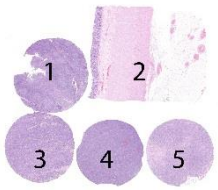
## Assessment Run 48 2016

# Multiple Myeloma Oncogene 1 (MUM1)

**Material**
The slide to be stained for Multiple Myeloma Oncogene 1 (MUM1) comprised:




1. Tonsil, 2. Colon, 3. Diffuse Large B-Cell Lymphoma (DLBCL) Germinal centre B-cell like (GCB), 4. DLBCL non-GCB like 5. Mb Hodgkin (Classical type).

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 late stage germinal centre B-cells and plasma cells in the tonsil.
- A strong, distinct nuclear staining reaction of virtual all plasma cells in lamina propria of the colon.
- An at least moderate, distinct nuclear staining reaction of the vast majority of the neoplastic cells in the non-GCB DLBCL tissue core no. 4.
- A strong, distinct nuclear staining reaction of the neoplastic cells (Reed-Sternberg cells) in Hodgkin lymphoma, classical type.
- No or only scattered nuclear staining reaction of the neoplastic cells in the GCB DLBCL tissue core no. 3. Normal plasma cells intermingling with the neoplastic cells of the DLBCL should be positive.
- A weak cytoplasmic staining reaction was accepted in the cells with a nuclear staining for MUM1
- No staining reaction in other cellular structures including epithelial cells and smooth muscle cells of lamina muscularis propria of the colon.

**Participation**

| | |
|---|---|
| Number of laboratories registered for MUM1, run 48 | 232 |
| Number of laboratories returning slides | 211 (91%) |

**Results**
211 laboratories participated in this assessment. 127 (60%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful primary antibody - rmAb MRQ-43, mAb MRQ-8 and mAb BC5
- Insufficient HIER (too short heating time).
- Less sensitive detection systems

**Performance history**
This was the second NordiQC assessment of MUM1. The overall pass rate was low but comparable to the result obtained in run 32, 2011 (see table 2).

Table 2. **Proportion of sufficient results for MUM1 in the two NordiQC runs performed**

| | Run 32 2011 | Run 48 2016 |
|---|---|---|
| Participants, n= | 120 | 211 |
| Sufficient results | 58% | 60% |

**Conclusion**
The mAb clones MUM1p, EAU32 and the rmAb clone EP190 could all be used to obtain optimal staining for MUM1. HIER in alkaline buffer, precise calibration of the primary Ab and use of a 3-step polymer or multimer based detection system were the main prerequisites for an optimal result. Protocols based on the rmAb MRQ-43, the mAb MRQ-8 and the mAb BC5 all produced insufficient results. Tonsil is recommendable as positive tissue control for MUM1 in which both plasma cells and more important late stage germinal centre B-cells must show at least a moderate nuclear staining. Colon can be used as negative tissue control in which no staining of epithelial and smooth muscle cells should be seen.

Table 1. **Antibodies and assessment marks for MUM1, run 48**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **MUMp1** | 84<br>1<br>1 | Agilent/Dako<br>Diagnostic Biosystem<br>GeneMed | 52 | 19 | 11 | 4 | 83% | 86 % |
| mAb clone **MRQ-8** | 3 | Cell Marque | 0 | 0 | 2 | 1 | - | - |
| mAb clone **BC5** | 3 | Biocare Medical | 0 | 0 | 3 | 0 | - | - |
| mAb clone **EAU32** | 3 | Leica/Novocastra | 0 | 2 | 1 | 0 | - | - |
| rmAb clone **MRQ-43** | 5<br>1<br>1 | Cell Marque<br>Menarini<br>Zeta | 0 | 0 | 3 | 4 | - | - |
| rmAb clone **SP114** | 1 | Thermo S./ LabVision | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **MUMp1 GA644** | 18 | Agilent/Dako | 8 | 7 | 2 | 1 | 83% | 88 % |
| mAb clone **MUMp1 IR/IS644** | 28 | Agilent/Dako | 13 | 12 | 3 | 0 | 89% | 88 % |
| mAb clone **MUMp1 GA644, IR/IS644**[3] | 5 | Agilent/Dako | 3 | 0 | 2 | 0 | - | - |
| mAb clone **MUMp1 MAD-000470QD** | 3 | Master Diagnostica | 1 | 1 | 1 | 0 | - | - |
| mAb clone **MUMp1 MAB-0573** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| mAb clone **EAU32 PA0129** | 6 | Leica Biosystems | 5 | 1 | 0 | 0 | 100% | 100% |
| rmAb clone **MRQ-43 760-4529** | 31 | Ventana/Roche | 0 | 0 | 25 | 6 | 0% | 0% |
| rmAb clone **MRQ-43 358R-77/78** | 15 | Cell Marque | 0 | 0 | 13 | 2 | 0% | 0% |
| rmAb clone **EP190 358R-17/18** | 1 | Cell Marque | 1 | 0 | 0 | 0 | - | - |
| Total | 211 | | 84 | 43 | 66 | 18 | - | |
| Proportion | | | 40% | 20% | 31% | 9% | 60% | |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only (see below).
3) RTU systems developed for Agilent/Dako`s automatic systems (Omnis/Autostainer) but used by laboratories off-label on the platforms Ventana Benchmark/Ultra or Leica BOND III.

## Detailed analysis of MUM1, Run 48

The following protocol parameters were central to obtain optimal staining.

### Concentrated antibodies

mAb **MUMp1**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (BenchMark, Ventana) (25/42) *, TRS High pH (3-1) (Dako/Agilent) (11/16), Bond Epitope Retrieval Solution 2 (Bond, Leica) (10/12), Epitope Retrieval Solution pH 9 (RE7119, Leica/Novocastra) (1/1) or TRIS-EDTA pH 9 (3/8) as retrieval buffer. Two laboratories obtained an optimal mark performing HIER in acidic buffers using Epitope Retrieval Solution pH 6 (RE7113, Leica/Novocastra) (1/1) and TRS Low pH (3-1) (Dako/Agilent) (1/1). The mAb was typically diluted in the range of 1:20 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 59 of 69 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Table 3. **Proportion of optimal results for MUM1 for the most commonly used antibody as concentrate on the 3 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic/ Omnis** | | **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 |
| mAb clone **MUMp1** | 8/12 ** (67%) | 1/1 | 24/39 (62%) | - | 7/7 (100%) | - |

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

**Ready-To-Use antibodies and corresponding systems**
mAb clone **MUMp1,** product no. **GA644,** Agilent/Dako Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (3-1) (efficient heating time 24-30 min. at 97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 14 of 16 (88%) laboratories produced a sufficient result. One laboratory obtained an optimal result using same protocol settings as above but without performing any pre-treatment at all.

mAb clone **MUMp1,** product no. **IR/IS644,** Agilent/Dako Autostainer+ /Autostainer Link:
Protocols with optimal results were based on HIER using TRS pH 9 (3-1) (efficient heating time 10-40 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision Flex/Flex+ (K8000/K8002) as detection system. Using these protocol settings, 22 of 25 (88%) laboratories produced a sufficient result. One laboratory obtained an optimal result using same protocol settings as above but without performing any pre-treatment at all.

mAb clone **EAU32,** product no. **PA0129,** Leica/Novocastra BOND III/BOND MAX:
Protocols with optimal results were based on HIER using BERS-2 or Epitope Retrieval Solution pH 9 (RE7119) (efficient heating time 20-40 min. at 99-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient result.

rmAb clone **EP190,** product no. **358R-17/18,** Cell Marque, Ventana Benchmark Ultra:
One protocol with an optimal result was based on HIER using CC1 (efficient heating time 64 min. at 95°C), 16 min. incubation time of primary Ab and UltraView (Ventana,760-500) as detection system.

**Comments**
In this second NordiQC assessment of MUM1, the prevalent feature of an insufficient result was either a generally too weak staining reaction of cells expected to be demonstrated and/or a false positive staining compromising the interpretation. Too weak or false negative staining reaction was seen in 36% of the insufficient results (30 of 84). The majority of the laboratories were able to stain for MUM1 in plasma cells (all specimens) and the neoplastic cells of the Hodgkin Lymphoma, tissue core no. 5, whereas demonstration of MUM1 in late stage germinal centre B-cells in the tonsil and the neoplastic B-cells of the non-GCB DLBCL, tissue core no. 4 was significantly more challenging and required a carefully calibrated protocol. In 64% (54 of 84) of the insufficient results, a poor signal-to-noise ratio and/or false positive staining reaction was seen. This high proportion of false positive staining results was mainly observed by protocols based on the rmAb MRQ-43 either as Ready-To-Use format from Ventana/Roche or as concentrated format from Cell Marque. All protocols based on this clone (53 of 53) were assessed as insufficient (borderline or poor).

mAb clone MUMp1was the most widely used antibody for the demonstration of MUM1. Applied as a concentrate in a laboratory developed (LD) assay, mAb clone MUMp1 gave an overall pass rate of 83% (71 of 86). As shown in table 3, optimal results could be obtained on all three main IHC platforms from Agilent/Dako, Leica/Novocastra and Ventana/ Roche. Best performance was obtained on the BOND III/MAX as all protocols (7 of 7) were assessed as optimal. Efficient HIER in alkaline buffer, careful calibration of the primary Ab and use of a sensitive detection system were the most central parameters for optimal results. Choosing the correct titre of the primary Ab is important and can significantly influence the analytical sensitivity of the assay. For protocols based on the concentrate of mAb clone MUMp1, HIER in alkaline buffer applied in combination with a standard 2- or 3-step multimer/polymer detection system, the Median Dilution Value (MDV) for optimal results was 1:164 (range 1:20-1:1500), whereas a MDV of 1:496 (range 1:20-1:2000) was seen in protocols with insufficient results. Consequently, titre must be carefully calibrated to provide an IHC protocol, that is able to demonstrate MUM1 in structures with both low- and high-level MUM1 expression, which is the range seen in different haematological neoplasia`s (e.g. subtypes of DLBCL).

The outcome was also influenced by the choice of the detection system. Using HIER in alkaline buffer and optimal dilution in the range 1:20-1:200 of the mAb clone MUMp1 as concentrate, the pass rate for participants using a 2-step multimer/polymer detection system was 71% (22 out of 31) of which 32% (10 of 31) were optimal. In comparison using same titre range and HIER settings, the pass rate for laboratories using a 3-step multimer/polymer system was 100 % (37 out of 37) of which 87% (32 out of 37) were optimal.

In this assessment the Leica/Novocastra RTU system PA0129 based on the mAb EAU32 and the Agilent/ Dako RTU systems IR/IS644 or GA644 based on the mAb clone MUMp1 provided the highest number of sufficient and optimal results (see table 1). Best performance was obtained with the RTU system PA0129 from Leica/Novocastra as all protocols were assessed as sufficient (6 of 6) of which 83% (5 of 6) were optimal. Optimal results were primarily obtained by using the RTU system accordingly to the vendor protocol recommendations. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) also provided sufficient and optimal results.
For laboratories using one of the RTU systems IS/IR644 or GA644 from Agilent/Dako, an overall pass rate of 86% was obtained of which 46% (21 out of 46) were optimal. Optimal results could be obtained by using vendor protocol recommendations or by using laboratory modified protocol settings (typically adjusting HIER time, incubation time of the primary Ab or applying the 3 step-polymer system FLEX+). For the RTU system GA644 (Omnis), the vendor recommendation for the detection system is to use FLEX+ (used by all laboratories producing an optimal result), whereas for the RTU system IS/IR644 the recommendation is to use FLEX as a starting point. For laboratories using the RTU system IS/IR644 in combination with FLEX as the detection system, 83 % (15 of 18) of the protocols were assessed as sufficient (optimal or good) of which 39% (7 out of 18) were optimal. In comparison, 100% (7 of 7) of the protocols based on FLEX+ as detection system produced a sufficient result of which 71% (5 of 7) were optimal.

In this assessment, the RTU system from Ventana based on the rmAb MRQ-43, prod. no. 760-4529 gave an inferior performance compared to the RTU systems from Leica/Novocastra or Agilent/Dako. None of the 31 protocols were assessed as sufficient (optimal or good) despite using vendor recommended protocol settings and/or general best practice settings producing optimal results with the mAb MUM1p1 within a LD assay (efficient HIER in CC1 for 32-64 min. and a sensitive detection system as OptiView or UltraView with amplification). The same observation was seen for the Cell Marque RTU format 358R-77/78, as all 15 protocols gave an insufficient result. In general MRQ-43 gave an aberrant and false positive staining reaction in both the nuclei and cytoplasmic compartment of epithelial cells and smooth muscle cells in lamina muscularis propria of the colon. It was also observed that in some of the protocols, stromal fibroblast-like cells (stellate cells) intermingling with smooth muscle cells in lamina muscularis propria of the colon showed an aberrant and false positive cytoplasmic staining reaction. In the assessment run 32, 2011 for MUM1, all protocols (5 out of 5) based on RTU format of the rmAb MRQ-43 (Ventana) were assessed as sufficient. The discrepancy in performance between run 32 and this run 48, is probably related to the low number of participants using this system in the first run and different composition of tissue material included in the two different assessments (colon tissue was not included in the run 32, 2011). Not only the RTU formats of MRQ-43 gave these aberrant staining patterns, but also the corresponding concentrated format of rmAb clone MRQ-43 and the mAb clone BC5 showed similar results. In addition to the patterns described above, the concentrated format of the rmAb MRQ-43 from Cell Marque occasionally gave a false positive membranous staining reaction in normal lymphocytes (mostly T-cells). This aberrant reaction pattern was seen in 43% (3 out of 7) of the protocols. There was no clear correlation to the use of certain lot numbers or protocol settings in general. All protocols based on the concentrate of the mAb BC5 showed aberrant cytoplasmic staining reaction of goblet and luminal epithelia cells in the colon. The different types of aberrant staining patterns are illustrated in Figs. 6a-6f.

The newly launched rmAb clone EP190 as RTU system from 358R-17/18 from Cell Marque seems promising for the demonstration of MUM1 and could be an alternative to the RTU system based on the rmAb MRQ-43 (see table 1 and description above). However, this antibody needs further validation as only one protocol was submitted for assessment.

The use of Ready-To-Use (RTU) formats is consistently increasing and in this run 51% of all protocols (108 of 211) were based on RTU formats, compared to 23% (28 of 120) in run 32, 2011. Ideally, a RTU format of a primary Ab should be used within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected. Therefore, it is not advisable to use a RTU system on another vendor platform, although it might produce optimal results (see table 1).

This was the second assessment of MUM1 in NordiQC and a pass rate of 60% was obtained, which is comparable to the result obtained in run 32, 2011 (table 2). In this run an overall pass rate of 83% (117

of 141) was obtained with the mAb clone MUMp1 used either as a concentrate within a LD assay or as a RTU system. The RTU system PA0129 (Leica/Novocastra) based on the mAb EAU32 provided a high pass rate (100%) and was in this assessment the overall best system.
Several parameters influenced the overall performance (e.g. inefficient HIER, too low concentration of the primary Ab and the use of a low sensitive detection system), but the use of the less successful rmAb MRQ-43, mAbs MRQ-8 and BC5 contributes significantly to the relative low pass rate obtained in this assessment. All protocols (56 out of 56) based on these 3 primary Abs were assessed as insufficient. Thus, to improve the general performance of the individual assays, it is advisable to change the primary antibody to e.g. MUMp1 or EP190.

**Controls**
Tonsil is recommendable as positive tissue control, where the late stage germinal centre B-cells must display a moderate to strong distinct nuclear staining. Plasma cells are strongly stained and a weak cytoplasmic staining should be accepted. By highly sensitive protocols, weak nuclear staining may be seen in a fraction of B-cells located in the mantle zone.
Colon is recommended as negative tissue control. Epithelia cells and smooth muscle cells (lamina muscularis propria) should be negative and only plasma cells located to mucosa (lamina propria) should display a strong nuclear reaction.

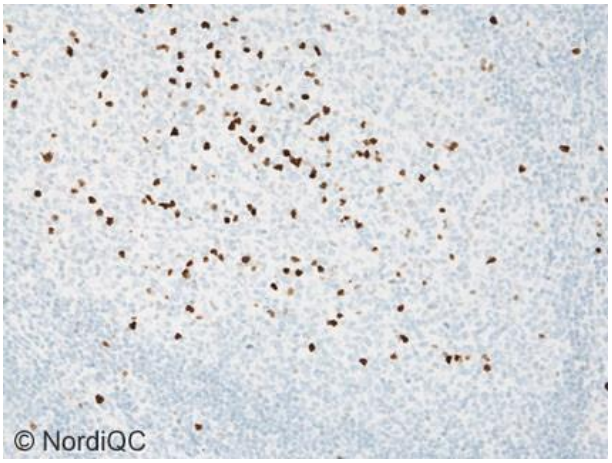



Fig. 1a (x200)
Optimal staining for MUM1 of the tonsil using the mAb MUMp1 as a concentrate, HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 5a. The late stage germinal centre B-cells show a distinct, moderate to strong nuclear staining reaction.

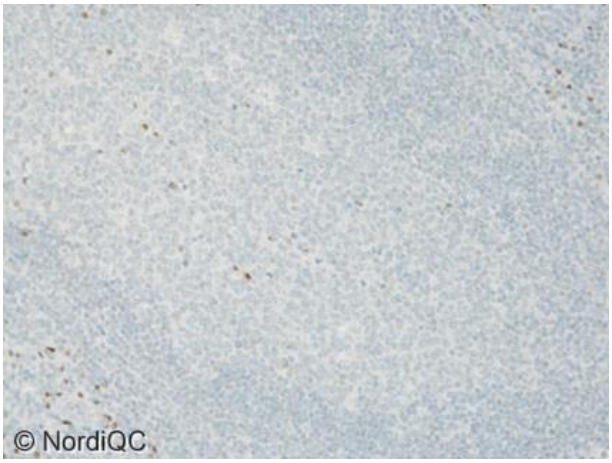



Fig. 1b (x200)
Insufficient staining for MUM1 of the tonsil using the mAb clone MUMp1 as concentrate (too diluted), HIER in an alkaline buffer (CC1) and a less sensitive multimer based detection system (Ultraview, Ventana) - same protocol used in Figs. 2b – 5b. The proportion of positive cells and the intensity of the staining reaction is significantly reduced - compare with Fig. 1a (same field).

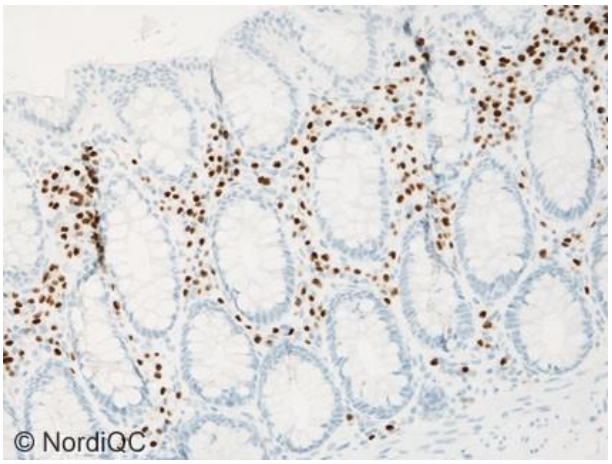



Fig. 2a (x200)
Optimal staining for MUM1 in the colon using same protocol as in Fig. 1a. Plasma cells show a distinct and strong nuclear staining, while all other structures are negative.

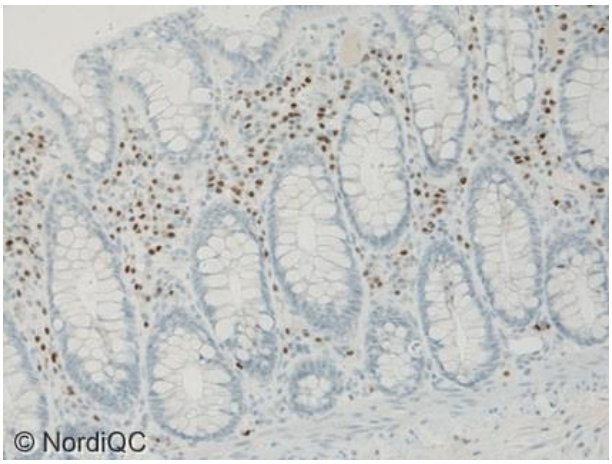



Fig. 2b (x200)
Insufficient staining for MUM1 in the colon using same protocol as in Fig 1b. Staining intensity of plasma cells is reduced - compare with Fig. 2a (same field).

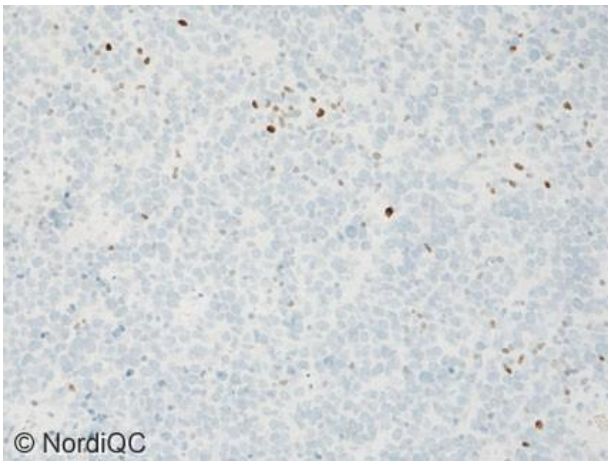



Fig. 3a (x200)
Optimal staining for MUM1 of the GCB DLBCL, tissue core 3 using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells are negative and only scattered normal plasma cells shows a moderate to strong but distinct nuclear staining reaction.

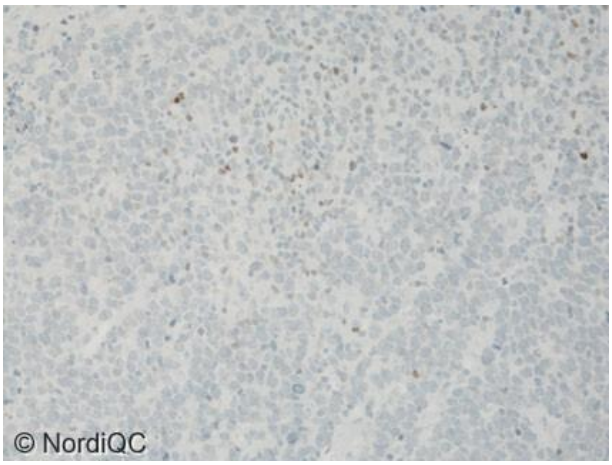



Fig. 3b (x200)
Insufficient staining for MUM1 of the GCB DLBCL, tissue core 3 using same protocol as in Figs. 1b & 2b. Intensity and proportion of stained normal plasma cells intermingling with the negative neoplastic cells of the DLBCL is reduced - compare with Fig. 3a (same field).

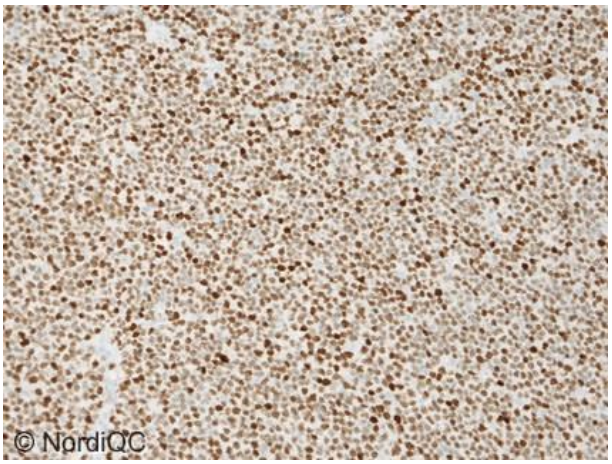



Fig. 4a (x200)
Optimal staining for MUM1 of the non-GCB DLBCL, tissue core 4 using same protocol as in Figs. 1a - 3a. Virtual all the neoplastic cells show a moderate to strong nuclear staining reaction.

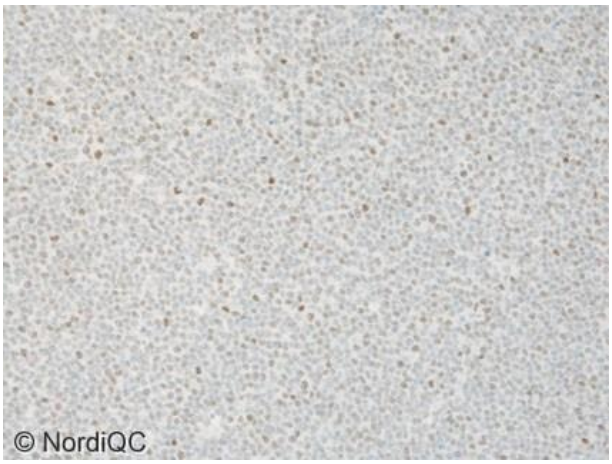



Fig. 4b (x200)
Insufficient staining for MUM1 of the non-GCB DLBCL, tissue core 4 using same protocol as in Figs. 1b -3b. Intensity and proportion of stained neoplastic cells is significantly reduced - compare with Fig. 4a (same field).

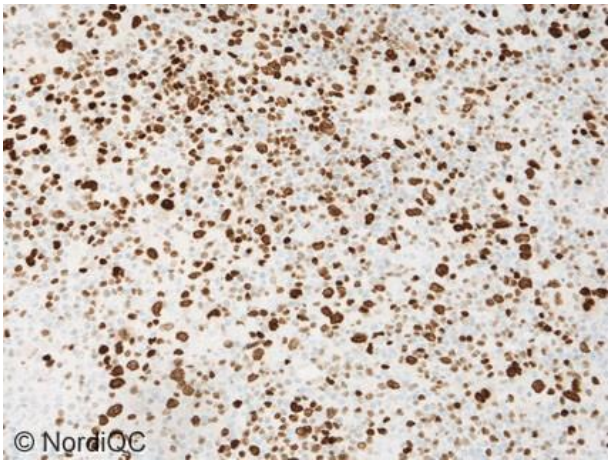



Fig. 5a (x200)
Optimal staining for MUM1 of the Hodgkin lymphoma (classical type) using same protocol as in Figs. 1a - 4a. The majority of the neoplastic cells show a strong and distinct nuclear staining reaction.

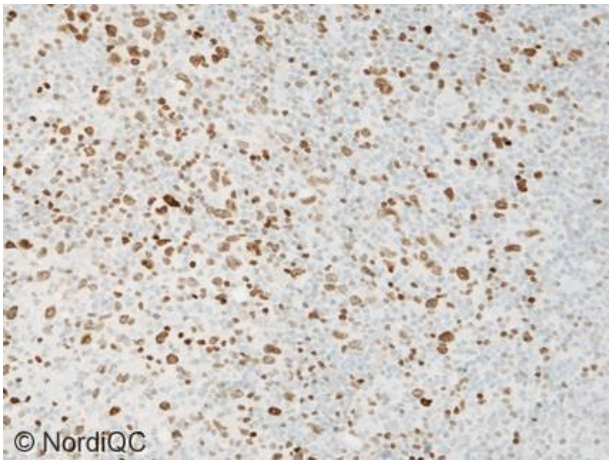



Fig. 5b (x200)
Insufficient staining for MUM1 of Hodgkin lymphoma (classical type) using same protocol as in Figs. 1b - 4b. The vast majority of neoplastic cells shows reduced intensity and only displays weak to moderate nuclear staining reaction - compare with Fig. 5a (same field).

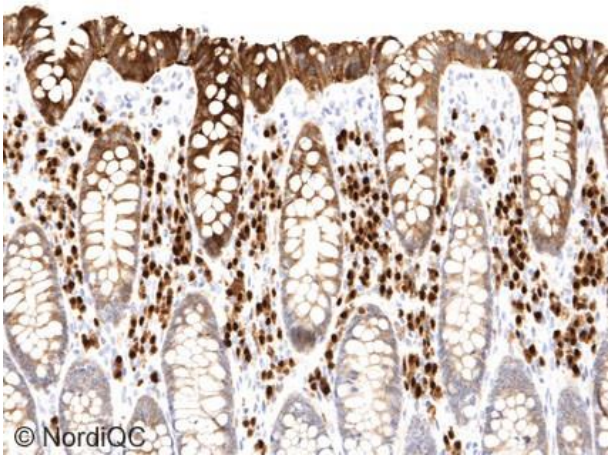



Fig. 6a (x200)
Insufficient staining for MUM1 using the rmAb MRQ-43 as Ready-To-Use format (760-4529, Ventana/Roche), with HIER in CC1 for 48 min. at 100°C and 3-step multimer OptiView, 760-700 (Ventana/Roche) as detection system. The epithelial cells in the colon are false positive displaying strong cytoplasmic reaction compromising the interpretation - compare with optimal protocol in Fig. 2a.

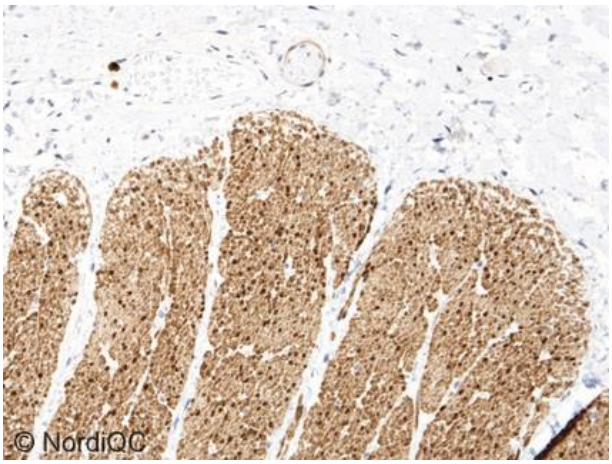



Fig. 6b (x200)
Insufficient and aberrant staining for MUM1 of the colon using the same protocol settings as in Fig. 6a. The smooth muscle cells in lamina muscularis propria are false positive displaying a distinct cytoplasmic but also strong nuclear staining reaction. In addition, smooth muscle cells surrounding the vessels are weakly labelled.

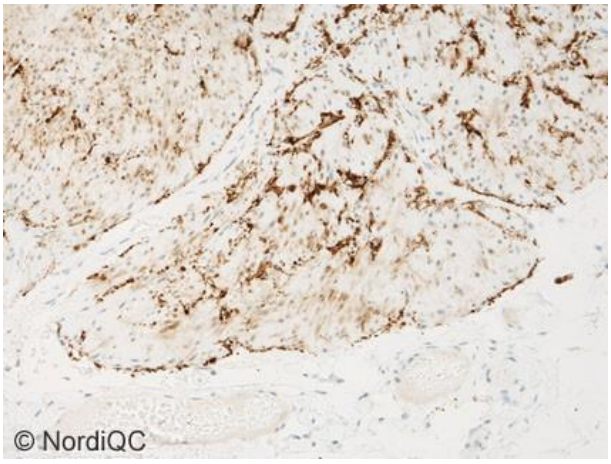



Fig. 6c (x200)
Insufficient staining for MUM1 of the colon using rmAb MRQ-43 as Ready-To-Use format (760-4529, Ventana/Roche), with HIER in CC1 for 60 min. at 100°C and 3-step multimer UltraView with amplification (Ventana/Roche) as detection system. The stellate cells (stromal fibroblast-like cells) intermingling with smooth muscle cells in lamina muscularis propria of the colon are aberrantly stained and displays a moderate to strong cytoplasmic reaction. The smooth muscle cells are only weakly labelled - compare with Fig. 6b.

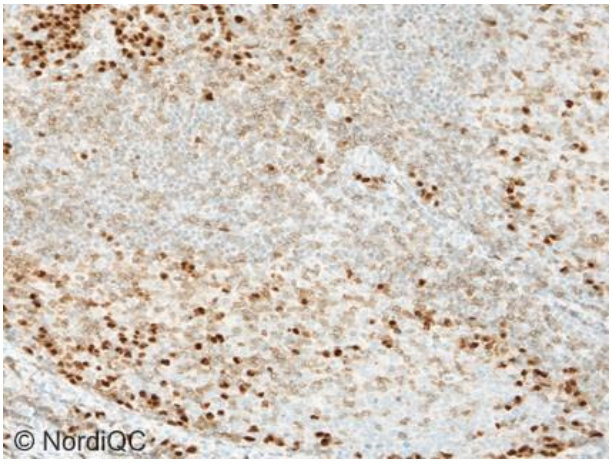



Fig. 6d (X200)
Insufficient staining for MUM1 of the tonsil using the rmAb MRQ-43 as concentrate, HIER in alkaline buffer (CC1) and a multimer based detection system (UltraView, Ventana/Roche). The late stage germinal centre B-cells show a moderate to strong nuclear staining reaction, but the lymphocytes (mostly T-cells) are aberrantly labelled displaying a weak to moderate membranous staning reaction – compare with optimal protocol in Fig. 1a.

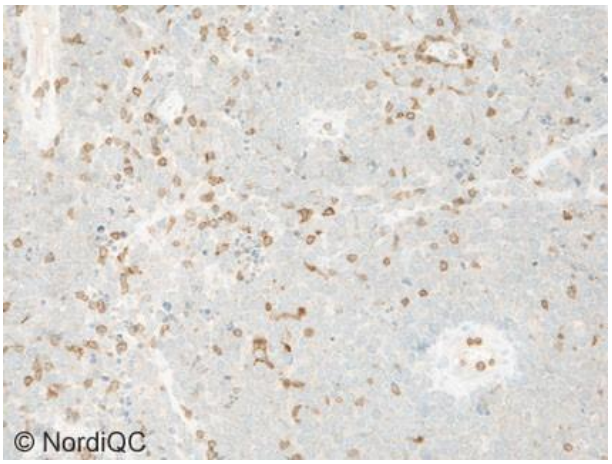



Fig. 6e (x200)
Insufficient staining for MUM1 of the GCB DLBCL, tissue core 3 using the same protocol as in Fig. 6d. T-cells are aberrantly stained compromising interpretation and it is difficult to identify normal plasma cells intermingling with the neoplastic cells of the DLBCL - compare with optimal protocol in Fig. 3a.

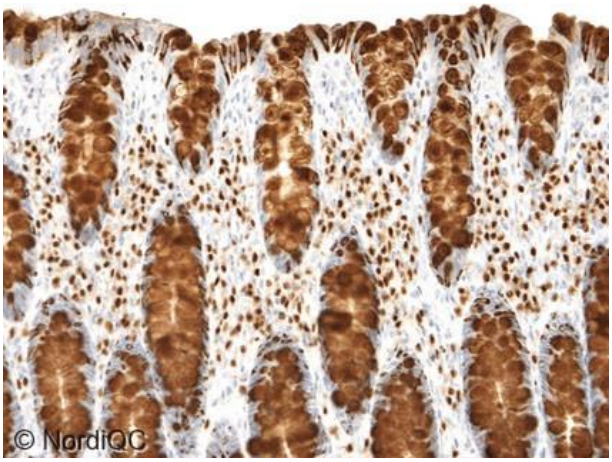



Fig. 6f (X200)
Insufficient staining for MUM1 of the colon using the mAb BC5 as concentrate. Plasma cells show a distinct and strong nuclear staining, but goblet and luminal epithelial cells of the colon are aberrantly stained displaying strong cytoplasmic reaction – compare with optimal protocol in Fig. 2a.

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