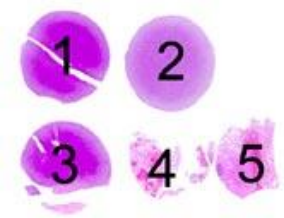
Assessment Run 19 2007
Ki67



The slide to be stained for Ki67 comprised:
1. Chronic lymphocytic leukaemia (B-CLL), 2. Burkitt lymphoma, 3. Tonsil fixed 24h, 4. Tonsil fixed 72h, 5. Tonsil fixed 168h. (unfortunately, 4 and 5 contained in most slides little or no lymphoid tissue). All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Ki67 staining as optimal included:

- A strong nuclear staining in 80-90% of the germinal centre lymphocytes in both the light and the dark zone.
- A strong nuclear staining in the basal and suprabasal squamous epithelial cells of the tonsil
- A strong nuclear staining in 20-30% of the neoplastic cells in the B-CLL and 90-100% of the neoplastic cells in the Burkitt lymphoma.

100 laboratories submitted stains. At the assessment 39 achieved optimal marks (39 %), 34 good (34 %), 19 borderline (19 %) and 8 (8 %) poor marks.

The following Abs were used:
mAb clone **7B11** (Zymed, n=2)
mAb clone **BGX-297** (BioGenex, n=1)
mAb clone **K-2** (Ventana, n=10)
mAb clone **MIB1** (Dako, n=74; Immunotech, n=1)
mAb clone **MM1** (Novocastra, n=4)
rmAb clone **30-9** (Ventana, n=2)
rmAb clone **SP6** (NeoMarkers, n=4)
pAb **A0047** (Dako, n=1)
Unknown (n=1)

Optimal staining for Ki67 in this assessment was obtained with the mAbs clones **K-2**, **MIB1**, and **MM1**, and the rmAb clones **30-9** and **SP6** as follows:

K-2: 8 out of 10 laboratories (80%) produced an optimal stain. Sufficient stains were seen in 9 cases (90%). The optimal protocols were all based on heat induced epitope retrieval (HIER) using Cell Conditioning 1 (CC1 Ventana). Clone K-2 was applied as a Ready-To-Use (RTU) product in all cases.

MIB1: 26 out of 75 laboratories (35%) using the mAb clone MIB1 produced an optimal stain. Sufficient stains were seen in 51 cases (68%). The optimal protocols were all based on HIER using either Tris-EDTA/EGTA pH 9, CC1, Cell Conditioning 2 (CC2 Ventana), Citrate pH 6 or Target Retrieval Solution pH 6.1 (Dako TRS). The Ab could both be applied as a RTU product and as a concentrate diluted in the range of 1:50 – 1:500 (the majority used 1:50 - 1:200) depending on the total sensitivity of the protocol employed. Using MWO (normally 10 - 20 min.) only 10 out of 46 (22%) produced optimal staining, while 4 out of 7 (57%) using a pressure cooker produced optimal staining. Laboratories using alternative ways of HIER (i.e. water bath, incubator, steamer or onboard HIER in immunostainer) were successful in producing optimal staining in 12 out of 22 cases (55%).

MM1: 1 out of 4 laboratories (25%) produced an optimal stain. Sufficient stains were seen in 3 cases (75%). The optimal protocol was based on HIER using citrate pH 6 and a 1:100 dilution of the concentrated Ab.

30-9: 2 out of 2 laboratories (100%) produced an optimal stain. Both protocols were based on HIER using CC1. Clone 30-9 was applied as a Ready-To-Use product.

SP6: 2 out of 4 laboratories (50%) produced an optimal stain. Sufficient stains were seen in 4 (100%). The optimal protocols were based on HIER using either Tris-EDTA/EGTA pH 9 or CC1, the Ab diluted 1:100 – 1:200.

The most frequent causes of an insufficient staining were:
- Too low concentration of the primary Ab
- Insufficient HIER (too short heating time)

The prevalent feature of the insufficient stains were a too weak or false negative reaction of both the B-CLL and the Burkitt lymphoma. All laboratories were able to demonstrate Ki67 in the germinal centre lymphocytes in the tonsil. However, the intensity of the reaction varied from weak to very strong and the proportion of labelled cells varied from 50% to 90%. A very strong reaction in approximately 90% of germinal centre cells could predict sufficient staining of the B-CLL and Burkitt lymphoma. If the germinal centre cells only showed a weak reaction including less than 80-90 % of the cells, the staining result was usually insufficient.
Normal tonsil can be used as an appropriate control tissue. However, to serve as a reliable control and to reduce the proportion of false negative reactions, the germinal centre cells must show a strong nuclear staining and no or only minimal cytoplasmic reaction.

The most frequently used Ab was clone **MIB1** (75%). The fact that MWO heating for "MIB1 retrieval" was found less successful than pressure-cooking and other heating methods, should inspire laboratories with borderline or poor marks to re-validate their MWO protocol for optimal performance. An efficient HIER is mandatory for an optimal result. The positive effect of prolonged MWO heating time is shown in Figs. 4a and 4b.

Conclusion
The mAb clones **K-2**, **MIB1** and **MM1** and the rmAb **30-9** and **SP6** are all useful for the demonstration of Ki67. Efficient HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated. Normal tonsil is an appropriate control tissue provided that 80-90% of the germinal centre cells show a **very** strong nuclear reaction with no or only a faint cytoplasmic reaction.

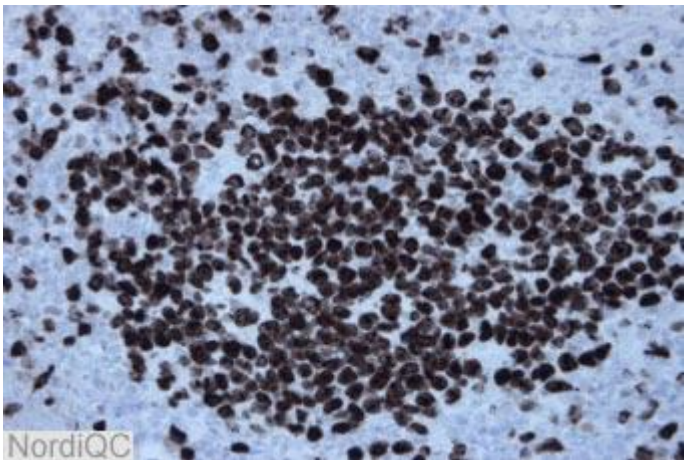


Fig. 1a
Optimal staining for Ki67 in the tonsil. A very strong nuclear staining is seen in 80-90% of the germinal centre lymphocytes.

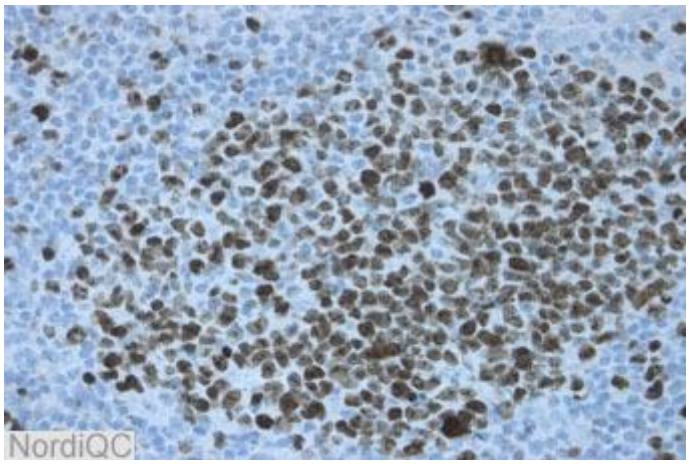


Fig. 1b
Staining for Ki67 in tonsil using an insufficient protocol, same field as in Fig. 1a. Most germinal centre lymphocytes are demonstrated, however compare with Fig. 2b and 3b - same protocol.

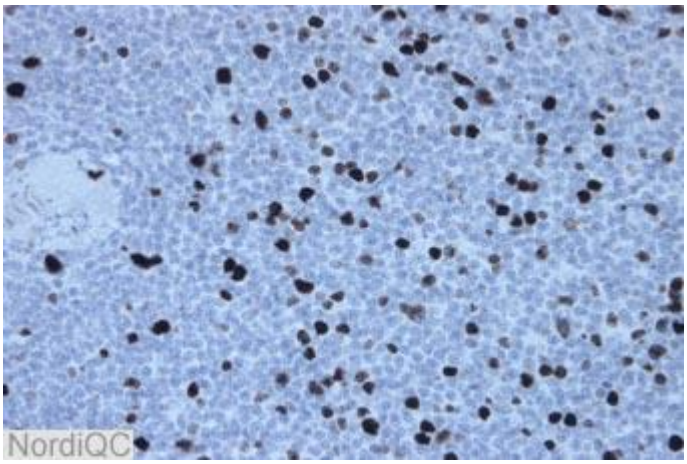


Fig. 2a
Optimal staining for Ki67 in the B-CLL. 20-30% of the neoplastic cells show a distinct strong nuclear staining . Same protocol as Fig. 1a.

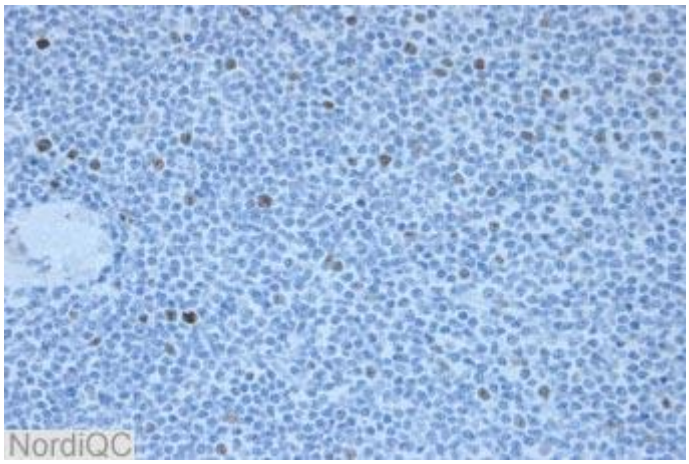


Fig. 2b
Staining for Ki67 in the B-CLL using the same insufficient protocol as in Fig. 1b. Same field as in Fig. 2a. Less than 5% of the neoplastic cells are stained, most of them weakly.

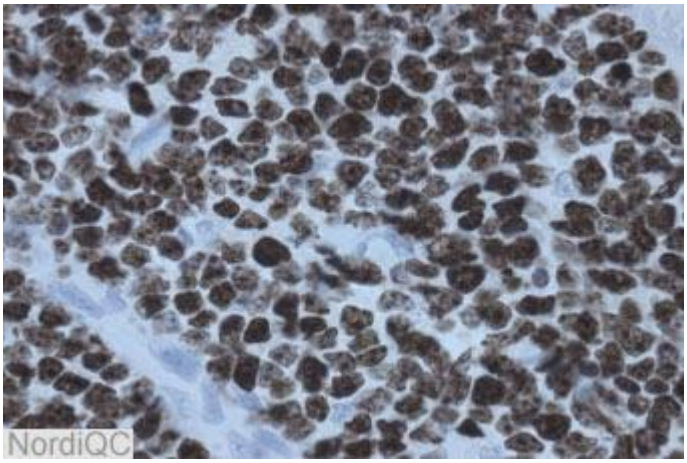


Fig. 3a
Optimal staining for Ki67 in the Burkitt lymphoma. Virtually all the neoplastic cells show a strong nuclear staining. Same protocol as in Fig. 1a and 2a.

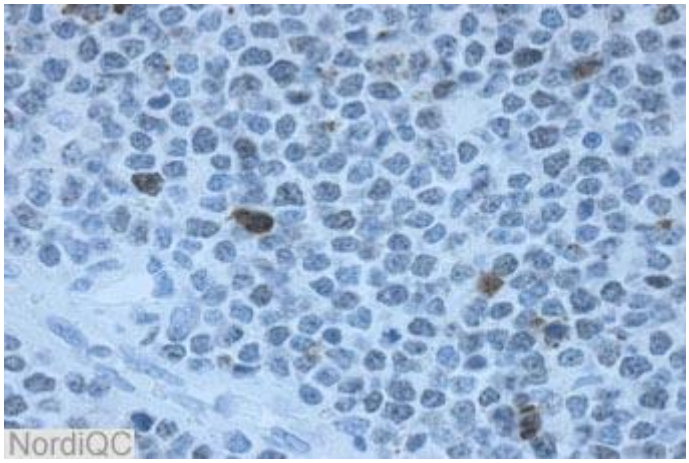


Fig. 3b
Staining for Ki67 in the Burkitt lymphoma using the same insufficient protocol as in Fig. 1b and 2b. Same field as in Fig. 3a. Less than 10% of the neoplastic cells are stained, most of them weakly.

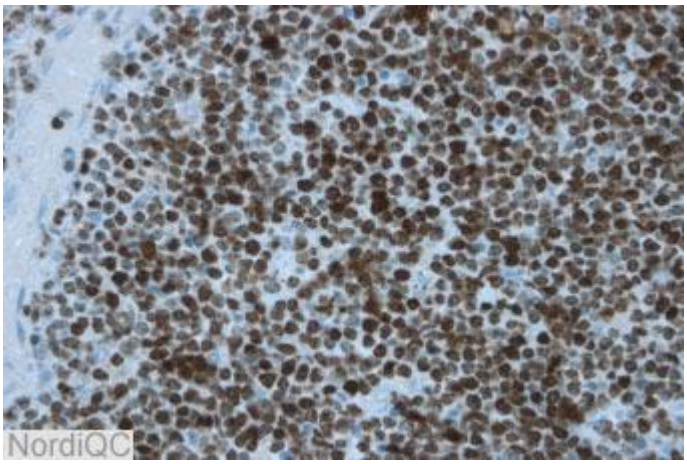


Fig. 4a
Staining for Ki67 in the Burkitt lymphoma. Same MIB1 protocol as in Fig. 4b except for a prolonged MWO heating time, i.e., efficient heating for 30 min in Tris-EGTA, pH 9 (10 min./**30 min.**/15 min.). Compare with Fig. 4b.

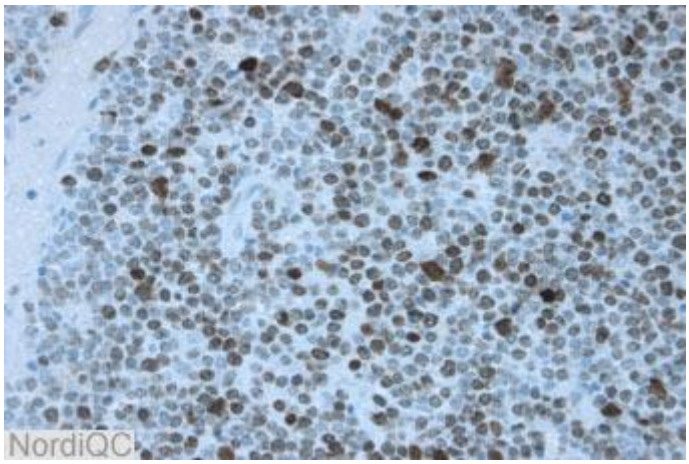


Fig. 4b
Staining for Ki67 in the Burkitt lymphoma. MIB1 protocol using "standard" MWO heating, with an efficient 15 min. heating time in Tris-EGTA pH 9 (10 min./**15 min.**/15 min.). Compare with prolonged MWO heating in Fig. 4a.

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