Assessment Run 18 2006
IgM

The slide to be stained for membranous IgM comprised:
1-2. B-Chronic Lymphatic Leukaemia (B-CLL),
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 IgM staining as optimal included:

- A strong distinct membranous staining of the majority of the mantle zone B-cells in the germinal centres of the tonsils.
- Distinct membraneous/cytoplasmic staining of activated B-cells, centroblasts and centrocytes in the germinal centres of the tonsils.
- A distinct membranous staining of the majority of the neoplastic cells in the two B-CLLs.
- A strong cytoplasmic reaction in all plasma cells and immunoblasts.

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

61 laboratories participated in the assessment. 13 achieved optimal marks (21 %), 6 good (10 %), 12 borderline (20 %) and 30 (49 %) poor marks.

The following Abs were used:
mAb clone **R1/69** (Dako n=5)
mAb clone **1D7-F10** (BioGenex n=1)
pAb **414-01** (Signet n=1)
pAb **760-2654** (Ventana n=3)
pAb **A0091** (Dako n=2)
pAb **A0425** (Dako n=39)
pAb **A0426** (Dako n=6)
pAb **N1509** (Dako n=1)
pAb **NCL-IgMp** (Novocastra n=2)
pAb **RB1434** (NeoMarkers n=1)

Optimal staining for IgM in this assessment was only obtained with the pAb **A0425** (13 out of 39 were optimal).

All the optimal protocols were based on heat induced epitope retrieval (HIER). As HIER buffer both Target Retrieval Solution pH 6.1 (S1699 Dako), Tris-EDTA/EGTA pH 9, Citrate pH 6.0 and Cell Conditioning 1 (CC1, Ventana) could be used to obtain an optimal result. In the optimal protocols the pAb A0425 was used in the range of 1:200 – 1:1,000 depending of the total sensitivity of the protocol employed.

With pAb A0425 after HIER in one of the above mentioned buffers and a primary Ab dilution between 1:200 – 1:1,000, 16 out of 19 obtained an sufficient mark (84 %), of which 13 (68 %) were optimal.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Omission of epitope retrieval
- Proteolytic pre-treatment

In the assessment almost all laboratories were able to demonstrate the IgM 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 IgM of the neoplastic B-cells in the two B-CLL. A too weak or false negative staining was seen in 95 % of the insufficient results (40 out of 42) and in only 5 % (2 out of 42) a too strong staining was observed.

In all the insufficient results, weak or false negative staining of the two B-CLLs were seen in parallel with weak or false negative staining in all three tonsil specimens. If the staining was assessed as too weak, the mantle zone B-cells also showed a too weak membranous staining and if the staining was too strong the background staining in

the interfollicular areas and the squamous epithelium showed a too strong and a false positive staining, respectively.

Normal tonsil seems to a reliable positive control in which virtually all the peripheral mantle zone B-cells shall show a strong distinct membranous reaction with a minimal background reaction in the interfollicular areas (only circulating peripheral B-cells and plasma cells should be demonstrated in these areas).

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 IgK.

Conclusions:

In this assessment, pAb **A0425** (Dako) was the most useful Ab for the demonstration of membranous IgM. HIER was the only appropriate pre-treatment. The concentration of the primary Ab should be carefully calibrated. Normal tonsil is an appropriate control tissue in which the mantle zone B-cells should show a distinct membranous staining with only a minimal background reaction.

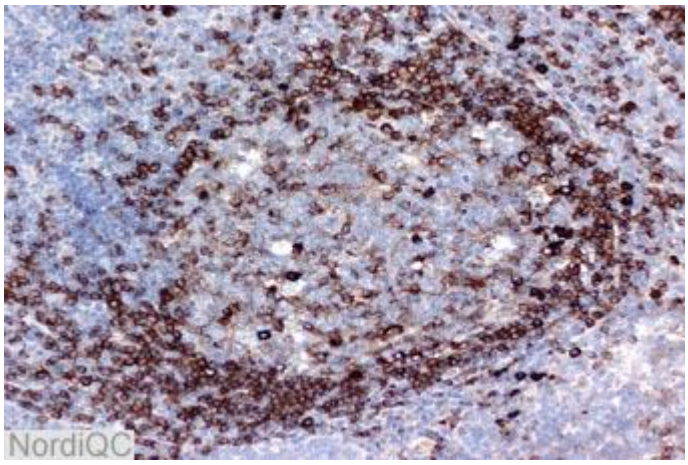

Fig. 1a
Optimal staining for IgM of the tonsil. The mantle zone B-cells show a distinct membranous reaction and in the germinal centre plasma cells and immunoblasts show an intense cytoplasmic reaction. The background is only weak positive.

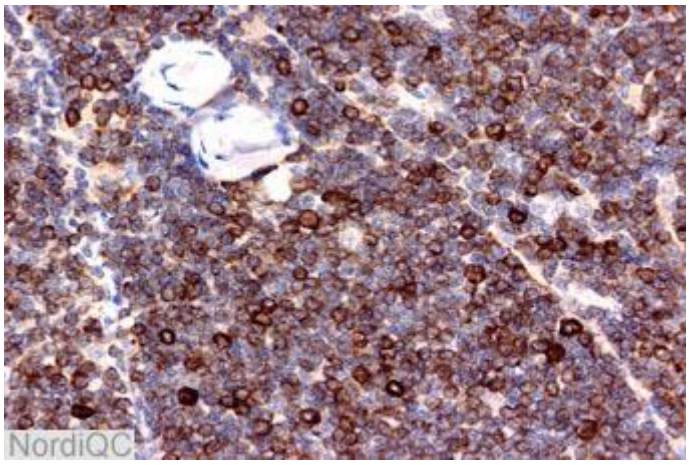

Fig. 1b
Optimal staining for IgM of the B-CLL. Virtually all the neoplastic cells show a distinct moderate to strong membranous reaction. Same protocol as Fig 1a, using the pAb A0425 and HIER.

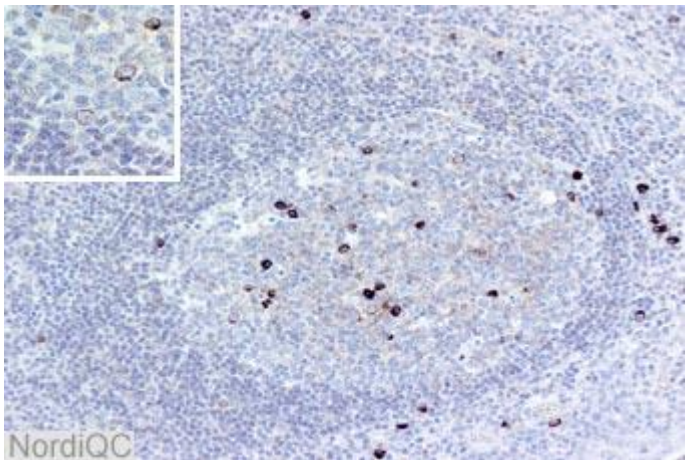

Fig. 2a
Insufficient staining for IgM of the tonsil, same field as Fig 1a. The mantle zone B-cells are negative and only the plasma cells and immunoblasts (insert) show a positive reaction.

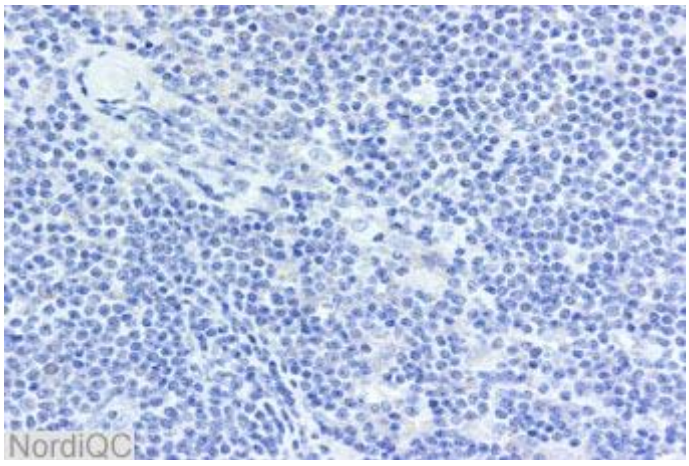

Fig. 2b
Insufficient staining for IgM of the B-CLL, same field as Fig 1b. All the neoplastic cells are false negative. Same protocol as Fig 2a, using the pAb A0425 and HIER, but using a too low concentration of the primary Ab.

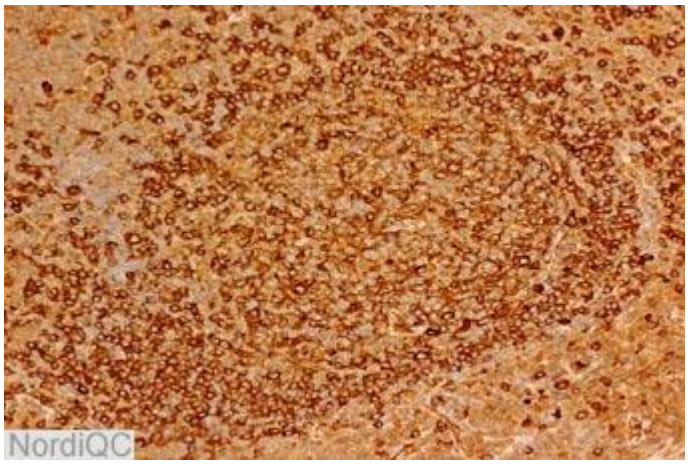

Fig. 3a
Insufficient staining for IgM of the tonsil, same field as Fig 1a. The mantle zone B-cells are strongly stained, but the background reaction, due to a too high concentration of the primary Ab, compromises the interpretation.

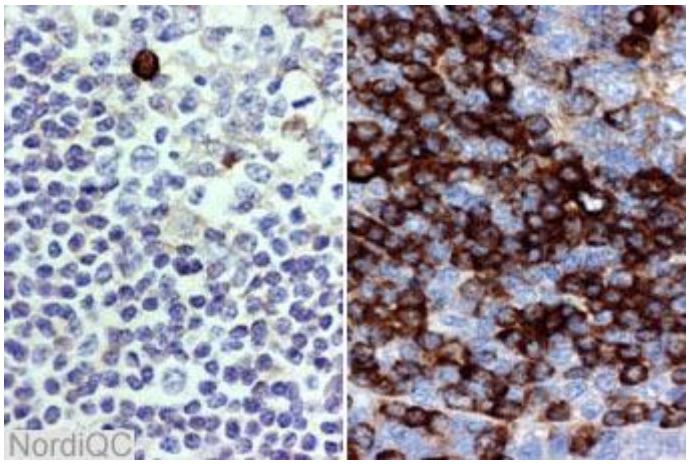

Fig. 3b
Left: Insufficient staining for IgM of the tonsil using the pAb A0425 and proteolytic pretreatment. The membranes of the mantle zone B-cells are digested giving a false negative reaction.
Right: Optimal staining for IgM of the tonsil using the pAb A0425 and HIER, same field as the left photo.

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