## Assessment Run 58 2020

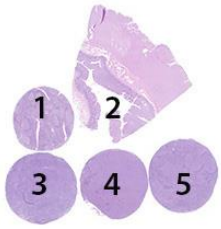
# Multiple Myeloma Oncogene 1 (MUM1)

**Purpose**
Evaluation of the technical performance, and in particular the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for MUM1, discriminating Diffuse Large B-Cell Lymphoma (DLBCL) of Germinal centre B-cell like (GCB) from non-GCB subtype. Relevant clinical tissues, both normal and neoplastic, were selected for a broad spectrum of antigen densities for MUM1 (see below). Cases diagnosed with DLBCL were classified according to Hans[1] algorithm in which the non-GCB phenotype is characterized with a cut-off value ≥30% MUM1 positive neoplastic B-cells (in absence of CD10 and presence of BCL6).

*[1]Hans CP, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275-82.*

**Material**
The slide to be stained for MUM1 comprised:

1. Tonsil, 2. Colon, 3. Mb Hodgkin (Classical type), 4. DLBCL (non-GCB subtype), 5. DLBCL (GCB subtype).




All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MUM1 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all plasma cells in lamina propria mucosae of the colon.
- A moderate to strong, distinct nuclear staining reaction of late stage germinal centre B-cells and plasma cells in the tonsil.
- An at least weak, distinct nuclear staining reaction of dispersed mantle zone lymphocytes in the tonsil.
- A strong, distinct nuclear staining reaction of the neoplastic cells (Reed-Sternberg cells) in the Hodgkin lymphoma, classical type.
- A weak to strong, distinct nuclear staining reaction of virtually all neoplastic B-cells accounting for 70% of the total cell population in the DLBCL, non-GCB subtype.
- No or only nuclear staining reaction of scattered neoplastic cells in the DLBCL, GCB subtype. Activated lymphocytes and normal plasma cells intermingling with the neoplastic cells should be positive.
- No staining reaction in other cellular structures including epithelial cells, endothelial cells and smooth muscle cells.

In this assessment, the DLBCLs were assessed based on Hans classification. In optimally calibrated protocols, approximately 70% of the neoplastic B-cells in the DLBCL, non-GCB subtype were positive displaying a weak to strong nuclear staining reaction. If the fraction of positive neoplastic B-cells was significantly reduced but still ≥30%, protocols were assessed as good.

**Participation**

| | |
|---|---|
| Number of laboratories registered for MUM1, run 58 | 277 |
| Number of laboratories returning slides | 259 (94%) |

**Results**
259 laboratories participated in this assessment. 189 (73%) 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 or use of inappropriate buffer).
- Less sensitive detection systems.
- Unexplained technical issues.

**Performance history**
This was the third NordiQC assessment of MUM1. The overall pass rate was relatively low but increased significantly compared to the result obtained in the recent run 48, 2016 (see Table 2).

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

| | Run 32 2011 | Run 48 2016 | Run 58 2020 |
|---|---|---|---|
| Participants, n= | 120 | 211 | 259 |
| Sufficient results | 58% | 60% | 73% |

**Conclusion**
The mAb clones MUM1p, EAU32 and the rmAb clone EP190 are all recommendable antibodies for demonstration of 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. Over the two latest assessments, protocols based on the rmAb MRQ-43 and the mAb BC5 have consistently produced inferior results, providing poor-signal-to-noise ratio, false positive and/or false negative results. Tonsil is recommended as positive tissue control for MUM1 in which both plasma cells and late stage germinal centre B-cells must show a moderate to strong nuclear staining reaction. Importantly, dispersed lymphocytes in the mantle zones of germinal centres must at least display a weak distinct nuclear staining reaction. Colon can be used as negative tissue control in which no staining of epithelial, endothelial and smooth muscle cells should be seen.

Table 1. **Antibodies and assessment marks for MUM1, run 58**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **MUMp1** | 93<br>3<br>1 | Agilent/Dako<br>Diagnostic Biosystem<br>Thermo Scientific | 67 | 17 | 10 | 3 | 87% | 69 % |
| mAb clone **MRQ-8** | 3 | Cell Marque | 1 | 1 | 0 | 1 | - | - |
| mAb clone **EAU32** | 5 | Leica/Novocastra | 2 | 1 | 1 | 1 | 60% | 40% |
| rmAb clone **MRQ-43** | 4 | Cell Marque | 0 | 0 | 3 | 1 | - | - |
| rmAb clone **EP190** | 2<br>1 | Cell Marque<br>PathnSitu Biotech. | 0 | 2 | 0 | 1 | - | - |
| rmAb clone **BC5** | 2 | Biocare Medical | 0 | 0 | 2 | 0 | - | - |
| rmAb clone **SP114** | 1 | Zytomed Systems | 0 | 1 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | Suff.[1] | OR[2] |
| mAb clone **MUMp1 GA644 (VRPS)**[3] | 32 | Agilent/Dako | 19 | 12 | 1 | 0 | 97% | 59 % |
| mAb clone **MUMp1 GA644 (LMPS)**[4] | 13 | Agilent/Dako | 6 | 5 | 2 | 0 | 85% | 46% |
| mAb clone **MUMp1 IR/IS644 (VRPS)**[3] | 10 | Agilent/Dako | 2 | 8 | 0 | 0 | 100% | 20% |
| mAb clone **MUMp1 IR/IS644 (LMPS)**[4] | 19 | Agilent/Dako | 12 | 6 | 1 | 0 | 95% | 63% |
| mAb clone **MUMp1 MAD-000470QD** | 1 | Master Diagnostica | 0 | 1 | 0 | 0 | - | - |
| mAb clone **EAU32 PA0129 (VRPS)**[3] | 6 | Leica Biosystems | 1 | 3 | 2 | 0 | 67% | 17% |
| mAb clone **EAU32 PA0129 (LMPS)**[4] | 7 | Leica Biosystems | 0 | 4 | 2 | 1 | 57% | 0% |
| mAb clone **MRQ-8 358M-17/18** | 1 | Cell Marque | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **MRQ-43 760-4529 (VRPS)**[3] | 3 | Ventana/Roche | 0 | 0 | 3 | 0 | - | - |
| rmAb clone **MRQ-43 760-4529 (LMPS)**[4] | 23 | Ventana/Roche | 1 | 0 | 22 | 0 | 4% | 4% |
| rmAb clone **MRQ-43 358R-77/78** | 4 | Cell Marque | 1 | 0 | 3 | 0 | - | - |
| rmAb clone **EP190 760-6082 (VRPS)**[3] | 8 | Ventana/Roche | 4 | 3 | 1 | 0 | 88% | 50% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| rmAb clone **EP190 760-6082 (LMPS)**[4] | 9 | Ventana/Roche | 1 | 4 | 4 | 0 | 56% | 11% |
| rmAb clone **EP190 358R-17/18** | 5 | Cell Marque | 1 | 1 | 3 | 0 | 40% | 20% |
| rmAb clone **EP190 8420-C010** | 1 | Sakura Finetek | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP114 AN750-5M** | 1 | BioGenex | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **BC5 PRM352** | 1 | Biocare Medical | 0 | 0 | 1 | 0 | - | - |
| Total | 259 | | 119 | 70 | 61 | 9 | - | |
| Proportion | | | 46% | 27% | 24% | 3% | 73% | |

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols)
2) Proportion of Optimal Results (OR)
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

**Detailed analysis of MUM1, Run 58**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
mAb **MUMp1**: Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (CC1) (Ventana) (42/58)*, Target Retrieval Solution (TRS) High pH (3-in-1) (Dako) (11/18) or Bond Epitope Retrieval Solution 2 (BERS2) (Leica) (13/16), as retrieval buffer. One laboratory obtained an optimal mark performing HIER in acidic buffer using Bond Epitope Retrieval Solution 1 (BERS1) (Leica) (1/2). The mAb was typically diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 69 of 78 (88%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb **EAU32**: Protocols with optimal results were based on HIER using BERS1 (Leica) (2/2) as retrieval buffer. The mAb was diluted in the range of 1:300 – 1:400 and BOND Refine (Leica) was used as detection system.

mAb **MRQ-8**: One protocol with an optimal result was based on HIER using BERS2 (Leica) as retrieval buffer. The mAb was diluted 1:100 and BOND Refine (Leica) was used as detection system.

Table 3. **Proportion of optimal results for MUM1 for the most commonly used antibody as concentrate on the 4 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link/Classic** | | **Dako Omnis** | | **Ventana BenchMark GX /XT/ Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | 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** | 4/8** (50%) | - | 6/8 (75%) | - | 36/47 (77%) | - | 10/12 (83%) | 1/2 |

* 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,** Dako, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30-40 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, 33 of 34 (97%) laboratories produced a sufficient result. Applying vendor recommended protocol settings (VRPS), the proportion of sufficient results (good or optimal) was 97% (31/32) and 59% (19/32) of the laboratories produced an optimal staining result (see Table 1 and 4).

mAb clone **MUMp1,** product no. **IR/IS644,** Dako, Autostainer+ /Autostainer Link:
Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-97°C), 15-30 min. incubation of the primary Ab and EnVision Flex/Flex+ (K8000/K8002) as detection system. Using these protocol settings, 21 of 21 (100%) laboratories produced a sufficient result.

Applying VRPS, the proportion of sufficient results was 100% (10/10) and 20% (2/10) of the laboratories produced an optimal staining result.

mAb clone **EAU32,** product no. **PA0129,** Leica, BOND III/BOND MAX:
One protocol with an optimal result was based on HIER using BERS2 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 2 of 4 (50%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 67% (4/6) and 17% (1/6) of the laboratories produced an optimal staining result.

rmAb clone **MRQ-43**, product no. **760-4529**, Ventana, BenchMark GX/XT/Ultra:
One protocol with an optimal result was based on HIER in CC1 (efficient heating time 52 min. at 100°C), 32 min. incubation of the primary Ab and UltraView (760-500) as detection system. 11% (3/27) of the laboratories applied VRPS of which none were assessed as sufficient.

rmAb clone **EP190**, product no. **760-6082**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were based on HIER in CC1 (efficient heating time 32-64 min. at 94-100°C), 32 min. incubation of the primary Ab and UltraView with or without amplification (760-500/760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 9 of 11 (82%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 88% (7/8) and 50% (4/8) of the laboratories produced an optimal staining result.

rmAb clone **EP190,** product no. **8420-C010,** Sakura FineTek, Tissue-Tek Genie Advanced Stainer:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45 min. at 98°C), 30 min. incubation time of primary Ab and Tissue-Tek Genie Pro Detection Kit DAB (8826-K250) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included (in Table 1 Laboratory Modified Protocol Settings (LMPS) also includes off label use on deviant IHC stainers).

Table 4. **Proportion of sufficient and optimal results for MUM1 for the most commonly used RTU IHC systems**

| RTU systems | **Recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako Omnis<br>mAb MUMp1<br>**GA644** | 97% (31/32) | 59% (19/32) | 78% (7/9) | 44% (4/9) |
| Dako AS<br>mAb MUMp1<br>**IR644** | 100% (10/10) | 20% (2/10) | 92% (11/12) | 58% (7/12) |
| VMS Ultra/XT/GX<br>rmAb MRQ-43<br>**760-4529** | 0/3 | 0/3 | 4% (1/23) | 4% (1/23) |
| VMS Ultra/XT/GX<br>rmAb EP190<br>**760-6082** | 88% (7/8) | 50% (4/8) | 56% (5/9) | 11% (1/9) |
| Leica Bond III<br>mAb EAU32<br>**PA0129** | 67% (4/6) | 17% (1/6) | 40% (2/5) | 0% (0/5) |

* Protocol settings recommended by vendor – retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

**Comments**
In this third 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 poor signal-to-noise ratio/false positive staining reaction compromising the interpretation. Too weak or false negative staining reaction was seen in 36% of the insufficient results (25 of 70). The majority of these laboratories were able to demonstrate MUM1 in plasma cells (all specimens), whereas demonstration of MUM1 in late stage germinal centre B-cells, dispersed lymphocytes in the mantle zones of the tonsil, the neoplastic cells of the Hodgkin Lymphoma and the neoplastic B-cells of the DLBCL (non-GCB subtype) was significantly more challenging and required a carefully calibrated protocol. In 56% (39 of 70) of the insufficient results, a poor signal-to-noise ratio and/or false positive staining reaction was seen. In the remaining insufficient

results, a combination of a too weak/false negative and background/false positive staining result was observed. The high proportion of poor signal-to-noise and false positive staining results was primarily related to the use of the rmAbs clones MRQ-43 and BC5 but could also be seen with other clones as e.g. mAb clone EAU32 and MUMp1.

mAb clone MUMp1 was the most widely used antibody for the demonstration of MUM1. Used as a concentrate in a laboratory developed (LD) assay, mAb clone MUMp1 gave an overall pass rate of 87% (84/97). As shown in Table 3, a high proportion of optimal results could be obtained on the three fully automated IHC platforms from Dako, Leica and Ventana. The most common cause of an insufficient staining result was use of a protocol with too low analytical sensitivity, typically applying several inadequate protocol settings in combination (e.g. inefficient HIER, too diluted primary Ab and a less sensitive detection system). However, it was observed that especially the choice of detection system impacted the overall performance of the assays. Applying HIER in an alkaline buffer, use of the primary antibody in the optimal dilution range (1:25-1:200) and using a 2-step multimer/polymer detection system, the pass rate was 79% (15/19) of which 37% (7/19) of the protocols were assessed as optimal. In comparison and using the same conditions except for applying a 3-step multimer/polymer detection system, the pass rate and proportion of optimal result increased significantly to 93% (54/58) and 84% (49/58), respectively. Also, the HIER conditions and especially the heating time was a critical parameter for a sufficient and optimal result. It was observed that for protocols providing an insufficient result, the average HIER time in an alkaline buffer was 33 min. (range 20-64 min.) compared to an average of 40 min. for protocols with optimal results.
Consequently, all these parameters must be optimized and carefully calibrated to provide an IHC protocol that is able to demonstrate MUM1 in cellular structures with both low- and high-level MUM1 expression and validated according to the IHC based classification algorithm for subtyping DLBCL providing both prognostic and predictive information.

In this assessment, the Agilent/Dako Ready-To-Use (RTU) systems IR/IS644 or GA644 based on the mAb clone MUMp1 provided the highest numbers of sufficient and optimal results (see Table 1 and 4). Optimal results could be obtained using both vendor and laboratory modified protocol settings (typically adjusting HIER settings, incubation time of the primary Ab or change of the recommended detection system).
For the RTU systems GA644 (Omnis) and IS/IR644 (Autostainer), the vendor recommended detection systems are Flex+ (3-step polymer system) and Flex (2-step polymer system), respectively. For all laboratories using the RTU system IS/IR644 in combination with FLEX as the detection system, 100 % (17/17) of the protocols were assessed as sufficient (optimal or good) of which 29% (5/17) were optimal. In comparison, 100% (4/4) of the protocols based on FLEX+ as detection system were assessed as optimal. Therefore, and as described for LD protocols based on the same clone, the use of a 3-step polymer detection system (Flex+) could improve performance of these assays. This is also indicated by the superior performance of GA644 using vendor recommended protocol settings and Flex+, providing the highest proportion of optimal results (59%) among RTU systems (see Table 4).

In the former run 48 (2016), best performance was obtained with the RTU system PA0129 based on the mAb clone EAU32 from Leica as all protocols were assessed as sufficient (6/6) of which 83% (5/6) were optimal. Using VRPS in this run, the pass rate declined to 67% (4/6) and 17% (1/6) optimal. The explanation for this decrease in performance is difficult to elucidate upon, but results assessed as insufficient were characterized by poor-signal-to noise ratio and/or false positive staining reactions. The same pattern was seen in assays applying laboratory modified protocol settings for the RTU system. Using mAb EAU32 within a LD-assay, the two protocols assessed as optimal was based on relatively low sensitivity protocol settings - HIER in acidic buffer (BERS1) and a low concentration of the primary Ab (see above), and thus, avoiding problems with poor-signal-to noise ratio and/or false positive staining reactions. However, the observations and conclusions must be interpreted with caution due to the low number of data.

In this assessment, the RTU system from Ventana based on the rmAb MRQ-43 (760-4529) gave inferior results and only 4% (1/26) of the protocols were assessed as sufficient. As shown in Table 4, only 12% (3/26) of the protocols were based on VRPS, whereas 88% (23/26) of the laboratories applied LMPS. Protocols based on this clone, both concentrates and RTU formats, were challenged by false positive or an aberrant cytoplasmic staining reaction of epithelial cells and smooth muscle cells in lamina muscularis propria of the colon compromising the interpretation and identification of the specific nuclear expression of MUM1. Thus, to improve the general performance of the individual assays for the immunohistochemical demonstration of MUM1, it is advisable to change the primary antibody to e.g. MUMp1 or EP190 and recalibrate and validate the protocol. For detailed description and representative illustrations of the problems, see report Run 48, 2016.

The RTU system based on the rmAb clone EP190, prod.no. 760-6082 (Ventana), seems to be a superior alternative compared to the RTU system based on the rmAb MRQ-43. Using VRPS, the pass rate was 88% (7/8) of which 50% (4/8) of the protocols were assessed as optimal (see Table 4). LMPS could also be used but pass rate and proportion of optimal results declined to 56% (5/9) and 11% (1/9), respectively. The main feature of an insufficient result was a too weak staining reaction of cells expected to be positive.

The use of RTU formats increased by 5% to 56% (144/259) compared to 51% (108/211) in run 48, 2016. Applying LMPS for the RTU products from the three major vendors, twelve laboratories used an RTU format off-label on a non-validated IHC platform. Of these, 77% (10/13) applied the RTU products from Dako, IR644 or GA644 developed for Autostainer and Omnis, on the Ventana Benchmark platform. All were assessed as sufficient of which 70% (7/10) provided an optimal mark. Although the quality and staining performance was high for these assays, it is in general not recommendable to use an RTU format of-label as these formats has been calibrated within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected.

This was the third assessment of MUM1 in NordiQC and a pass rate of 73 % was obtained, which is a significant increase compared to the result obtained in run 48, 2016 (see Table 2). The most important parameters causing insufficient results were:
1) Use of the rmAb clone MRQ-43 typically provided poor-signal-to-noise and/or false positive staining results. In addition, protocols were also challenged by weak and/or false negative staining results. In total for the two last runs, only 2% (2/87) of the protocols based on the rmAb MRQ-43 has been assessed as sufficient.
2) Use of LD assays with too low analytical sensitivity typically applying more than one problematic protocol parameter at the same time e.g. inefficient HIER in combination with a too diluted primary Ab.
3) Use of a less sensitive 2-step multimer/polymer detection system e.g. UltraView (Ventana) or Flex (Dako).
Importantly, laboratories should use a robust Ab, calibrate the protocols correctly and verify the results accordingly to the expected antigen level of the recommended control materials (see below).

**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 reaction. Plasma cells are strongly stained, and a weak cytoplasmic staining should be accepted. A weak nuclear staining reaction should be seen in dispersed lymphocytes situated in the mantle zones of germinal centres.
Colon is recommended as negative tissue control. Epithelial cells, endothelial and smooth muscle cells (lamina muscularis propria) should be negative, whereas plasma cells located to mucosa (lamina propria) should display a strong nuclear reaction.

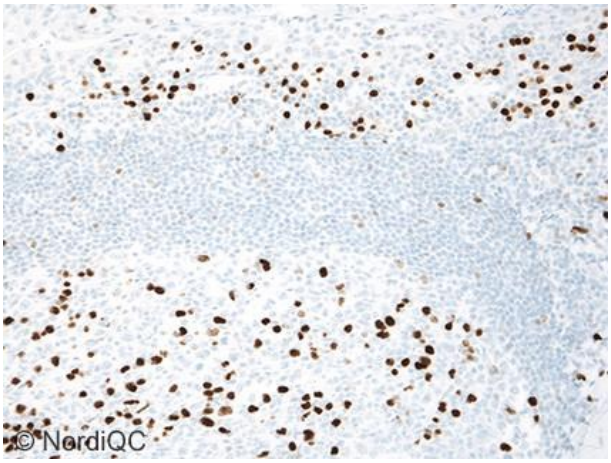



Fig. 1a (x200)
Optimal staining for MUM1 of the tonsil using the mAb MUMp1 as a concentrate, HIER in an alkaline buffer (CC1) and a 3-step multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 5a. The late stage germinal centre B-cells show a distinct, moderate to strong nuclear staining reaction and dispersed lymphocytes in the mantle zone display the expected weak intensity.

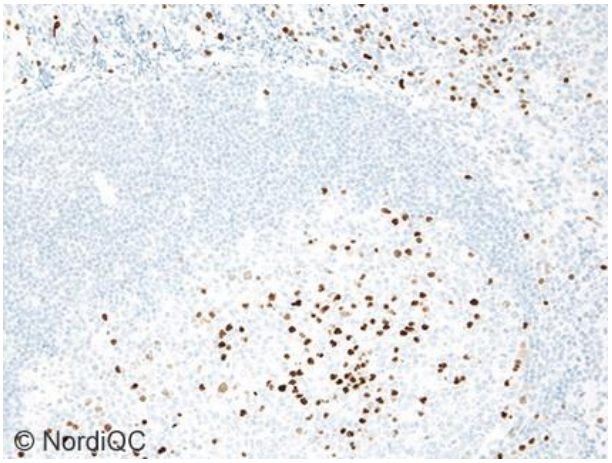



Fig. 1b (x200)
Insufficient staining for MUM1 of the tonsil using the mAb clone MUMp1 as concentrate (too diluted), inefficient HIER in CC1 (too short time) and OptiView as the detection system - same protocol used in Figs. 2b – 5b. Intensity of the staining reaction is weaker and importantly, the proportion of dispersed lymphocytes in the mantle zone of the germinal centre is reduced and positive cells are only faintly demonstrated - compare with Fig. 1a (same field).

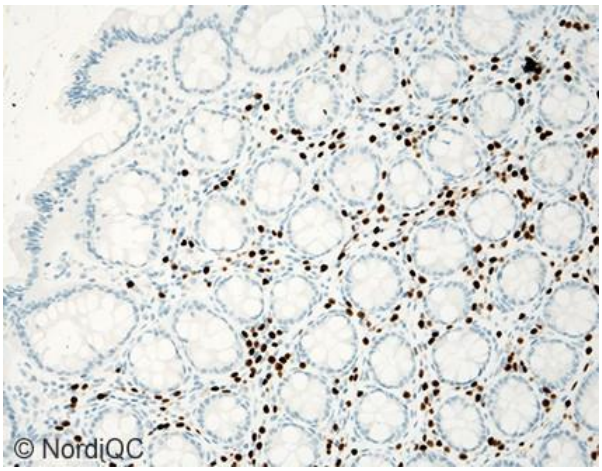



Fig. 2a (x200)
Optimal staining for MUM1 in the colon using same protocol as in Fig. 1a. Plasma cells show a distinct and strong nuclear staining reaction, while all other structures are negative.

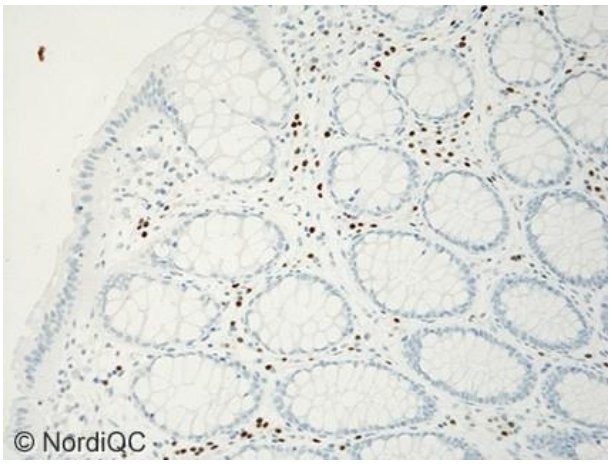



Fig. 2b (x200)
Insufficient staining for MUM1 in the colon using same protocol as in Fig 1b. The number and staining intensity of plasma cells is reduced - compare with Fig. 2a (same field).

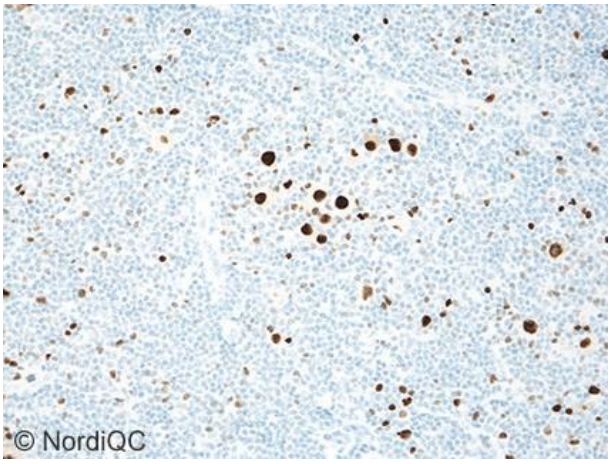



Fig. 3a (x200)
Optimal staining for MUM1 of the Hodgkin Lymphoma using same protocol as in Figs. 1a and 2a. Virtually all the neoplastic cells (Reed Sternberg cells) display a strong nuclear staining intensity, whereas scattered activated lymphocytes/normal plasma cells show a weak to strong but distinct nuclear staining reaction.

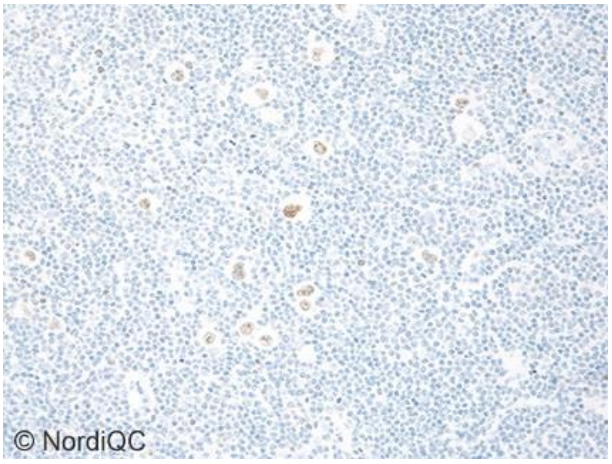



Fig. 3b (x200)
Insufficient staining for MUM1 of the of the Hodgkin Lymphoma using same protocol as in Figs. 1b and 2b. The Reed Sternberg cells only display a faint to weak nuclear staining intensity and activated lymphocytes/normal plasma cells intermingling with the neoplastic cells are not demonstrated - compare with Fig. 3a (same field).

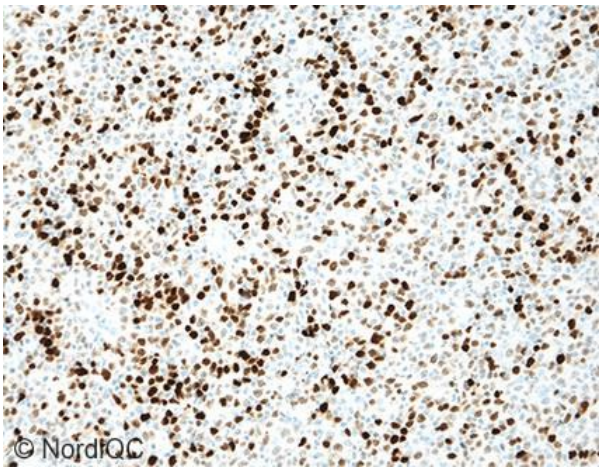



Fig. 4a (x200)
Optimal staining for MUM1 of the DLBCL, non-GCB subtype using same protocol as in Figs. 1a - 3a. Approximately 70% of the neoplastic B-cells show a weak to strong nuclear staining reaction.

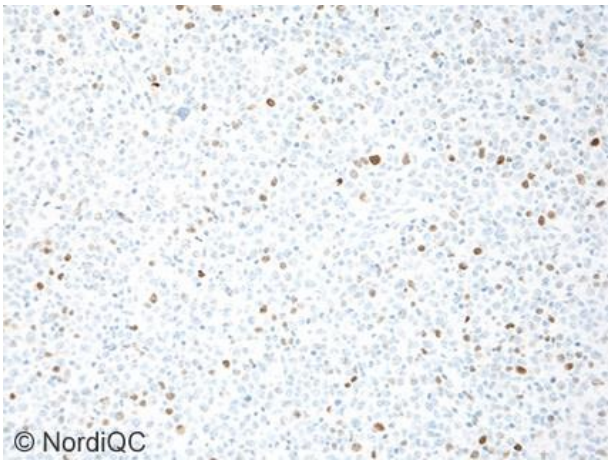



Fig. 4b (x200)
False negative staining for MUM1 of the DLBCL, non-GCB subtype using same protocol as in Figs. 1b -3b. The proportion and intensity of positive neoplastic B-cells is significantly reduced and below the cut-off of 30% using Hans algorithm - compare with Fig. 4a (same field).

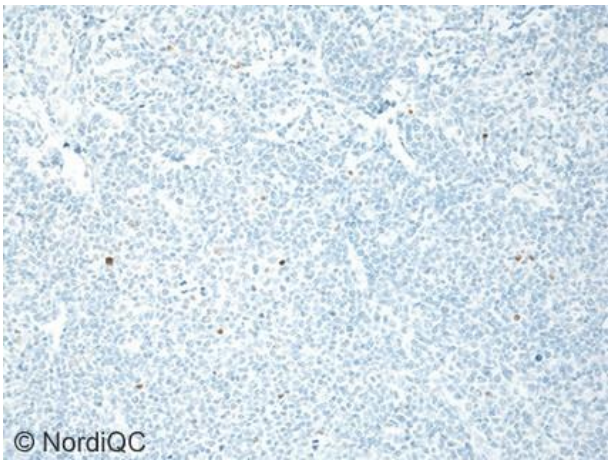



Fig. 5a (x200)
Optimal staining for MUM1 of the DLBCL, GCB subtype using same protocol as in Figs. 1a - 4a. Virtually all the neoplastic cells are negative and thus, was classified as DLBCL, GCB subtype according to Hans algorithm. Only scattered activated lymphocytes and plasma cells display weak to strong but distinct nuclear staining reaction.

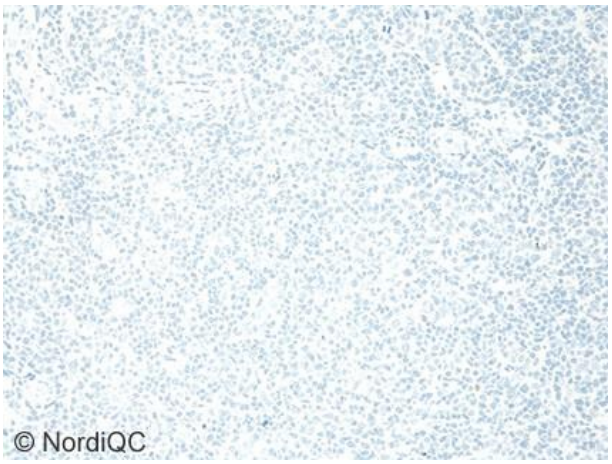



Fig. 5b (x200)
Staining for MUM1 of the DLBCL, GCB subtype using same insufficient protocol as in Figs.1b - 4b. Although this DLBCL can be classified correctly using Hans algorithm, the protocol provides a general reduced analytical sensitivity and false negative staining result of the DLBCL, non-GCB subtype (see Figs. 4a-4b). In the DLBCL, GCB subtype, activated lymphocytes and plasma cells intermingling between the neoplastic B-cells are completely negative - compare with Fig. 5a (same field).

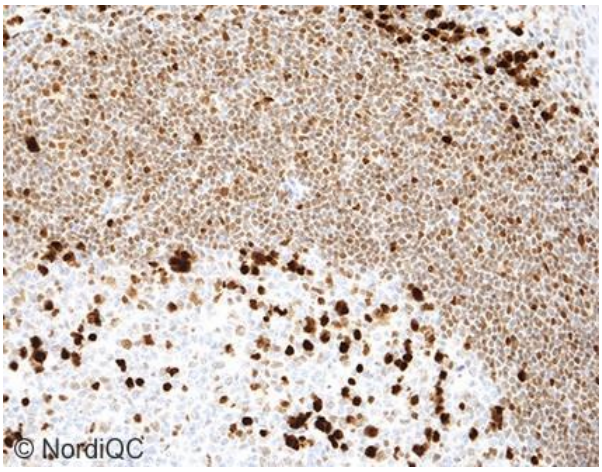



Fig. 6a (x200)
Insufficient staining for MUM1 of the tonsil using the mAb EAU32 with a protocol providing a too high level of analytical sensitivity (too high concentration and too long incubation time of the primary Ab, HIER in BERS1 and Bond Refine (Leica/Novocastra) as the detection system). The late stage germinal centre B-cells and plasma cells show the expected reaction pattern, but virtually all lymphocytes in the mantle zone and an increased number of B-cells within the germinal centre are positive. The impact of this aberrant reaction pattern can be seen in fig. 6b - also compare with optimal protocol in Fig. 1a.

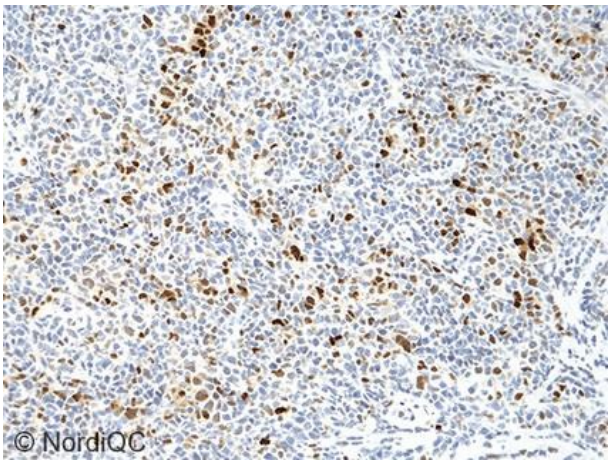



Fig. 6b (x200)
Aberrant staining for MUM1 of the DLBCL, GCB subtype using the same protocol settings as in Fig. 6a. The proportion of positive cells was assessed to be higher than the cut-off value of 30% and thereby could be misclassified as a DLBCL of non-GCB subtype according to Hans algorithm – compare with optimal protocol in Fig 5a. In addition, a cytoplasmic staining reaction is seen compromising the interpretation.

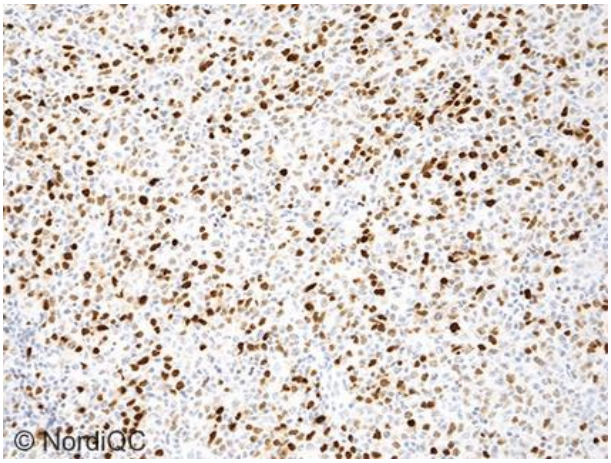



Fig. 7a (x200)
Optimal staining for MUM1 of the DLBCL, non-GCB subtype using mAb MUMp1 as RTU format (GA644, Dako/Agilent) and VRPS based on HIER in TRS pH 9 (3-in-1) for 30 min. (97°), 20 min. incubation time in primary Ab and Flex+ as the detection system. The assay provided the expected reaction pattern and proportion of neoplastic B-cells to be positive - compare with Fig. 4a.

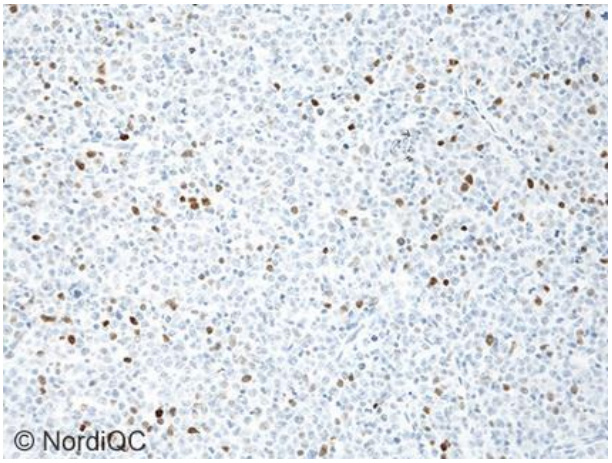



Fig. 7b (X200)
Insufficient staining for MUM1 of the DLBCL, non-GCB subtype using exactly the same system as in Fig. 7a, but with LMPS based on HIER in TRS pH 6 (3-in-1) and reduced time in the antigen retrieval buffer (24 min.). The intensity and proportion of positive neoplastic B-cells is significantly reduced, and below the cut-off of 30% using Hans algorithm, thus risking misclassification as a DLBCL of GCB subtype – compare with optimal protocol in Fig. 7a.

MB/RR/LE/SN 24.03.2020