Assessment Run 30 2010
IgM

The slide to be stained for IgM comprised:

1. Tonsil fixed 24 h, 2. Tonsil fixed 48 h, 3. B-CLL, 4. Mantle cell lymphoma, 5. Follicular lymphoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an IgM staining as optimal included:

- A strong and distinct membranous staining of virtually all the mantle zone B-cells of the germinal centres of the tonsils.
- A moderate to strong distinct membranous staining of virtually all the neoplastic cells in the mantle cell lymphoma and the follicular lymphoma.
- An at least weak to moderate predominantly membranous and dot-like cytoplasmic staining of the majority of the neoplastic cells of the B-CLL.
- A strong cytoplasmic reaction in the plasma cells, immunoblasts and follicular dendritic network in the germinal centres of the tonsils.

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

110 laboratories participated in this assessment. 61 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for IgM, run 30**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8H6**	2	Novocastra/Leica	0	0	1	1	-	-
mAb clone **IgM88**	1	BioGenex	0	0	0	1	-	-
pAb **A0425**	74	Dako	18	28	14	14	62 %	96 %
pAb **A0091**	2	Dako	1	0	1	0	-	-
pAb **RB-1434**	2	NeoMarkers	0	1	1	0	-	-
pAb **NCL-IgMp**	2	Novocastra/Leica	0	1	1	0	-	-
pAb **MAD-005029QD**	1	Master Diagnostica	0	0	0	1	-	-
Ready-To-Use Abs								
pAb **IS513/IR513**	11	Dako	3	7	1	0	91 %	83 %
pAb **760-2654**	11	Ventana/Cell Marque	3	2	4	2	45 %	100 %
pAb **270A-18/CMA011**	2	Cell Marque	1	1	0	0	-	-
pAb **N1509**	2	Dako	0	1	0	1	-	-
Total	110		26	41	23	20	-	-
Proportion	-		24 %	37 %	21 %	18 %	61 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
pAb **A0425**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either TRS pH 9 (Dako) (1/3)*, TRS pH 6.1 (Dako) (8/14), Cell Conditioning 1 (BenchMark, Ventana) (4/9), Bond Epitope Retrieval Solution 1 (Bond, Leica) (2/6) or Citrate pH 6 (3/12) as the retrieval buffer. The pAb was typically diluted in the range of 1:400– 1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 25 out of 26 (96 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

pAb **A0091**: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using TRS pH 6.1 (Dako) (1/2) as the retrieval buffer. The pAb was diluted 1:1000.

Ready-To-Use Abs
pAb **IS513/IR513** (Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) and an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 5 out of 6 (83 %) laboratories produced a sufficient staining (optimal or good). 1 lab used an incubation time of 24 min, HIER in standard Cell Conditioning 1 (Benchmark, Ventana) and IView (760-091) as the detection system.

pAb **760-2654** (Ventana): The protocols giving an optimal result were based on HIER using standard Cell Conditioning 1, an incubation time of 32-48 min in the primary Ab and UltraView (760-500) as the detection system. Using these protocol settings all of 4 labs produced a sufficient staining (optimal or good).

pAb clone **270A-18/CMA011** (Cell Marque): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) and an incubation time of 16 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system.

The most frequent causes of insufficient stainings were:

- Inappropriate epitope retrieval (proteolytic pre-treatment or no pre-treatment)
- Too low concentration of the primary antibody
- Less successful primary antibody

In this assessment and in accordance to the previous assessments of IgM, run 18 and 23, 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 B-cell lymphomas and the normal mantle zone B-cells, whereas virtually all participants could demonstrate the cytoplasmic IgM in plasma cells and immunoblasts. A too weak or false negative staining was seen in 95 % of the insufficient results (41 out of 43) and in only 5 % a too strong or false positive staining was seen (2 out of 43).
The most widely used Ab was the pAb A0425, Dako, which gave a very high proportion of sufficient results, 96 %, when the Ab was used with HIER and a titre in the range of 1:400 - 2.000. 25 out of 26 laboratories applying these basic protocol settings obtained a sufficient result despite different IHC platforms, detection systems etc were used.
Also for the Ready-To-Use pAbs IS513/IR513 Dako and 760-2654 Ventana/Cell Marque a very high pass-rate and proportion of sufficient results, respectively 83 % and 100 %, was obtained, when HIER was used as epitope retrieval.
None of 9 protocols based on proteolytic pre-treatment gave a sufficient result primarily due to an excessive digestion of the fragile membranes of both the normal and neoplastic lymphocytes giving a false negative membranous staining (only giving a positive staining for IgM in the cytoplasmic compartment of plasma cells, immunoblasts and the follicular dendritic network). This pattern was seen for all Abs used with proteolytic pre-treatment (760-2654 Ventana/Cell Marque, A0425 and N1509 Dako).
In concordance with the previous IgM assessments, normal tonsil seems to a reliable positive control for the demonstration of membranous IgM: Virtually all the peripheral mantle zone B-cells must 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). If only plasma cells and immunoblasts are demonstrated, the protocol can exclusively be used for demonstration of cytoplasmic IgM in normal and neoplastic plasma cells, not for the demonstration of membranous IgM in lymphomas.
This was the 3rd assessment of IgM in NordiQC, as IgM also was assessed in run 18, 2006 and run 23, 2008 and a constant increase in the proportion of sufficient results have been seen as shown in table 2:

Table 2. **Proportion of sufficient results for IgM in the three NordiQC runs performed**

	Run 18 2006	Run 23 2008	Run 30 2010
Participants, n=	61	80	110
Sufficient results	31 %	50 %	61 %

Many factors contribute to this increase of sufficient results, but the identification of the mantle zone B-cells as critical staining quality indicator (CQSI) for IgM and the tailored recommendations given to participants previously obtaining an insufficient mark seem to be central for the improvement. In run 23, 34 laboratories were given a tailored recommendation and subsequently submitted a staining in run 30. 15 laboratories followed the

recommendations of which 13 (87 %) improved to a sufficient result, while 13 did not change their protocol and only 2 (15 %) improved. 6 laboratories changed their entire system and 4 of these (66 %) improved to sufficient. The recommendations given were typically: 1. Increase the concentration of the primary Ab and 2. Use HIER.

Conclusion

In this assessment the pAb A0425, Dako and the Ready-To-Use pAbs 760-2654 Ventana/Cell Marque and IR513 were the most robust Abs for the demonstration of membranous IgM. HIER was mandatory to obtain a sufficient result. The concentration of the primary Ab must be carefully calibrated. Normal tonsil is an appropriate control tissue: Virtually all the mantle zone B-cells must show a distinct membranous staining with only a minimal background reaction.

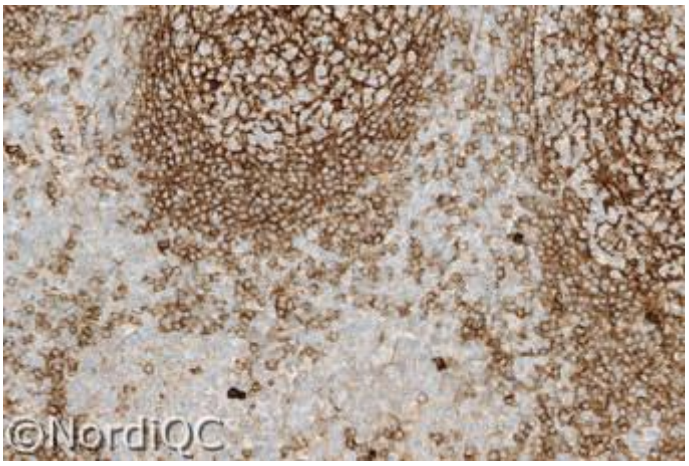

Fig. 1a
Optimal IgM staining of the tonsil using HIER and the pAb A0425, Dako, optimally calibrated. Virtually all the mantle zone B-cells show a distinct membranous staining. In the germinal centre scattered immunoblasts and the follicular dendritic network is stained. Only a weak reaction is seen in the background. Also compare with Figs. 2a & 3a, same protocol.

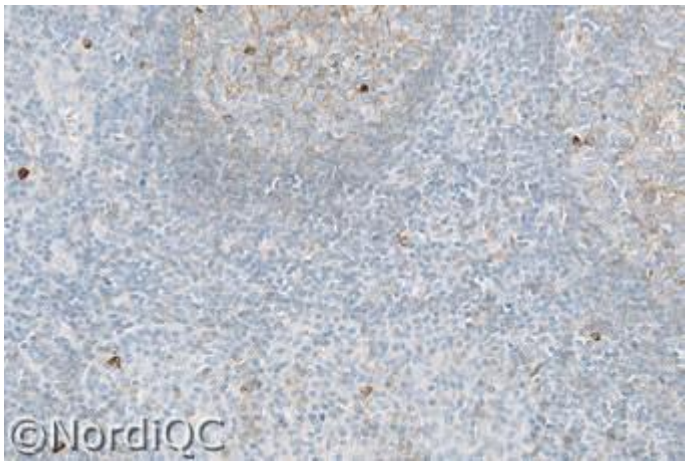

Fig. 1b
Insufficient IgM staining of the tonsil using HIER and the pAb A0425, Dako, in a too low concentration - same field as in Fig. 1a. The mantle zone B-cells are negative, and only the plasma cells and immunoblasts show a positive cytoplasmic reaction. Also compare with Figs. 2b & 3b, same protocol.

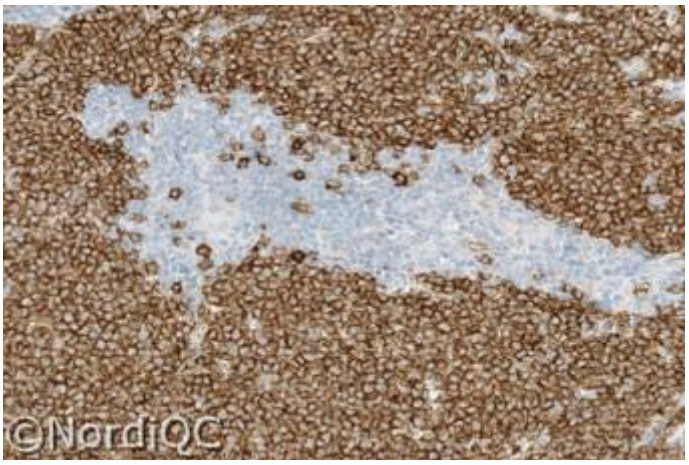

Fig. 2a
Optimal IgM staining of the mantle cell lymphoma. Virtually all the neoplastic cells show a distinct and strong membranous reaction. Same protocol as Fig. 1a.

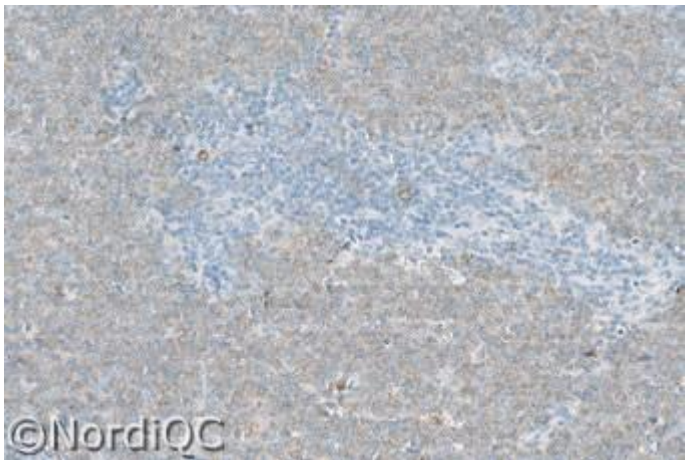

Fig. 2b
Insufficient IgM staining of the mantle cell lymphoma - same field as Fig. 2a. The neoplastic cells only show a diffuse and equivocal staining. Same protocol as Fig. 1b.

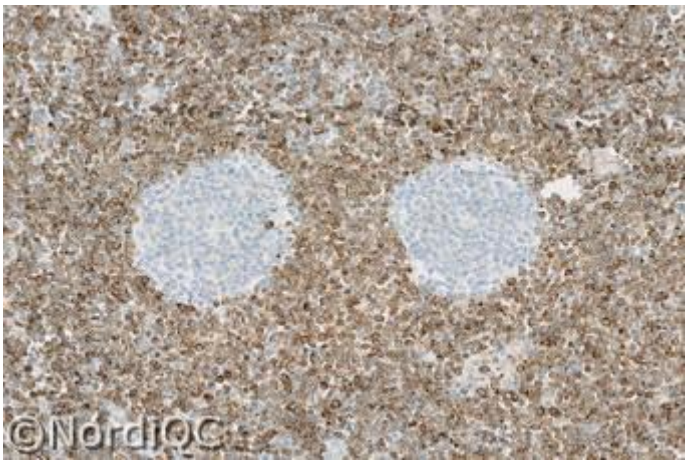

Fig. 3a
Optimal IgM staining of the B-CLL. Virtually all the neoplastic cells show a distinct strong and predominantly membranous reaction. Also a dot-like cytoplasmic staining is seen. Remnants of normal follicles are negative. Same protocol as Figs. 1a & 2a.

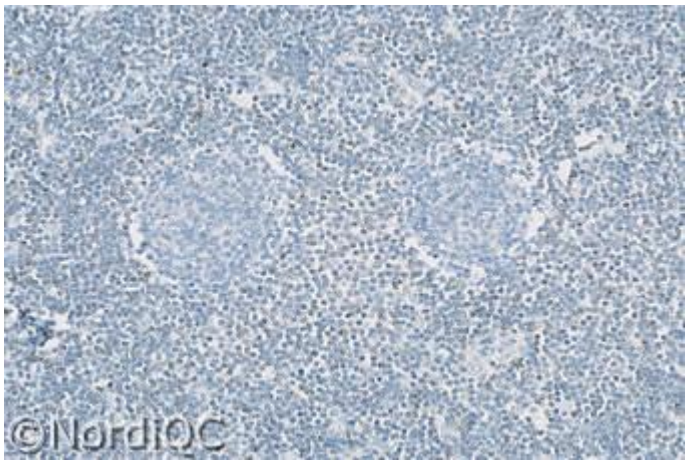

Fig. 3b
Insufficient IgM staining of the B-CLL - same field as Fig. 3a. The neoplastic cells are virtually negative. Same protocol as Fig. 1b and 2b.

Fig. 4a
Insufficient IgM staining of the tonsil using the pAb A0425 with proteolytic pretreatment. The membranes of the mantle zone B-cells are digested giving a false negative staining, while the follicular dendritic network still show a strong staining. Also compare with Fig. 4b, same protocol.

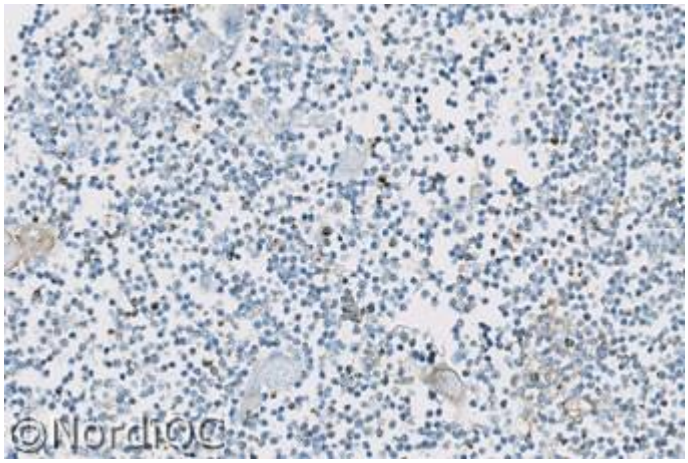

Fig. 4b
Insufficient IgM staining of the B-CLL using same protocol as in Fig. 4a. The neoplastic cells are virtually negative due to excessive digestion of the fragile membranes. Only scattered cells show a weak cytoplasmic dot-like staining. Also compare with Fig. 3a - same tissue.

SN/MV/LE 5-12-2010