Assessment Run 18 2006

IgKappa (IgK)

The slide to be stained for membranous IgK comprised:
1. B-Chronic Lymphatic Leukaemia (B-CLL), IgK positive, 2. B-CLL, IgL positive,
3. Tonsil fixed 4 hours, 4. Tonsil fixed 72 hours, 5. Tonsil fixed 168 hours.
All specimens were fixed in 10 % NBF.

Criteria for assessing a membranous IgK staining as optimal included:

- A strong and distinct membranous staining of approximately half of the normal peripheral B-cells in the mantle zones in the tonsils.
- A strong and distinct membranous reaction of the majority of the neoplastic cells in the CLL of IgK subtype (verified by IHC and flow cytometry).
- No staining of the neoplastic cells of the B-CLL of IgL subtype (verified by IHC and flow cytometry).
- A strong cytoplasmic reaction in approximately half of the plasma cells and the immunoblasts in the germinal centres.

A weak background reaction was accepted, as long as the interpretation was not compromised.

80 laboratories participated in the assessment. 11 achieved optimal marks (14 %), 22 good (27 %), 17 borderline (21 %) and 30 (38 %) poor marks.

The following Abs were used:
mAb clone **R10-21-F3** (Dako, n=4)
mAb clone **A8B5** (Dako, n=2)
mAb clone **HP6053** (Zymed, n=1)
mAb clone **kp-53** (Novocastra, n=1)
mAb clone **L1C1** (Biogenex, n=1)
pAb **A0191** (Dako, n=61)
pAb **A0192** (Dako, n=6)
pAb **N1510** (Dako, n=1)
pAb **760-2514** (Ventana, n=2)
pAb **RB-333** (Neomarkers n=1)

Optimal staining for IgK in this assessment was only obtained with the pAb **A0191** (11 out of 61).

All 11 optimal protocols were based on heat induced epitope retrieval (HIER) using either citrate pH 6.0, Target Retrieval Solution pH 6.1 (Dako TRS, S1699/1700) or citraconic anhydride pH 6.0 as the heating buffer. The pAb A0191 was typically used in range of 1:3,000 - 1:12,000 depending on the total sensitivity of the protocol employed.
With the pAb A0191 after HIER in one of the above mentioned buffers and a Ab dilution in the range of 1:3,000 – 12,000, 27 laboratories out of 38 obtained an sufficient mark (72 %), 11 (29 %) were marked as optimal. The most robust and reproducible procedure was based on HIER in TRS pH 6.1 as 11 laboratories out of 14 (78 %) obtained a sufficient mark, whereas using citrate pH 6.0 15 out of 23 laboratories (65 %) had a sufficient mark.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Inappropriate epitope retrieval (proteolytic pre-treatment)
- No pretreatment.

Almost all laboratories were able to demonstrate the IgK in the cytoplasm of the plasma cells and the immunoblasts in the germinal centres, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the membranous IgK in the mantle zone lymphocytes and the B-CLL of IgK subtype. A too weak or false negative staining was seen in 89 % of the insufficient results (43 out of 47).
In 11 % (4 out of 47) a too strong staining was observed giving a false positive staining of IgK in the B-CLL of IgL subtype.

In all the insufficient results due to weak or false negative staining this feature was seen in both the two B-CLLs and in all three tonsil specimens.
In most stains (also when optimal) a non-specific background reaction was observed in the tonsil fixed for 4 hours, whereas in the tonsils fixed for 72 and 168 hours the signal-to-noise ratio was improved and the identification of the IgK positive B-cells facilitated, indicating that a short fixation time in NBF may impede the interpretation of the IgK reactivity. The same feature was noted with IgM.

IgK was also assessed in run 15. In that run 79 laboratories participated out of which 74 % (58 laboratories) obtained an insufficient mark. Each laboratory was given a specific recommendation to improve their protocol. 49 laboratories, which obtained insufficient results in run 15, submitted a new IgK stain in run 18. 22 of the laboratories followed the recommendation and 11 of these improved to either good or optimal marks (50 %). 27 laboratories did not follow the recommendation. Only 2 of these (14 %) obtained a sufficient staining in run 18.

Conclusion:
In this assessment, the pAb A0191 (Dako) was the most useful Ab for the demonstration of membranous IgK. HIER in citrate pH 6.0, Target Retrieval Solution pH 6.1 or citraconic anhydride was the most appropriate pre-treatment methods. The concentration of the primary Ab should be carefully calibrated. Normal tonsil is an appropriate control tissue: approximately half of the peripheral mantle zone B-cells should show a distinct membranous staining reaction for IgK, while the remaining mantle zone B-cells (which are IgL producing) should be unstained.

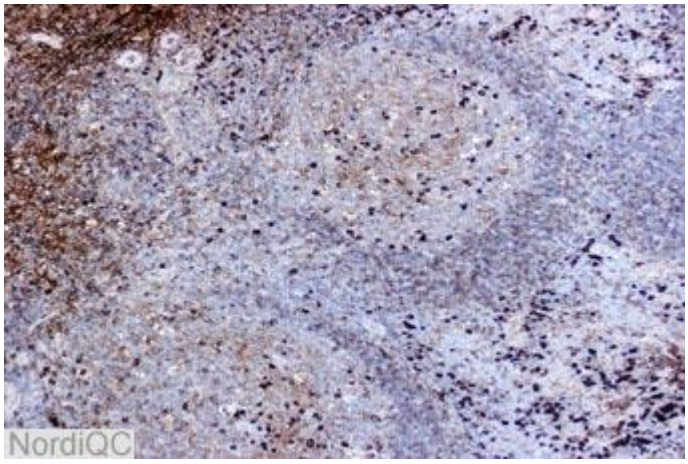

Fig. 1a
Optimal staining for IgK of the tonsil. Even at a low magnification x10 a proportion of the B-cells in the mantle zone of the secondary follicles are demonstrated (compare with Fig. 2a). The protocol was based on the pAb A0191 correctly calibrated and HIER in TRS pH 6.1.

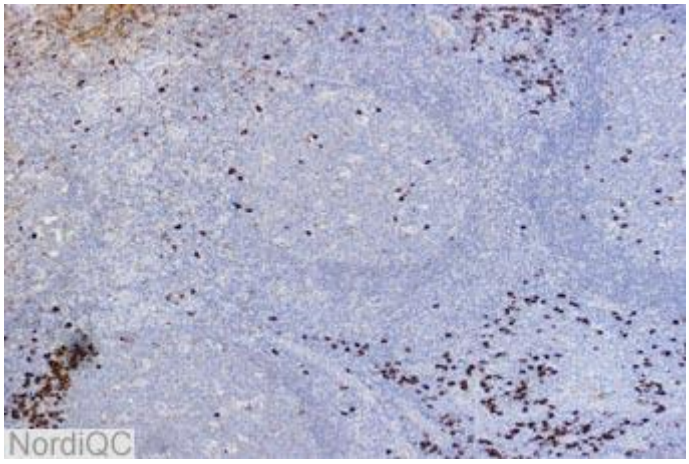

Fig. 1b
Insufficient staining for IgK of the tonsil, same field as in Fig. 1a. At the low magnification x10 the B-cells in the mantle zone of the secondary follicles are negative and only the plasma cells are identified (compare with Fig. 2b). The protocol was based on HIER in citrate pH 6.0 and the pAb A0191 but in a too low concentration.

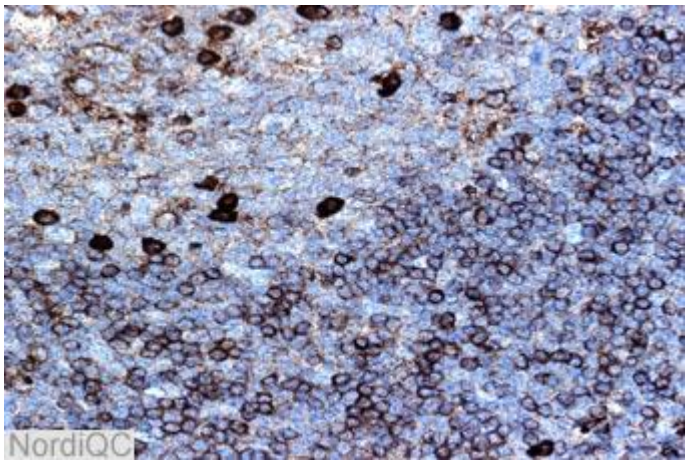

Fig. 2a
High magnification x40 of the optimal staining of a secondary follicle in the tonsil. Approximately 50–60 % of the mantle zone B-cells show a distinct membranous reaction and only a minimal background reaction.

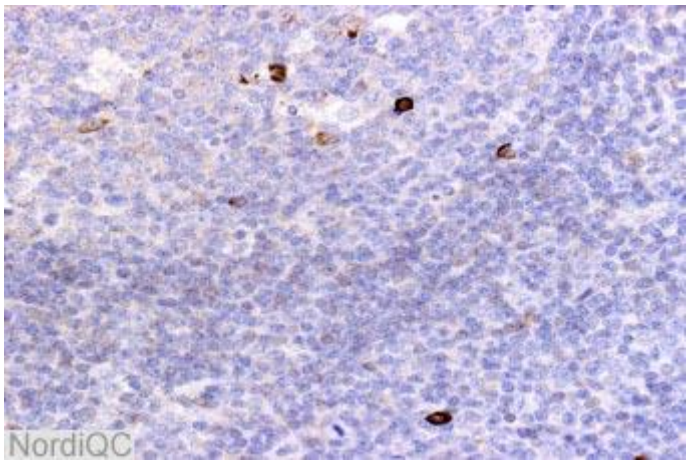

Fig. 2b
High magnification x40 of the insufficient staining of a secondary follicle in the tonsil, same field as in Fig. 2a. Only the plasma cells and immunoblasts are positive, while all the mantle zone B-cells are false negative. Same protocol as in Fig. 1b.

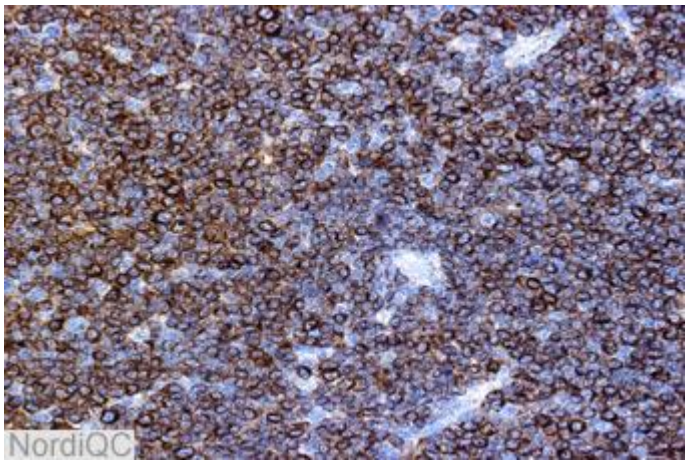

Fig. 3a
Optimal staining for IgK of the IgK positive CLL. All the neoplastic cells show a distinct membranous reaction, same protocol as in Figs. 1a and 2a.

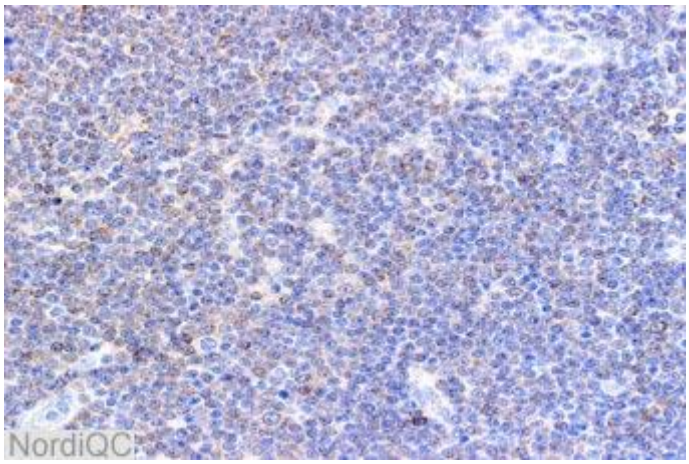

Fig. 3b
Insufficient staining for IgK of the IgK positive CLL. The majority of the neoplastic cells are negative or only weakly demonstrated, same protocol as in Figs. 1b and 2b.

SN/MV/LE 29-11-2006