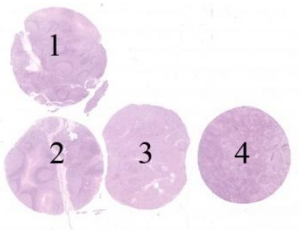
Membrane IgM (mIgM)

Assessment Run 39 2013

The slide to be stained for mIgM comprised:



1: Tonsil, fixed 24 hours, 2: Tonsil, fixed 48 hours, 3: Mantle cell lymphoma, 4: Follicular lymphoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing the mIgM staining as optimal were:

- A strong, distinct membranous staining reaction of virtually all mantle zone B-cells of the germinal centres in the tonsils.
- An at least weak to moderate distinct, predominantly membranous staining reaction of virtually all neoplastic cells in the mantle cell lymphoma and the follicular lymphoma.
- A strong cytoplasmic reaction in plasma cells, immunoblasts and follicular dendritic network in the germinal centres of the tonsils.
- No staining of T-cells.
- No more than a weak background staining.

140 laboratories participated in this assessment. 63 % achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for mIgM, run 39**

Concentrated antibodies	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8H6**	6	Leica/Novocastra	0	0	3	3	0 %	-
mAb clone **IgM88**	1	BioGenex	0	0	0	1	-	-
pAb **A0425**	75	Dako	35	15	11	14	67 %	95 %
pAb **A0091***	2	Dako	0	0	2	0	-	-
pAb **NCL-IgMp***	1	Leica/Novocastra	0	0	1	0	-	-
pAb **PU427-UP**	1	BioGenex	0	1	0	0	-	-
pAb **RaHu/IgMFC**	1	Nordic MUbio	0	0	0	1	-	-
pAb **RB-1434**	5	Thermo/NeoMarkers	0	1	1	3	20 %	-
Ready-To-Use antibodies	N							
pAb **270A-17/18**	2	Cell Marque	0	2	0	0	-	-
pAb **760-2654**	21	Ventana/Cell Marque	6	9	2	4	71 %	92 %
pAb **AR427-5R**	1	BioGenex	0	1	0	0	-	-
pAb **GA04250**	1	Gene Tech	0	0	0	1	-	-
pAb **IR/IS513**	21	Dako	7	9	4	1	76 %	93 %
pAb **MAD-005029QD**	1	Master Diagnostica	1	0	0	0	-	-
pAb **N1509***	1	Dako	1	0	0	0	-	-
Total	140		50	38	24	28	-	
Proportion			36 %	27 %	17 %	20 %	63 %	

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

Detailed analysis of mIgM, Run 39

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
pAb **A0425**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/7)*, TRS pH 9 (Dako) (1/3), TRS low pH 6.1, 3-in-1 (Dako) (2/4), TRS pH 6.1 (Dako) (5/11), Cell Conditioning 1 (CC1; Ventana) (12/22), CC2 (BenchMark, Ventana) (1/1), Bond Epitope Retrieval Solution 1 (BERS1; Leica) (6/7), Tris-EDTA/EGTA pH 9 (3/5) or Citrate pH 6 (2/5) as retrieval buffer. The mAb was typically diluted in the range of 1:500-1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 42 of 44 (95 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Table 2. **Optimal results for mIgM using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
pAb A0425 **Dako**	40 % 4/10**	56 % 5/9	55 % 12/22	100 % 1/1	0 % 0/7	86 % 6/7

* Ab concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
pAb product no. 760-2654 Ventana, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 24-44 min. incubation of the primary Ab and UltraView (760-500 + amplification kit) or OptiView (760-700) as detection system. Using these protocol settings 11 of 12 (92 %) laboratories produced a sufficient staining (optimal or good).

pAb product no. IS/IR513 Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 15-20 min at 95-97°C), 20 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings 14 of 15 (93 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient staining reactions were:

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

In this assessment and in concordance with the previous NordiQC assessments of IgM, the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction of the mIgM 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 reaction was seen in 90 % of the insufficient results (47 of 52). In the remaining insufficient results poor signal-to-noise ratio and/or a false positive staining were seen.

The pAb A0425 (Dako) was the most widely used Ab for IgM. Provided that an optimal titre and appropriate epitope retrieval were used, a high proportion of sufficient results were seen when applied as a concentrate. Used in the range of 1:500-2.000 with HIER, 95 % of the stains evaluated were assessed as sufficient. Optimal staining results could be obtained on all 3 main IHC platforms from (Dako, Ventana and Leica, see table 2). HIER in a non-alkaline buffer provided a slightly higher proportion of optimal results compared to an alkaline buffer, as the former in general improved the signal-to-noise ratio while the extracellular background staining was reduced, see table 2. However both HIER based on non-alkaline and alkaline buffers could be used to obtain an optimal result. Omission of epitope retrieval or use of proteolytic pre-treatment resulted in insufficient result in 8 of 9 protocols (none were assessed as optimal).

The Dako ready-to-use (RTU) system based on a rabbit pAb, prod. no. IS/IR513 gave an overall pass rate of 76 %, which is slightly higher compared to the corresponding concentrated format (67 %). If the RTU system was applied with the protocol settings recommended by Dako (HIER in TRS High pH for 20 min. at 95-97°C, 20 min. incubation of the primary Ab EnVision FLEX as detection system and performed on the Autostainer), a pass rate of 93 % was seen (n=13/14). 33 % were evaluated as optimal.

The Ventana RTU system also based on a rabbit pAb, prod.no, 760-2654 gave an overall pass rate of 71 %. If the RTU system was applied with the protocol settings recommended by Ventana (HIER in CC1 mild, 32 min incubation of the primary Ab, UltraView as detection system and performed on the BenchMark XT/Ultra), a pass rate of 80 % was seen (n=4/5). 0 % was evaluated as optimal. Optimal staining results were only seen with

modifications to the officially recommended protocol such as application of a more sensitive detection system as OptiView.

Controls
Tonsil is recommendable as control for the demonstration of membranous IgM. Virtually all mantle zone B-cells of the germinal centres must show a moderate to strong, distinct and predominantly membranous staining reaction, while plasma cells and immunoblasts must show a strong cytoplasmic staining reaction. T-cells must be negative and no or only a weak general background staining must be seen.

Performance history
This was the 4th NordiQC assessment of IgM. A small but consistent increase in the pass rate has been observed from run 18 to run 39, although the pass rate is still relatively low (see table 3).

Table 3. **Proportion of sufficient results for IgM in four NordiQC runs**

	Run 18 2006	**Run 23 2008**	**Run 30 2010**	Run 39 2013
Participants, n=	61	80	110	140
Sufficient results	31 %	50 %	61 %	63 %

Several parameters may contribute to the low pass rate throughout the assessments. Inappropriate epitope retrieval (proteolysis or omission of retrieval), has consistently given insufficient results and has been applied in 7-15 % of the protocols used in the assessments. However the main parameter giving insufficient results seems to be related to the choice of the primary Ab and the titre applied. The vast majority of protocols giving an insufficient result have been evaluated to provide a false negative staining reaction of the mIgM in normal and neoplastic B-cells, whereas the cytoplasmic IgM in plasma cells consistently have been demonstrated in virtually all protocols assessed. In this context it has to be emphasized that the NordiQC assessments consistently have been focused on the identification of mIgM in B-cell lymphomas and the identification of the monoclonal population of the neoplastic cells.
If the NordiQC assessment was focused on cytoplasmic IgM in e.g., normal and plasma cell disorders both the content of the material circulated and assessment criteria would have been different.

Conclusion
In this assessment the rabbit pAb A0425 (Dako) was the most widely used Ab. A high proportion of sufficient results was seen provided HIER and an appropriate titre was applied. Omission of retrieval and proteolysis gave insufficient results. The RTU systems from Dako and Ventana also gave a high proportion of sufficient results. Tonsil is recommendable as control tissue for the demonstration of mIgM. Virtually all mantle zone B-cells must show an at least moderate distinct membranous staining reaction, while only a minimal background staining must be seen. No staining must be seen in T-cells.

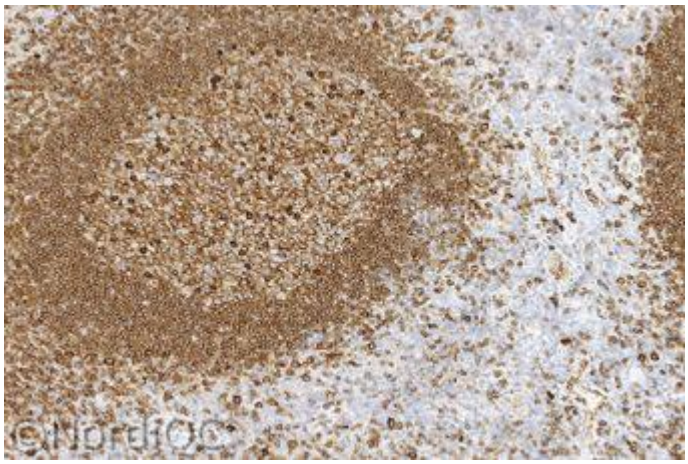


Fig. 1a
Optimal mIgM staining of the tonsil using the pAb A0425, Dako optimally calibrated and with HIER. Virtually all the mantle zone B-cells show a distinct membranous staining reaction. In the germinal centre both scattered immunoblasts and the follicular dendritic network are demonstrated. Only a weak background reaction is seen. Also compare with Figs. 2a - 4a, same protocol.

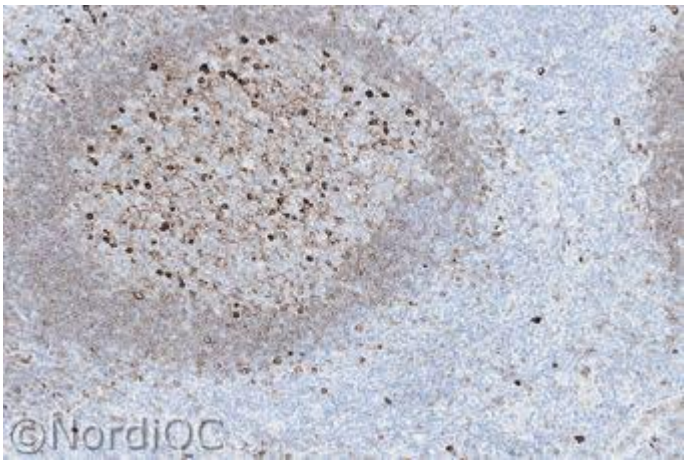


Fig. 1b
Insufficient mIgM staining of the tonsil using the pAb A0425, Dako with HIER, but using a too low concentration of the primary Ab. - same field as in Fig. 1a. The mantle zone B-cells show an equivocal membranous staining reaction and only the plasma cells and immunoblasts show a distinct positive cytoplasmic reaction. Also compare with Figs. 2b - 4b, same protocol.

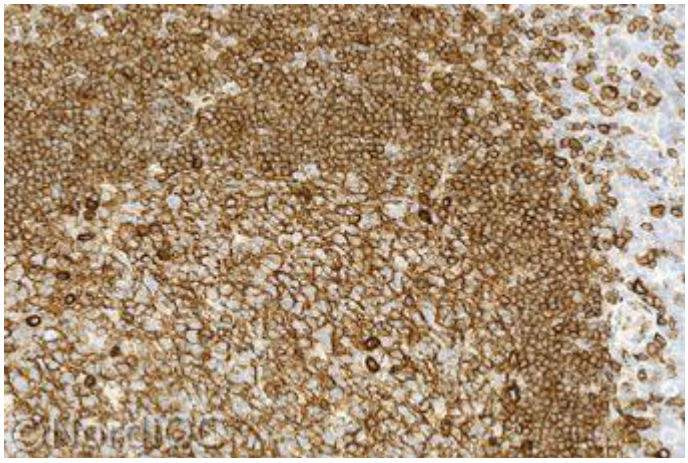


Fig. 2a
Optimal mIgM staining of the tonsil using same protocol as in Fig. 1a, high magnification (x 200). The mantle zone B-cells show a moderate to strong and distinct membranous staining reaction.

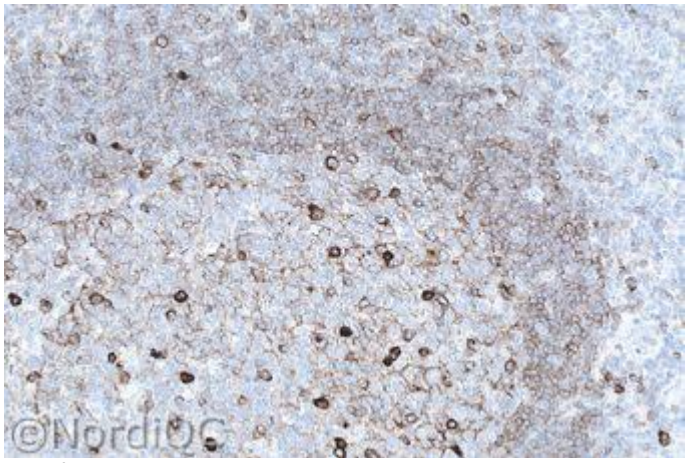


Fig. 2b
Insufficient mIgM staining of the tonsil using same protocol as in Fig. 1b, high magnification (x 200) - same field as Fig. 2a. The mantle zone B-cells show a weak and patchy membranous staining.

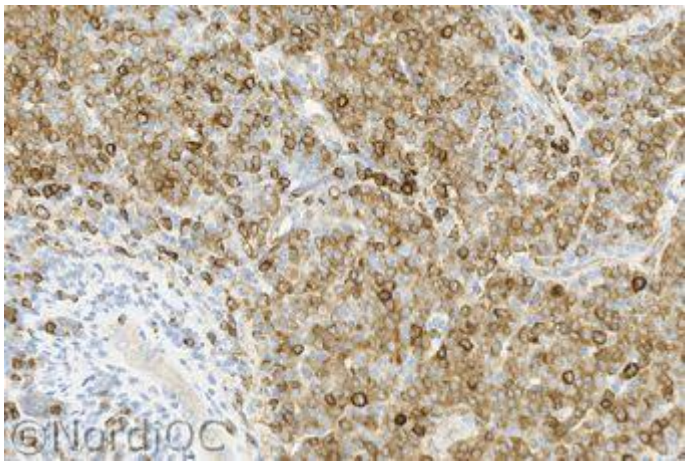


Fig. 3a
Optimal mIgM staining of the mantle cell lymphoma using same protocol as in Figs. 1a & 2a. The vast majority of the neoplastic cells show a distinct, moderate predominantly membranous staining reaction. No background staining is seen.

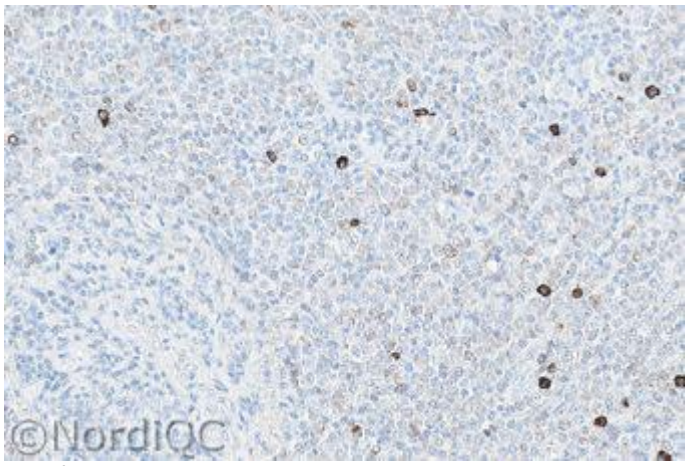


Fig. 3b
Insufficient mIgM staining of the mantle cell lymphoma using same protocol as in Figs. 1b & 2b - same field as Fig. 3a. The neoplastic cells are virtually negative and only plasma cells show a distinct cytoplasmic staining reaction.

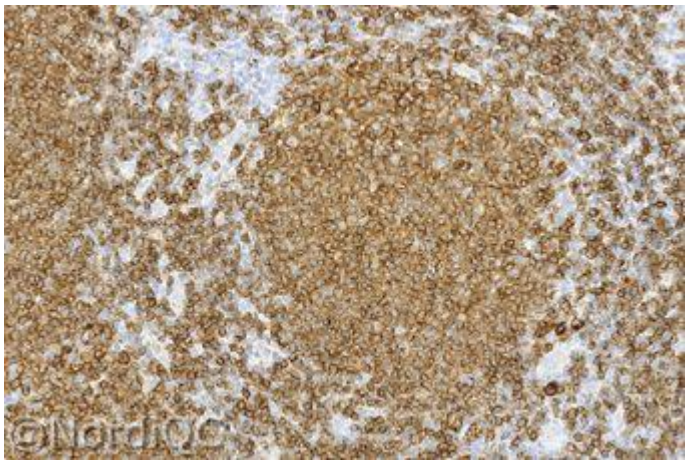


Fig. 4a
Optimal mIgM staining of the follicular lymphoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a distinct, moderate predominantly membranous staining reaction.

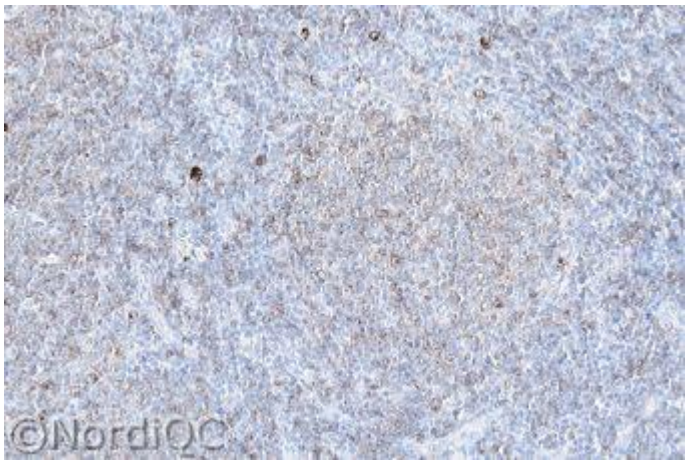


Fig. 4b
Insufficient mIgM staining of the follicular lymphoma using same protocol as in Figs. 1b - 3b - same field as Fig. 4a. The neoplastic cells only show a weak and equivocal staining reaction.

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