Assessment Run 23 2008

Immunoglobulin M (IgM)

The slide to be stained for membranous immunoglobulin M (IgM) comprised:
1. Mantle cell lymphoma, 2. B-Chronic lymphatic leukaemia (B-CLL),
3. Tonsil fixed 24 hours, 4. Tonsil fixed 48 hours, 5. Tonsil fixed 72 hours.
All specimens were fixed in 10 % neutral buffered formalin.

Criteria for assessing a membranous IgM staining as optimal included:

- A strong distinct membranous staining of virtually all the mantle zone B-cells of the germinal centres of the tonsils.
- A distinct membranous staining of the majority of the neoplastic cells in the mantle cell lymphoma and the B-CLL.
- A strong cytoplasmic reaction in plasma cells, immunoblasts and the follicular dendritic network in the germinal centres of the tonsils.

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

80 laboratories submitted stains. At the assessment 15 achieved optimal marks (19 %), 25 good (31 %), 19 borderline (24 %) and 21 poor marks (26 %).

The following Abs were used:
mAb clone **R1/69** (Dako, n=2)
mAb clone **IgM88** (BioGenex, n=1)
mAb clone **RVS-M** (Chemicon, n=1)
pAb **760-2654** (Ventana, n=4)
pAb **A0425** (Dako, n=58)
pAb **A0426** (Dako, n=5)
pAb **N1509** (Dako, n=2)
pAb **A0091** (Dako, n=1)
pAb **P0215** (Dako, n=1)
pAb **P0322** (Dako, n=1)
pAb **NCL-IgMp** (Novocastra, n=3)
pAb **RB-1434** (NeoMarkers, n=1)

Optimal staining for IgM in this assessment was only obtained with the pAb **A0425** (15 out of 58, 26%).

The 15 optimal protocols were based on heat induced epitope retrieval (HIER) - except for one laboratory which used a combination of proteolytic pre-treatment (Proteinase K) and HIER (Citrate pH 6.0). The HIER buffers used in the 14 protocols were Citrate pH 6.0 (5/22)*, Target Retrieval Solution pH 6.1 (Dako TRS, S1699/1700) (7/10)*, Bond Epitope Retrieval Solution 1 (Bond, Leica Microsystems) (1/2)*, Cell Conditioning1 (BenchMark, Ventana) (1/4)* and TRIS-EDTA/EDTA pH 9 (1/12)*.
The pAb A0425 was typically used in the range of 1:300 - 1:5.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 32 out of 43 laboratories (74%) produced a sufficient staining assessed as good or optimal.

* (number of optimal results/number of laboratories using this buffer)

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

In this assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the membranous IgM of the neoplastic cells in the two B-cell lymphomas and the normal mantle zone B-cells, whereas virtually all could demonstrate the cytoplasmic IgM in plasma cells. A too weak or false negative staining was seen in 98 % of the insufficient results (39 out of 40), which typically displayed by a diffuse or dot-like cytoplasmic reaction with no accentuation of the membranes of the neoplastic cells in the lymphomas.

None of 10 protocols based on proteolytic pre-treatment gave a sufficient result (with these protocols only the cytoplasmic IgM in plasma cells and immunoblasts were demonstrated, with no membranous reaction in the lymphatic B-cells (probably due to digestion of the membrane epitopes on these cells).

IgM was also assessed in Run 18 2006, in which 61 laboratories participated. The overall proportion of sufficient staining has increased from 31 % in run 18 to 50 % in the present run. In the assessment of IgM run 18, each of 42 laboratories, which obtained an insufficient result, was given a specific recommendation to improve their protocol. 35 of these laboratories submitted a new IgM stain in run 23. 20 followed the recommendations, of which 14 improved to good or optimal (70 %). 13 laboratories did not follow the recommendation, and 1 of these (8 %) obtained a sufficient staining in run 23. Three laboratories changed their entire IHC platform and one improved.

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 two runs have given an optimal result - irrespective of the protocols applied; second, usage of proteolytic pre-treatment for the pAb A0425 (Dako) instead of HIER; and third, many new laboratories participating in an IgM assessment for the first time. Though it cannot be excluded that other Abs than pAb A0425 would be able give optimal IgK stains with better protocols, the datasheets provided with these Abs have apparently not provided any good solutions. Some laboratories only use anti-IgM for plasma cells and plasma cell disorders. However, the material provided from NordiQC needs an IgM protocol aimed at demonstrating membranous IgM, not cytoplasmic, in order to diagnose lymphomas.

In concordance with the previous IgM assessment, 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). There was not noticed any significant difference in the reaction pattern in the tonsils fixed in the range of 24 – 72 hours in 10 % NBF, when optimal protocol settings (HIER) was applied.

Conclusions

In this assessment, pAb A0425 (Dako) was the most useful Ab for the demonstration of membranous IgM. HIER was the most robust 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.

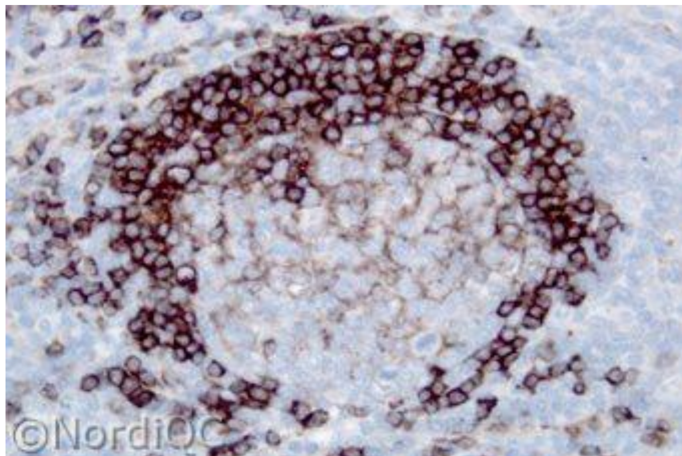

Fig. 1a
Optimal staining for IgM of the tonsil using the pAb A0425 and HIER. Virtually all the mantle zone B-cells show a distinct membranous reaction and in the germinal centre scattered immunoblasts and the follicular dendritic network is stained. The background is only weak positive. Also compare with Fig. 2a, same protocol.

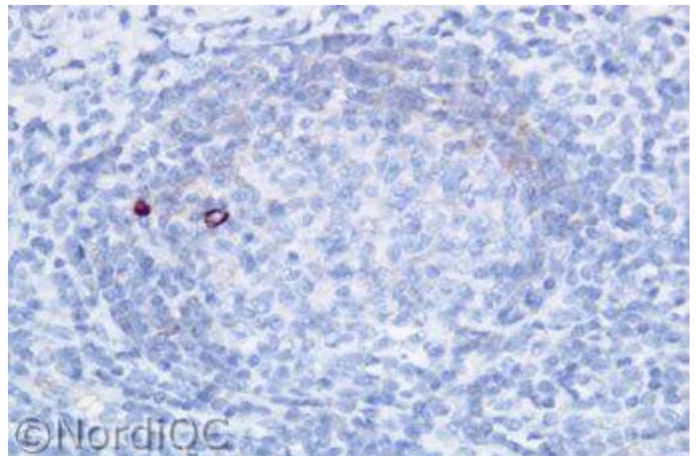

Fig. 1b
Insufficient staining for IgM of the tonsil, same field as Fig. 1a. The mantle zone B-cells are negative and only the plasma cells and immunoblasts show a positive reaction. Also compare with Fig. 2b, same protocol using the pAb A0425 and HIER, but using a too low concentration of the primary Ab.

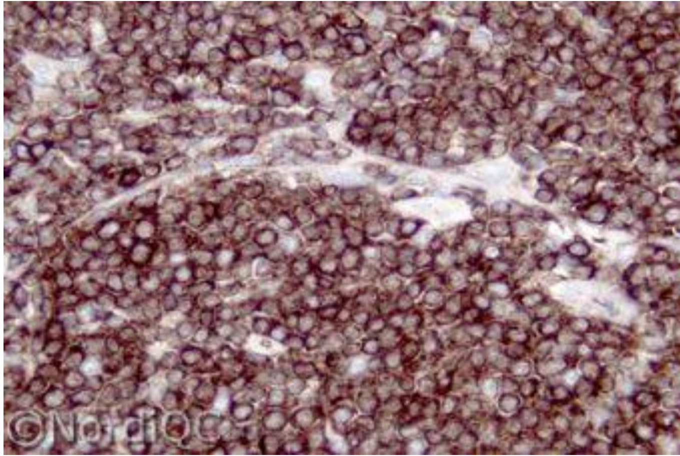

Fig. 2a
Optimal staining for IgM of the follicular B-cell lymphoma. Virtually all the neoplastic cells show a distinct strong membranous reaction. Same protocol as Fig. 1a.

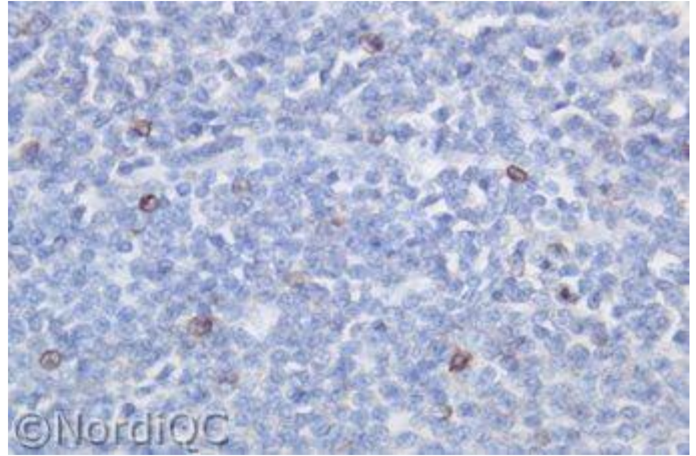

Fig. 2b
Insufficient staining for IgM of the follicular B-cell lymphoma, same field as Fig 1b. Only scattered cells show a diffuse staining. Same protocol as Fig. 1b.

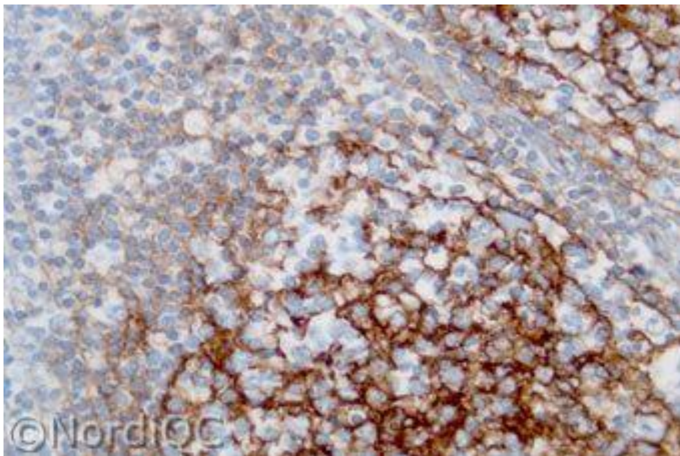

Fig. 3a
Insufficient staining for IgM of the tonsil using the pAb A0425 and proteolytic pre-treatment. The membranes of the mantle zone B-cells are digested giving a false negative reaction and only the follicular dendritic network show a strong staining. Also compare with Fig. 3b, left, same protocol.

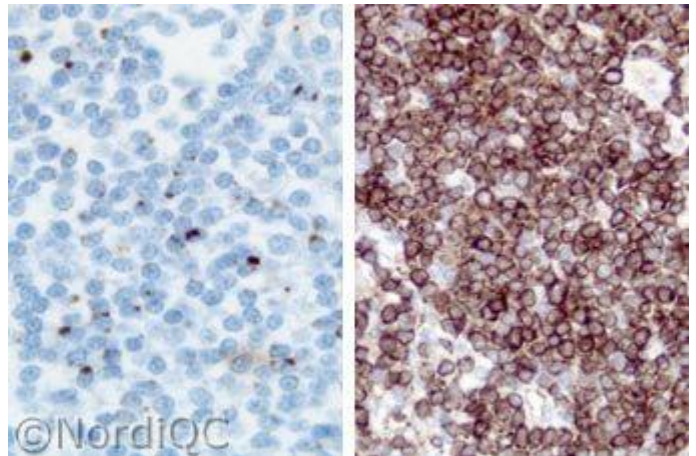

Fig. 3b
Left: Insufficient staining for IgM of the B-CLL using same protocol as in Fig. 3a. The neoplastic cells only show a dot-like cytoplasmic reaction – also compare with Fig. 3b right.
Right: Optimal staining for IgM of the B-CLL using same protocol as in Figs. 1a and 2a. Virtually all the neoplastic cells show a distinct strong membranous reaction.

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