Assessment Run 32 2011
MUM1

Material

Multiple Myeloma Oncogene 1 (MUM1)

The slide to be stained for MUM1 comprised:

1. Classical Hodgkin lymphoma, mixed cellularity, 2. Tonsil fixed 24 h, 3. Diffuse large B-cell lymphoma, germinal centre B-cell (GCB) type, 4. Tonsil fixed 4 h, 5. Diffuse large B-cell lymphoma, non-germinal centre (non-GCB) type, 6. Malignant melanoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MUM1 staining as optimal included:

- A moderate to strong and distinct nuclear staining of the plasma cells and the late stage germinal centre B-cells in the two tonsils.
- A moderate to strong nuclear staining in > 30 % of the neoplastic cells of the diffuse large B-cell lymphoma, non-GCB phenotype and in < 10 % of the neoplastic cells of the diffuse large B-cell lymphoma, GCB phenotype.
- An at least weak to moderate, distinct nuclear staining of the Reed-Sternberg cells of the Hodgkin lymphoma.
- An at least weak to moderate nuclear staining of the majority of the neoplastic cells of the melanoma.
- A weak cytoplasmic staining reaction was accepted in the cells with a nuclear staining for MUM1.

120 laboratories participated in this assessment. 58 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for MUM1, run 32**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **MUM1p**	82 1 1	Dako Master Diagnostica Zhongshan jinqiao	25	28	27	4	63 %	71 %
mAb clone **MRQ-8**	3 1	Cell Marque Immunologic	0	1	1	2	-	-
mAb clone **BC5**	1	Biocare	0	0	1	0	-	-
mAb clone **EAU32**	1	Leica/Novocastra	0	0	1	0	-	-
pAb **Ab27508**	1	Abcam	0	0	0	1	-	-
pAb **E18351**	1	Spring Bioscience	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **MUM1p IS/IR644**	16	Dako	5	3	7	1	50 %	75 %
mAb clone **MUM1p N1603**	1	Dako	0	1	0	0	-	-
mAb clone **EAU32 PA0129**	3	Leica/Novocastra	2	0	1	0	-	-
mAb clone **MRQ-8 358M-18**	2	Cell Marque	0	0	1	1	-	-
mAb clone **BC5 PRM352**	1	Biocare	0	0	1	0	-	-
rmAb clone **MRQ-43 760-4529**	4	Ventana/Cell Marque	2	2	0	0	-	-
rmAb clone **MRQ-**	1	Cell Marque	1	0	0	0	-	-

43 358R-77								
Total	120		35	35	41	9	-	
Proportion			29 %	29 %	34 %	8 %	58 %	

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
mAb clone **MUM1p**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Bond Epitope Retrieval Solution 2 8BERS2) (Bond, Leica) (8/10)*, Tris-EDTA/EGTA pH 9 (6/15), Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/11), TRS pH 9 (Dako) (3/9), Cell Conditioning 1 (CC1) (BenchMark, Ventana)(2/28), Diva Decloaker pH 6.2 (Biocare)(1/1) or Citrate pH 6 (2/5) as the retrieval buffer. The mAb was typically diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 46 out of 65 (71 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

Ready-To-Use Abs
mAb clone **MUM1p** (prod. no. IS/IR644, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using either TRS pH 9 (3-in-1) or TRS pH 9 and an incubation time of 20-40 min in the primary Ab and a 2-step polymer based detection system as EnVision Flex (K8000). Using these protocol settings 8 out of 12 (75 %) laboratories produced a sufficient staining.

mAb clone **EAU32** (product.no. PA0129, Leica/Novocastra): The protocols giving an optimal result were based on HIER using BERS2 (Bond, Leica), an incubation time of 15 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings 2 out of 3 laboratories produced a sufficient staining.

rmAb clone **MRQ-43** (prod. no. 760-4529, Ventana): The protocols giving an optimal result were based on HIER using standard CC1, an incubation time of 32 min in the primary Ab and UltraView (760-500) as the detection system. 1 laboratory used amplification kit.
Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining.

rmAb clone **MRQ-43** (prod. no. 358R-77, Cell Marque): The protocol giving an optimal result was based on HIER using standard CC1, an incubation time of 32 min in the primary Ab and UltraView (760-500) as the detection system.

The most frequent causes of insufficient stains were:

- Less successful Ab
- Insufficient HIER - too short HIER time and/or use of a non alkaline buffer for HIER
- Too low concentration of the primary antibody
- Use of low sensitivity detection systems.

In this first NordiQC assessment for MUM1, the prevalent feature of an insufficient staining was a generally too weak or completely false negative staining reaction of the cells expected to be demonstrated. The majority of the laboratories could demonstrate MUM1 in the plasma cells in the two tonsils and the Reed Sternberg cells of the Hodgkin lymphoma, whereas the demonstration of MUM1 in the activated and late stage germinal centre B-cells and in particular the neoplastic cells of the melanoma was much more challenging and required a correctly calibrated protocol.
The most widely used Ab for MUM1 was the mAb clone MUM1p, which gave a proportion of sufficient results of 71 and 75 % when used either as a concentrate or as a Ready-To-Use (RTU) format (Dako).
Applying the mAb clone MUM1p as a concentrate, the pass rate was highly influenced by the sensitivity of the detections systems used. If a 2-step polymer or multimer based detection system e.g., EnVision Flex, Dako or UltraView, Ventana was used, 23 out of 54 laboratories obtained a sufficient staining result (43%) out of which 6 (11%) were assessed as optimal. If a more sensitive 3-step polymer or multimer based detection system e.g., EnVision Flex+, Bond Refine (Leica) or UltraView + amplification was used, 20 out of 21 laboratories produced a sufficient staining result (95%) of which 14 (66%) were optimal. It was also observed that too short efficient HIER as e.g. mild CC1 1 combined with a too low titre of the primary Ab gave a too low sensitivity.
The newly launched rmAb clone MRQ-43 gave a pass rate of 100 % (5 out of 5 laboratories) applying the Ab as an RTU format from either Ventana or Cell Marque. An optimal result was obtained by HIER in an alkaline buffer,

Cell Conditioning 1 standard and UltraView as detection system.

Tonsil was found to be a recommendable control, where the late stage germinal centre B-cells must display a moderate to strong distinct nuclear staining. If these cells were negative or only faintly demonstrated, the proportion of positive neoplastic cells in the two diffuse large B-cell lymphomas were reduced and the neoplastic cells of the melanoma were false negative. A weak cytoplasmic staining should be accepted.
By some high sensitive protocols a weak nuclear staining was seen in the mantle zone B-cells. This staining pattern was accepted as long as a correct proportion of the neoplastic cells in the two diffuse large B-cell lymphomas were maintained.

Conclusion
The mAb clones MUM1p and EAU32 and the rmAb clone MRQ-43 could all be used to obtain an optimal staining for MUM1. HIER in an alkaline buffer and the use of a 3-step polymer or multimer based detection system gave the most robust protocol. Tonsil is a recommendable positive control for MUM1 in which both the plasma cells and more important the late stage germinal centre B-cells must show at least a moderate nuclear staining.

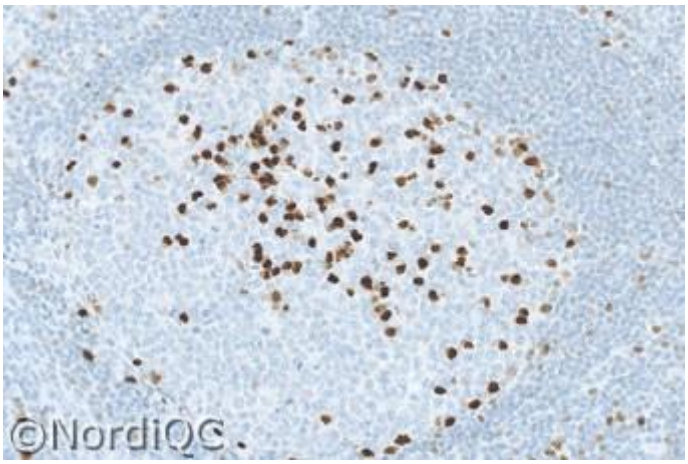

Fig. 1a
Optimal staining for MUM1 of the tonsil using the mAb clone MUM1p optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system. The late stage germinal centre B-cells show a distinct, moderate to strong nuclear staining.

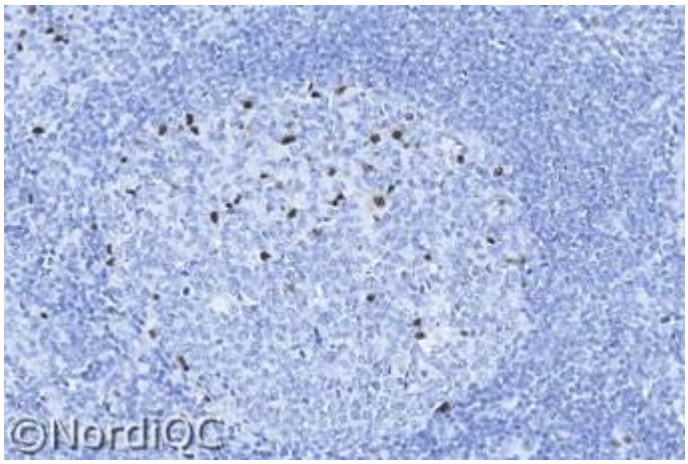

Fig. 1b
Insufficient staining for MUM1 of the tonsil using the mAb clone MUM1p. by a protocol with a too low sensitivity (2-step polymer and too low. conc. of the primary Ab), same field as in Fig. 1a.
The proportion of positive cells and the intensity of the staining reaction is significantly reduced compared to the result in Fig. 1a.
Also compare with Figs. 2b - 4b, same protocol.

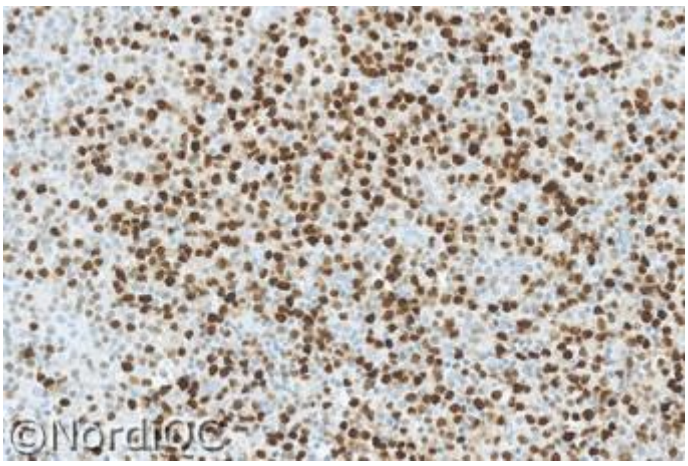

Fig. 2a
Optimal staining for MUM1 of the diffuse large B-cell lymphoma, non-GCB phenotype, using same protocol as in Fig. 1a. > 30 % of the neoplastic cells cells show a moderate to strong nuclear staining. No background staining is seen.

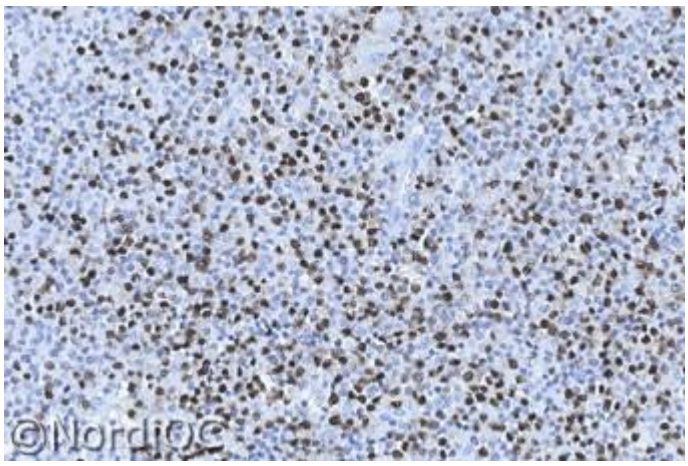

Fig. 2b
Staining for MUM1 of the diffuse large B-cell lymphoma, non-GCB phenotype, using same insufficient protocol as in Fig. 1b., same field as in Fig. 2a. > 30 % of the neoplastic cells cells show a moderate to strong nuclear staining. However, also compare with Figs. 3b & 4b, same protocol.

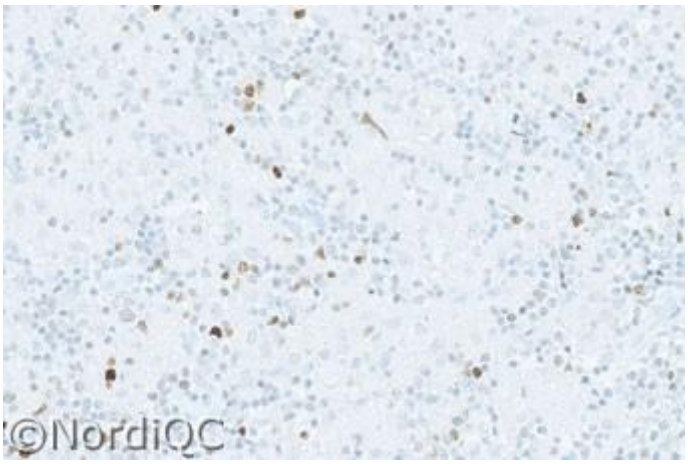

Fig. 3a
Optimal staining for MUM1 of the diffuse large B-cell lymphoma, GCB phenotype, using same protocol as in Figs. 1a & 2a.
< 10 % of the neoplastic cells cells show a moderate to strong nuclear staining.

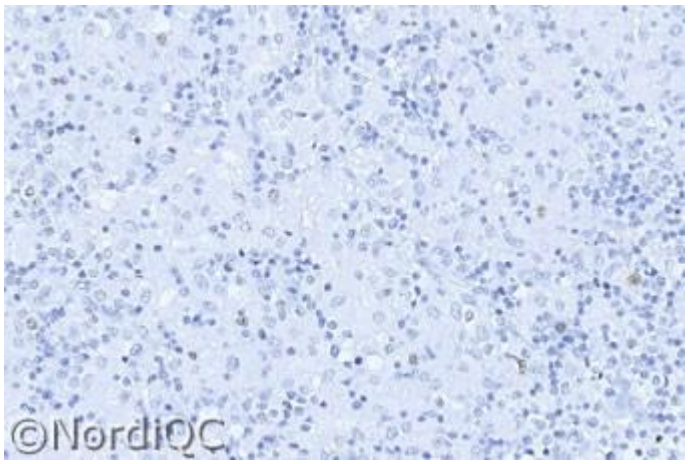

Fig. 3b
Insufficient staining for MUM1 of the diffuse large B-cell lymphoma, GCB phenotype, using same protocol as in Figs. 1b & 2b, same field as in Fig. 3a.
A false negative staining is seen in the neoplastic cells.

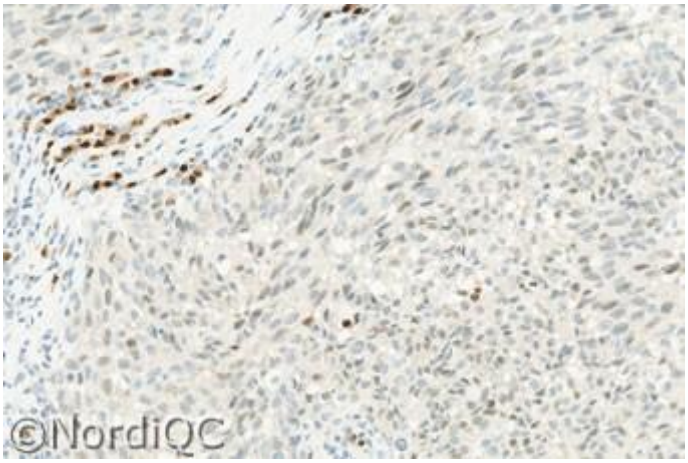

Fig. 4a
Optimal staining for MUM1 of the melanoma using same protocol as in Figs. 1a - 3a.
The majority of the neoplastic cells show a weak to moderate nuclear staining, while the entrapped plasma cells show a strong nuclear staining.

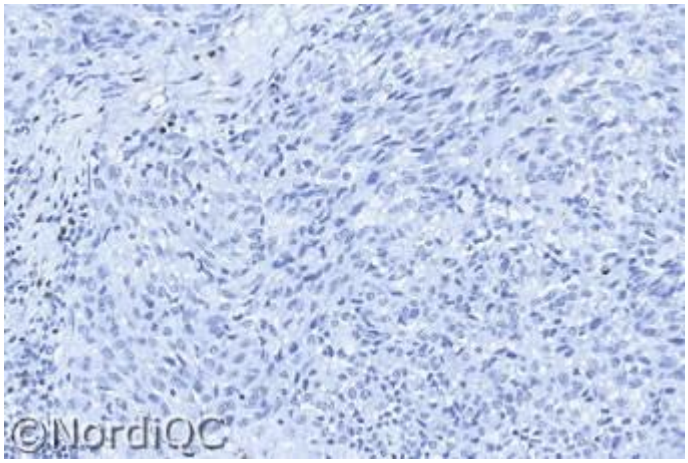

Fig. 4b
Insufficient staining for MUM1 of the melanoma using same protocol as in Figs. 1b – 3b, same field as in Fig. 4a.
Only the plasma cells show a nuclear staining, whereas the neoplastic cells of the melanoma are false negative.

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