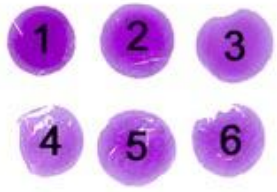
Assessment Run 23 2008

Immunoglobulin Kappa (IgK)

The slide to be stained for membranous Immunoglobulin Kappa (IgK) comprised: 1. B-Chronic lymphatic leukaemia (B-CLL), IgL positive, 2. Follicular lymphoma, IgK positive, 3. B-CLL, IgK positive, 4. Tonsil fixed 24 hours, 5. Tonsil fixed 48 hours, 6. Tonsil fixed 72 hours.
All specimens were fixed in 10 % neutral buffered formalin.



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 follicular lymphoma and 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 (it is not possible totally to eliminate background reaction with any protocol for membranous IgK while keeping an acceptable sensitivity).

91 laboratories submitted stains. At the assessment 11 achieved optimal marks (12 %), 27 good (30 %), 14 borderline (15 %) and 39 poor marks (43 %).

The following Abs were used:
mAb clone **A8B5** (Dako, n=2)
mAb clone **HP6053** (Zymed, n=1)
mAb clone **KDB-1** (Biocare, n=1)
mAb clone **kp-53** (Novocastra, n=2)
mAb clone **L1C1** (NeoMarkers, n=1; Novocastra, n=1)
pAb **760-2514** (Ventana, n=8)
pAb **A0191** (Dako, n=65)
pAb **A0192** (Dako, n=5)
pAb **N1510** (Dako, n=3)
pAb **NCL-KAPp** (Novocastra, n=2)

Optimal staining for IgK in this assessment was only obtained with the pAb **A0191** (11 out of 65).

All 11 optimal protocols were based on heat induced epitope retrieval (HIER) using either citrate pH 6.0 or Target Retrieval Solution pH 6.1 (Dako TRS, S1699/1700) as the heating buffer. The pAb A0191 was typically used in the range of 1:3,000 - 1:16,000 depending on the total sensitivity of the protocol employed.

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.

In this assessment as well as in the previous assessments (runs 15 and 18), almost all laboratories were able to demonstrate cytoplasmic IgK in the plasma cells and the immunoblasts in the germinal centres of the tonsils, whereas the prevalent feature of the insufficient staining was a too weak or false negative membranous staining of the benign mantle zone B-cells and the neoplastic cells of the follicular lymphoma and the B-CLL of IgK subtype. A too weak or false negative staining was seen in 47 out of 53 of the insufficient results (89 %). None of 13 laboratories using proteolytic pre-treatment obtained a sufficient result.

In 6 out of 53 cases (11 %) a too strong staining was observed giving a heavy background staining of IgK compromising the interpretation of the membranous staining.

As observed in the previous assessments for IgK proteolytic pre-treatment could not be used to obtain an optimal staining. Typically the membranes of both normal B-cells and neoplastic cells were digested causing a false negative reaction for IgK while at the same time enhancing the background reaction.
This was the third assessment of IgK. The proportion of sufficient results in the three runs are indicated in Table 1.

Table 1. **Proportion of sufficient results in three runs.**

	Run 15 2005	Run 18 2006	Run 23 2008
Participants, n=	79	80	91
Sufficient results	26%	41%	42%

There are several reasons for this constantly low proportion of sufficient results and the limited impact of specific recommendations to the laboratories with insufficient results. First, continuous use of less successful Abs, in particular mAbs, which in none of the three runs have given an optimal result - irrespective of the protocols applied (Table 2); second, usage of proteolytic pre-treatment for the pAb A0191 (Dako) instead of HIER (Table 3); and third, usage of inappropriate protocol settings for the pAb A0191 (Table 4). Though it cannot be excluded that other Abs than pAb A0191 would be able give optimal IgK stains with better protocols, the datasheets provided with these Abs have apparently not given any solutions.

Table 2. **Proportion of sufficient and optimal results with Abs used for membranous IgK in the three NordiQC assessments.**

	Sufficient	**Sufficient %**	**Optimal**	**Optimal %**
mAb clone **A8B5*)**	0/9	0	0/9	0
mAb clone **HP6053**	0/3	0	0/3	0
mAb clone **KDB-1**	0/2	0	0/2	0
mAb clone **kp-53**	0/2	0	0/3	0
mAb clone **L1C1**	0/3	0	0/3	0
mAb clone **R-10-21F3**	1/9	11	0/9	0
pAb **760-2514**	2/12	17	0/12	0
pAb **A0191**	85/181	47	30/181	17
pAb **A0192**	7/13	54	1/13	8
pAb **N1510**	0/3	0	0/3	0
pAb **NCL-KAPp**	0/2	0	0/2	0

*) Removed from the Dako portfolio before 2005. (Note added 10.12.09 /mv)

Table 3**. Proportion of sufficient results with HIER and proteolytic pre-treatment for the IgK pAb A0191 in the three NordiQC assessments:**

	HIER		**Proteolysis**	
	Sufficient	Optimal	Sufficient	Optimal
pAb A0191	52% (84/161)	19% (30/161)	5% (1/20)	0% (0/20)

Table 4. **Showing the difference in the proportion of sufficient results using pAb A0191 in its optimal protocol settings versus the general protocol settings.**

	All protocols Runs 15, 18 & 23		**Optimal protocol settings* Runs 15, 18 & 23**	
	Sufficient	Optimal	Sufficient	Optimal
pAb A0191	47% (85/181)	17% (30/181)	72% (75/104)	29% (30/104)

* HIER in citrate pH 6.0 or Target Retrieval Solution pH 6.1 (TRS, Dako, S1699/1700) and a dilution of A0191 in the range of 1:2.000 – 16.000.

Irrespective of the Ab and protocol applied for IgK, tonsil should be the preferred control material, in which a strong and distinct membranous staining should be seen in approximately half of the B-cells in the mantle zones. If only the plasma cells and immunoblasts are demonstrated, the protocol cannot be used for lymphoma diagnosis.

Conclusion
In this assessment and in accordance with the previous runs, the pAb A0191 (Dako) was the most useful Ab for the demonstration of membranous IgK. HIER in citrate pH 6.0 or Target Retrieval Solution pH 6.1 was the most robust pre-treatment method. The concentration of the primary Ab should be carefully calibrated. Tonsil is an appropriate control: Approximately half of the peripheral mantle zone B-cells must show a distinct membranous staining reaction for IgK, while the remaining mantle zone B-cells (which are IgL producing) should be unstained.

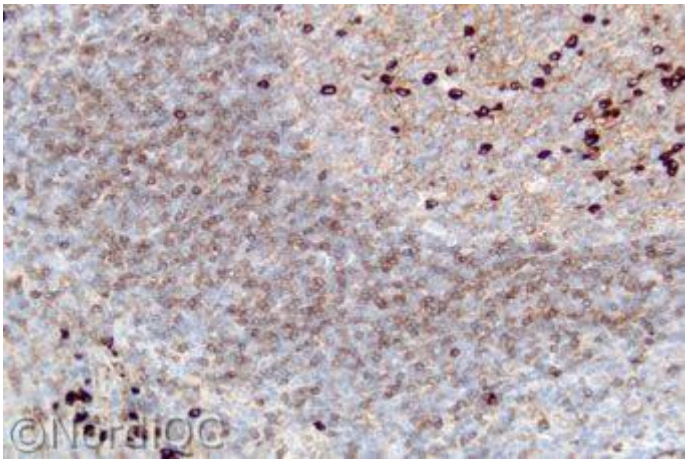


Fig. 1a
Optimal staining for membranous IgK of a secondary follicle in the tonsil using the pAb A0191 with HIER in Target Retrieval Solution low pH 6.1, Dako. Approximately 50–60 % of the mantle zone B-cells show a distinct membranous reaction with only a minimal background reaction. Also compare with Figs. 2a left and right, same protocol.

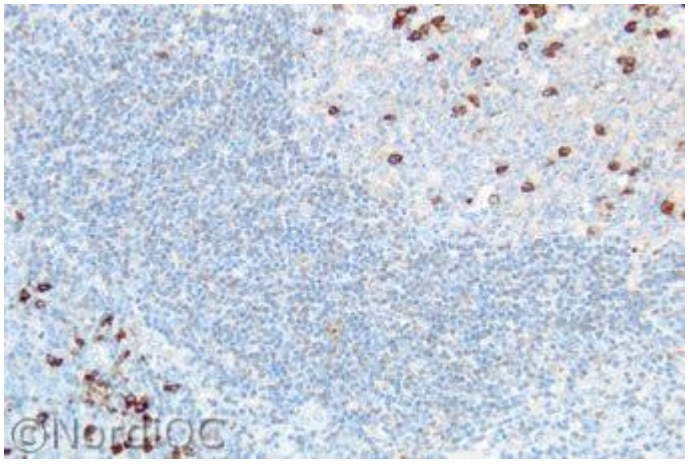


Fig. 1b
Insufficient staining for membranous IgK of a secondary follicle in the tonsil – same field as in Fig. 1a. Only plasma cells and immunoblasts are demonstrated showing a cytoplasmic reaction. Also compare with Figs. 2b left and right, same protocol.

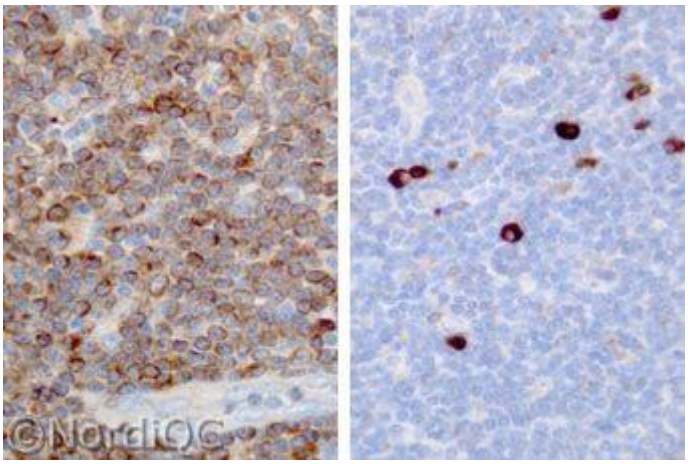


Fig. 2a
Optimal staining for membranous IgK in the two B-CLLs using same protocol as in Fig. 1a.
Left: A distinct staining is seen the majority of the neoplastic cells of the IgK positive B-CLL and only a minimal background reaction is seen.
Right: In the IgL positive B-CLL only plasma cells are stained, while the neoplastic cells are negative.

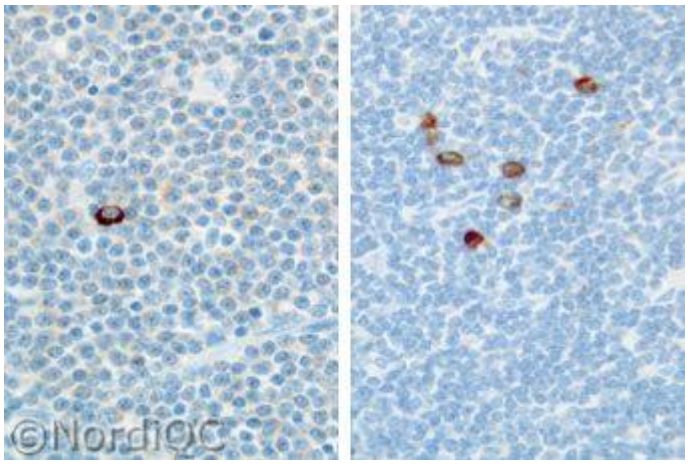


Fig. 2b
Insufficient staining for membranous IgK in the two B-CLLs using same protocol as in Fig. 1b.
Only plasma cells show a cytoplasmic reaction, and it is not possible to determine and differentiate the clonality of the two B-CLLs as seen in Figs 2a.

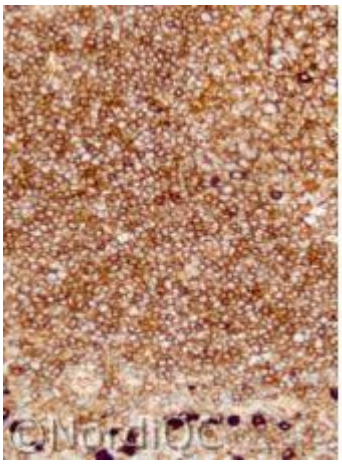
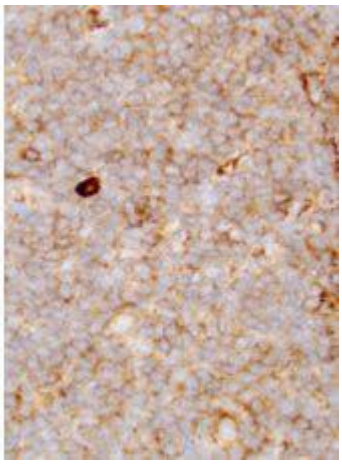



Fig. 3a
Insufficient staining for membranous IgK using the pAb A0191 too concentrated.
Left: In the tonsil a positive reaction is seen in all the mantle zone B-cells.
Right: The IgL positive B-CLL show a false positive staining – also compare with Figs. 2a left and right.

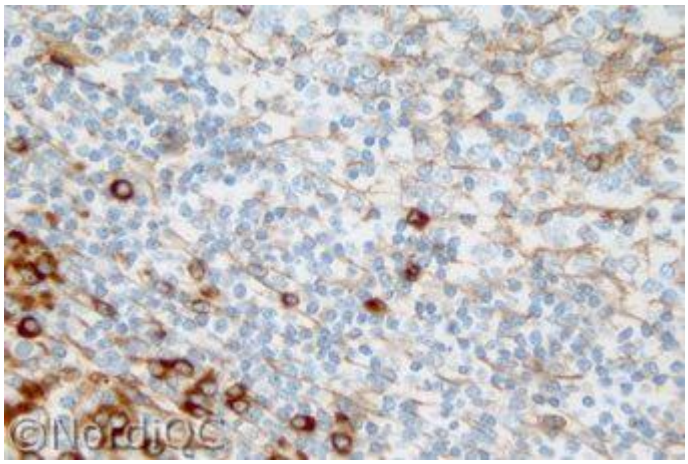


Fig. 3b
Insufficient staining for membranous IgK of the tonsil using the pAb A0191 and proteolytic pre-treatment. The membranes of the mantle zone B-cells are digested giving a false negative reaction and only plasma cells and the follicular dendritic network are demonstrated.

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