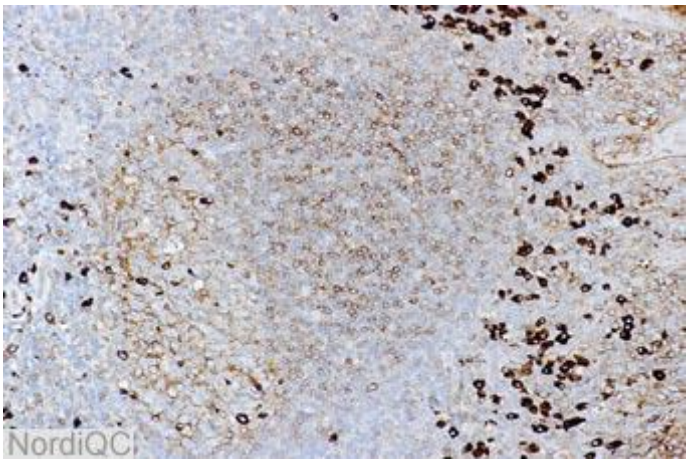

Fig. 1a
Optimal staining for IgK of the tonsil. Low magnification view shows cytoplasmic staining of the plasma cells and - more important - a membrane staining of the mantle zone B-cells.

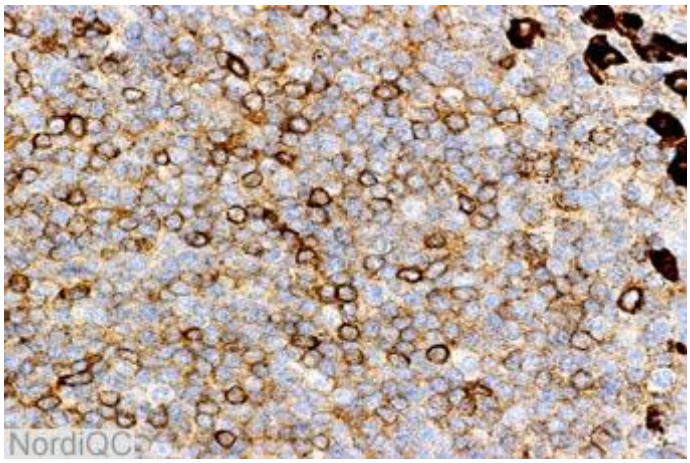

Fig. 1b
Optimal staining for IgK in the tonsil. High magnification view shows a distinct membranous staining reaction of approximately 50 % of the mantle zone B-cells with a minimal background reaction.

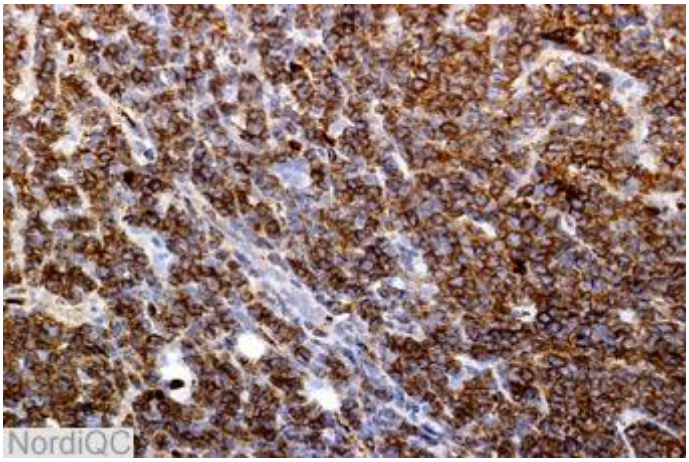

Fig. 1c
Optimal staining for IgK of the CLL. All the neoplastic cells show a distinct membranous reaction.

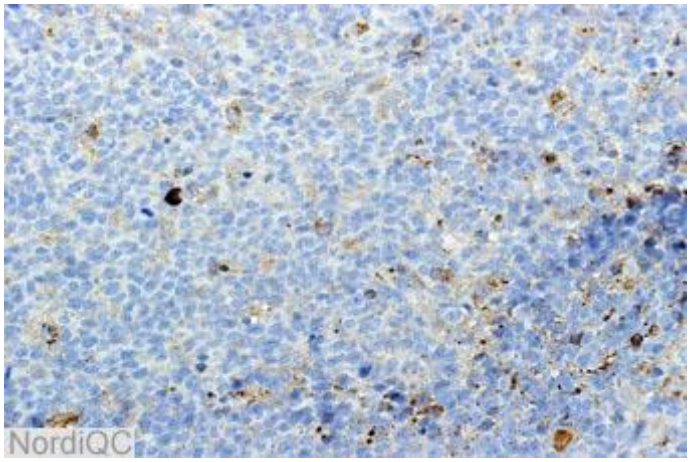

Fig. 1d
Optimal staining for IgK of the MCL. The neoplastic cells are negative. Some plasma cells are strongly stained, while macrophages are weakly stained.

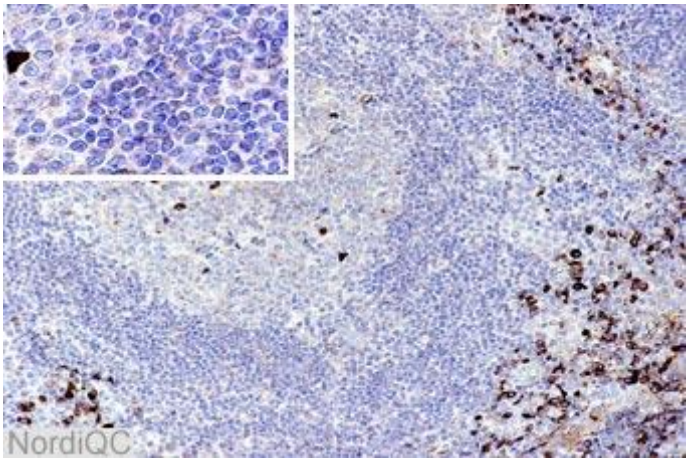

Fig. 2a
Insufficient staining for IgK in the tonsil. Low magnification shows rather strong staining of the plasma cells but only a weak staining of the mantle zone B-cells. Insert: high magnification of the mantle zone.

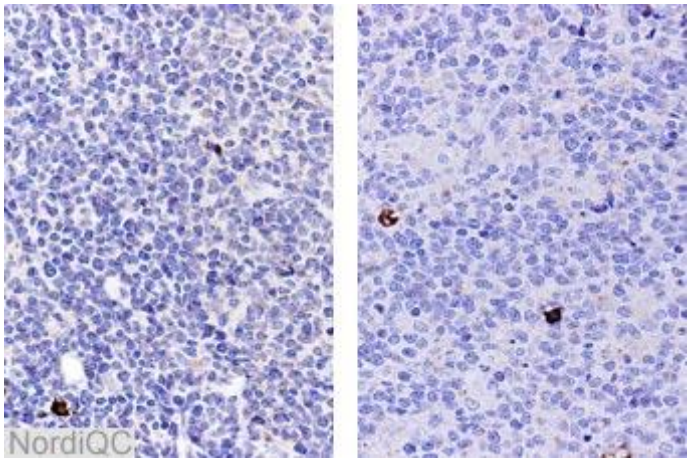

Fig. 2b
Insufficient staining for IgK of the two lymphomas using same protocol as in fig. 2a. The CLL (left) shows a false negative reaction for IgK, that does not allow a differentiation from the MCL (right). Only plasma cells are stained. Compare with Figs. 1c and 1d.

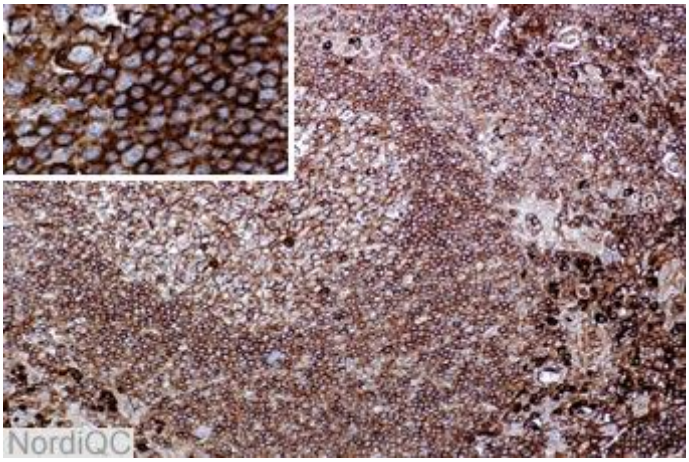

Fig. 3a
Insufficient staining for IgK in the tonsil. Low magnification shows positive reaction of almost all cells. Insert: high magnification of the mantle zone in which all B-cells are stained.

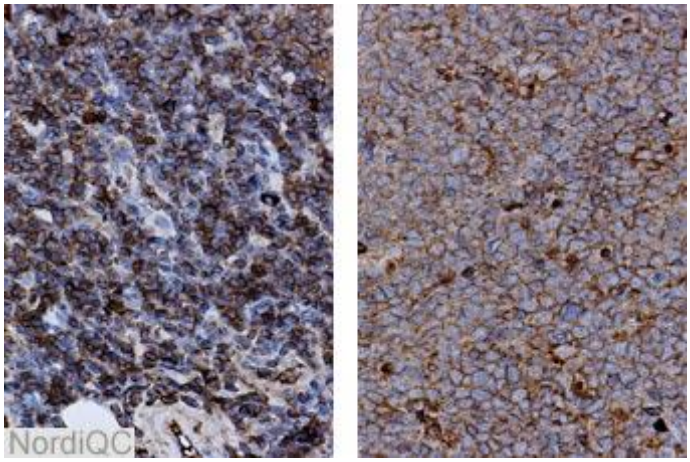

Fig. 3b
Insufficient staining for IgK in the two lymphomas using same protocol as in fig. 3a. The staining of the CLL (left) is difficult to distinguish from the false positive reaction of the MCL (right). Compare with Figs. 1c and 1d.

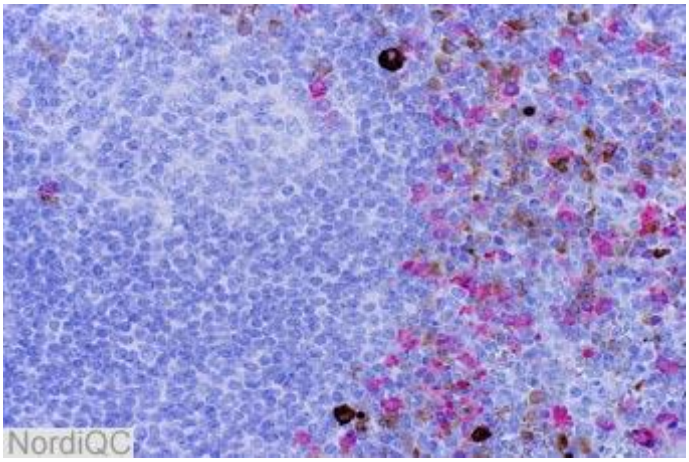

Fig. 4a
Insufficient staining for IgK in the tonsil using a double staining system for IgK and IgL. IgK (brown) and IgL (red) plasma cells are distinctively demonstrated simultaneously. However, the mantle zone B-cells are all negative.

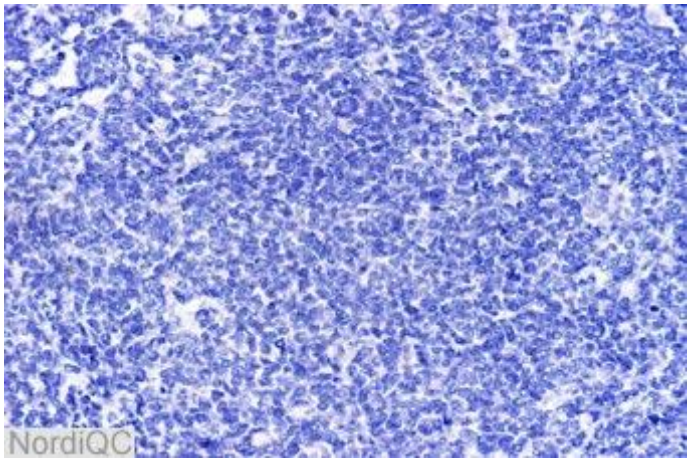

Fig. 4b
Insufficient staining for IgK in the CLL giving a false negative result. Same protocol as in fig. 4a.

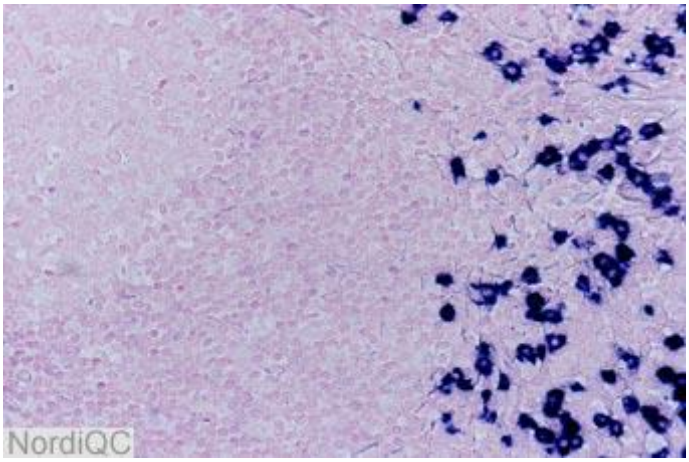

Fig. 5a
Insufficient staining for IgK in the tonsil using an ISH technique. The plasma cells are distinctively demonstrated. However, the mantle zone B-cells are all negative.

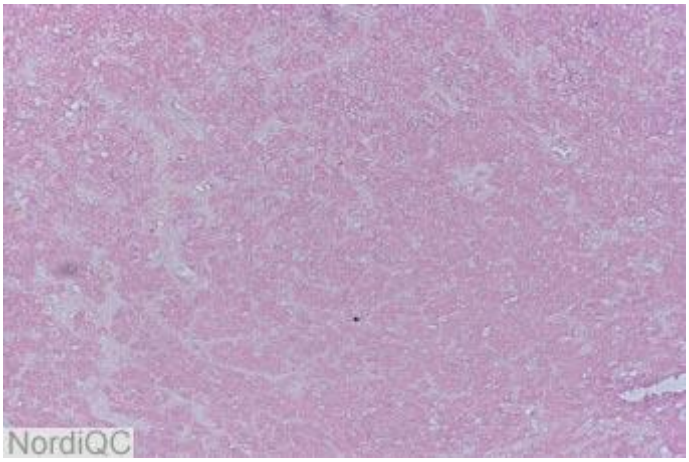

Fig. 5b
Insufficient staining for IgK in the CLL giving a false negative result. Same protocol as in fig. 5a.

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