Assessment run 77 2026

MUM1 (Multiple Myeloma Oncogene 1)

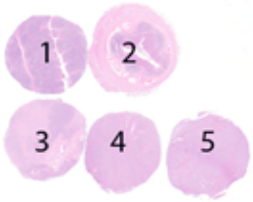
Purpose

Evaluation of the technical performance, analytical sensitivity and specificity of MUM1 IHC tests among the NordiQC participants, typically used to discriminate Diffuse Large B-Cell Lymphoma (DLBCL) of Germinal centre B-cell like (GCB) from non-GCB subtype.

Relevant clinical normal and neoplastic tissues were selected to include a broad spectrum of MUM1 antigen expression levels (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 cases of CD10 below 30% and presence of BCL6 in more than 30%).

Material

The slide to be stained for MUM1 comprised:



1. Tonsil, 2. Appendix, 3. Classical Hodgkin Lymphoma, 4. DLBCL (GCB subtype), 5. DLBCL (non-GCB subtype).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing MUM1 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all plasma cells in lamina propria mucosae of the appendix.
- A moderate to strong, distinct nuclear staining reaction of late-stage germinal centre B-cells and plasma cells in the tonsil.
- At least weak, distinct nuclear staining reaction of single, dispersed mantle zone lymphocytes (most likely late-stage B-cells) in the tonsil. The vast majority of resting B-cells in the mantle zones must remain negative.
- A strong, distinct nuclear staining reaction of the neoplastic cells (Reed-Sternberg cells) in the classical Hodgkin lymphoma.
- 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.

KEY POINTS FOR MUM1 IMMUNOASSAYS

- The mAb clone MUM1p was used by 52% of all laboratories.
- The VRPS assay **GA644** based on the mAb clone MUM1p produced 100% sufficient results, 98% optimal.
- In this assessment it was noted that 51% of all assessed protocols produced a cytoplasmic staining reaction. This reaction was downgraded to insufficient if the staining intensity interfered with the nuclear reaction.

Participation

Number of laboratories registered for MUM1, run 77	408
Number of laboratories returning slides	363 (89%)

Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results
363 laboratories participated in this assessment and 332 (91%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarise the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody.
- Insufficient HIER (too short heating time).
- Less sensitive detection systems (2-step detection e.g EnVision FLEX)

Controls
In concordance with previous assessments for MUM1, 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. Appendix may be used as negative tissue control, as epithelial, endothelial and smooth muscle cells should remain negative, however plasma cells and late-stage B-cells are expected to be positive.

Conclusion
The mAb clones **MUM1p** and **EAU32**, as well as the rmAb clone **EP190**, are all suitable antibodies for demonstrating MUM1. However, in this assessment, only **MUM1p** and **BC5** (n=1) produced optimal results, as the other clones showed an indistinct cytoplasmic reaction. MUM1p achieved optimal results on all automated IHC platforms. Despite the cytoplasmic reaction, the robust performance of these clones resulted in a high proportion of sufficient results. In all cases, HIER in alkaline buffer, precise calibration of the primary antibody, and the use of a multimer or a 3-step polymer-based detection system were key prerequisites for a sufficient result.

Table 1a. **Overall results for MUM1, run 77**

	n	Optimal	Good	Borderline	Poor	Sufficient results[1]	Optimal results
Concentrated antibodies	93	53	30	9	1	89%	56%
Ready-To-Use antibodies	270	96	153	19	2	92%	36%
Total	363	149	183	28	3		
Proportion		41%	50%	8%	1%	91%	

1) Optimal and good

Table 1b. **Concentrated antibodies and assessment marks for MUM1, run 77**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **MUM1p**	74	Dako/Agilent	50	20	4	-	94%	68%
	3	Diagnostic Biosystems	2	-	1	-		
mAb clone **MRQ-8**	1	Cell Marque	-	1	-	-	-	-
mAb clone **EAU32**	6	Leica Biosystems	-	4	2	-	67%	0%
rmAb clone **EP190**	2	Cell Marque	-	1	-	1	60%	0%
	2	PathNSitu	-	1	1	-		
	1	BioSB	-	1	-	-		
rmAb clone **BC5**	1	Biocare Medical	1	-	-	-	-	-
rmAb clone **GR28**	1	Genebio Solution	-	-	1	-	-	-
rmAb clone **QR075**	1	Quartett	-	1	-	-	-	-
rmAb clone **ZR411**	1	Zeta Corporation	-	1	-	-	-	-
Total	93		53	30	9	1		
Proportion			57%	32%	10%	1%	89%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for MUM1, run 77**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP190 760-6082 (VRPS)[3]**	53	Ventana/Roche	-	47	6	-	89%	0%
rmAb clone **EP190 760-6082 (LPMS)[4]**	47	Ventana/Roche	-	44	2	1	94%	0%
rmAb clone **MRQ-43 760-4529***	1	Ventana/Roche	-	1	-	-	-	-
mAb clone **MUM1p GA644 (VRPS)[3]**	63	Dako/Agilent	62	1	-	-	100%	98%
mAb clone **MUM1p GA644 (LPMS)[4]**	20	Dako/Agilent	18	2	-	-	100%	90%
mAb clone **MUM1p IR644 (VRPS)[3]**	9	Dako/Agilent	3	6	-	-	100%	33%
mAb clone **MUM1p IR644 (LPMS)[4]**	18	Dako/Agilent	13	3	2	-	89%	72%
mAb clone **EAU32 PA0129 (VRPS)[3]**	21	Leica Biosystems	-	20	1	-	95%	0%
mAb clone **EAU32 PA0129 (LPMS)[4]**	11	Leica Biosystems	-	7	4	-	64%	0%
mAb clone **MRQ-8 358M-17/18**	1	Cell Marque	-	1	-	-	-	-
mAb clone **MUM1p MAD-000470QDS**	2	Master Diagnostica/Vitro SA	-	2	-	-	-	-
mAb clone **MUM1p NOA-MSG125**	1	Zytomed Systems GmbH	-	1	-	-	-	-
mAb clone **C6H9 CMM-0303**	1	Celnovte	-	1	-	-	-	-
mAb clone **MX093 MAB-0885**	1	Fuzhou Maixin	-	-	1	-	-	-
mab clone **IHC627 IHC627-7**	1	GenomeMe	-	1	-	-	-	-
mAb clone **ABT080 BFM-0470**	1	Bioin Biotechnology	-	1	-	-	-	-
rmAb clone **EP190 358R-17/18**	14	Cell Marque	-	11	2	1	79%	0%
rmAb clone **EP190 HAR112**	1	PathNSitu	-	1	-	-	-	-
rmAb clone **EP190 GT2289**	1	Gene Tech	-	-	1	-	-	-
rmAb clone **RM352 BJ9014**	1	Guangzhou Biotron	-	1	-	-	-	-
rmAb clone **DY49557 4920582**	1	Immunologic	-	1	-	-	-	-
rmAb clone **990G3D2 PA198**	1	Abcarta	-	1	-	-	-	-
Total	270		96	153	19	2		
Proportion			35%	57%	7%	1%	92%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
*discontinued products

Detailed analysis of MUM1, run 77

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **MUM1p**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) High pH (5/9)*, Cell Conditioning 1 (CC1, Ventana/Roche) (43/49), BOND Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (4/14) as retrieval buffer. The mAb was typically diluted between 1:20-600. Using these protocol settings, 67 of 70 (96%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for MUM1 on the four main IHC systems by optimal titration settings as listed above**

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **MUM1p** 1:20-600	0/3	-	83% (5/6)	-	87% (41/47)	-	29% (4/14)	0/3

1) Autostainer Link 48.
2) BenchMark GX, Ultra, Ultra plus
3) BOND III, Prime

Ready-To-Use antibodies and corresponding systems

rmAb clone **EP190**, product no. **760-6082**, Ventana/Roche, BenchMark Ultra/Ultra Plus:
No optimal results were produced with this product on a BenchMark platform.
Sufficient protocols using UltraView (760-500) as detection system were typically based on HIER using CC1 (efficient heating time 32-64 min.) and 28-40 min. incubation of the primary Ab.
Sufficient protocols using OptiView (760-700) as detection system were typically based on HIER using CC1 (efficient heating time 32-64 min.) and 24-40 min. incubation of the primary Ab.

mAb clone **MUM1p,** product no. **GA644,** Dako, Omnis:
Optimal protocols were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-40 min. at 97°C), 20-40 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 78 of 78 (100%) laboratories produced a sufficient result.
The product was used by 3 laboratories on a non-intended platform. This data is not included.

mAb clone **MUM1p,** product no. **IR644,** Dako, Autostainer+ /Autostainer Link:
Optimal protocols were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 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, 11 of 11 (100%) laboratories produced a sufficient result.
The product was used by 13 laboratories on a non-intended platform. This data is not included.

mAb clone **EAU32,** product no. **PA0129,** Leica, BOND III/BOND Prime:
No optimal results were produced with this product on a BOND platform.
Sufficient protocols using BOND Refine (DS9800) or BOND-Prime polymer detection systems (DS9284) as detection system were typically based on HIER using BERS2 (efficient heating time 15-30 min.) and 15-30 min. incubation of the primary Ab.

Table 3 summarises the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems with changes to basic protocol settings. Only protocols performed on the intended IHC stainer are included.

Table 3. **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
VMS BenchMark rmAb **EP190** **760-6082** **UltraView**	93% (25/27)	0% (0/27)	89% (16/18)	0% (0/18)
VMS BenchMark rmAb **EP190** **760-6082** **OptiView**	84% (21/25)	0% (0/25)	96% (27/28)	0% (0/28)
Dako Omnis mAb **MUM1p** **GA644**	100% (63/63)	98% (62/63)	100% (17/17)	100% (17/17)
Dako AS mAb clone **MUM1p** **IR644**	100% (9/9)	33% (3/9)	80% (4/5)	60% (3/5)
Leica BOND mAb **EAU32** **PA0129**	95% (20/21)	0% (0/21)	64% (7/11)	0% (0/11)

*Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
**Modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this fourth NordiQC assessment of MUM1, the prevalent feature of an insufficient result was characterized by a poor signal-to-noise ratio and/or false positive staining reaction compromising the interpretation. This was seen in 52% of all insufficient results and was primarily related to the use of the mAb clone EAU32 and rmAb EP190 but could also be seen with other clones.

Too weak or false negative staining reaction was seen in 48% of the insufficient results (15 of 31). 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.

A general issue observed in this run was an additional cytoplasmic reaction in cells with a positive MUM1 nuclear staining reaction. This was seen in 51% (186/363) of all stained protocols. In many cases, cytoplasmic staining interfered with interpretation. Cores were therefore downgraded to insufficient when the cytoplasmic reaction was of equal intensity to the nuclear reaction, as seen in neoplastic tissue core no. 5 (non-GCB). Aberrant excessive cytoplasmic staining reaction was seen in more of the tissue samples included in the TMA used for this assessment run 77 for MUM1p and not isolated to one tissue sample.

mAb clone **MUM1p** was the most widely used antibody for the demonstration of MUM1. Used as a concentrate in a laboratory developed (LD) assay, mAb clone MUM1p gave an overall pass rate of 94% (72/77). As shown in Table 2 optimal results could be obtained on the three fully automated IHC platforms from Dako, Leica and Ventana. Optimal results were primarily produced in the dilution range of 1:300-600, with a 3-step detection system. However, the pass rate was significantly lower on the BOND platforms compared to both Ventana/Roche platforms and Dako Omnis. As mentioned, optimal results were obtained, however the BOND platforms were prone to increase the cytoplasmic reaction especially when using the antibody in a too concentrated format (less than 1:200).

The most common cause of insufficient staining results on the Ventana/Roche or Dako/Agilent platforms 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). 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 use of RTU formats increased from 56% (144/259) in the previous run 58 to 74% (270/363) in this run 77. The Agilent/Dako Ready-To-Use (RTU) systems **IR644** or **GA644** based on the mAb clone MUM1p provided the highest numbers of sufficient and optimal results (see Table 1c and 3). 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).

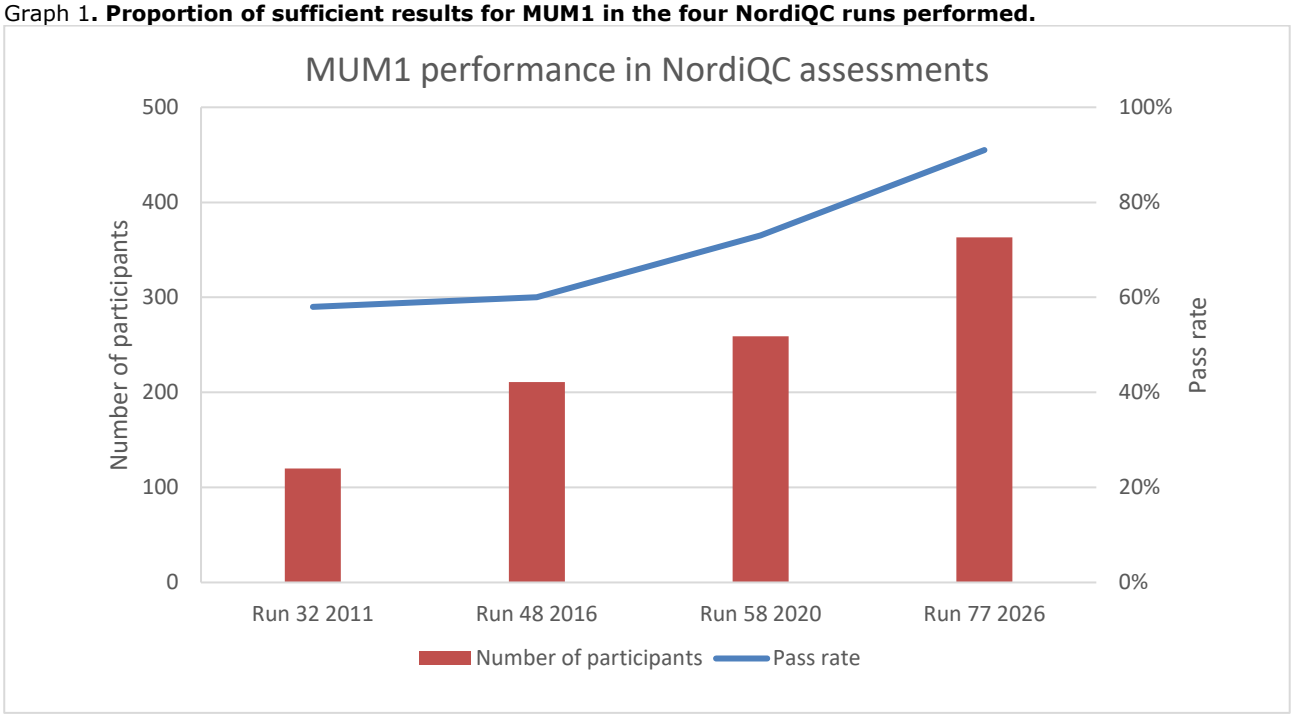
For the RTU systems **IR644** (Autostainer) and **GA644** (Omnis), the vendor recommended detection systems are FLEX (2-step polymer system) and FLEX+ (3-step polymer system) respectively. For all laboratories using the RTU system IR644 in combination with FLEX as the detection system, 100 % (11/11) of the protocols were assessed as sufficient (optimal or good) of which 36% (4/11) were optimal. In comparison, 83% (5/6) of the protocols based on FLEX+ as detection system for the IR644 format were assessed as optimal. Therefore, 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 (98%) among RTU systems (see Table 1c and 3).

In this assessment, the RTU system from Ventana/Roche based on the rmAb clone **MRQ-43** (**760-4529**) which was the most popular product from Ventana/Roche in the previous run 58 has been discontinued. It has been replaced with rmAb clone **EP190**, product no. **760-6082** (Ventana/Roche) which was already introduced in run 58 with promising results.
The rmAb clone EP190 product **760-6082** was in total used by 100 laboratories with a pass rate of 91%, however no optimal results. The rmAb clone EP190 did in general produce a cytoplasmic staining reaction being observed in 93% (93/100) of all protocols using this assay (see Figs 6a+b). In 7%, no interfering cytoplasmic staining reaction was observed, but the overall result was scored with a comment on a too weak specific signal. This demonstrates the challenge of reducing non-specific cytoplasmic staining and improving analytical specificity, without compromising the required analytical and diagnostic sensitivity.
When comparing 2- and 3-step detection system the vendor recommended protocol settings provided very similar pass rates with a high proportion of sufficient results e.g. 93% and 85% respectively. A total of 48 laboratories used the 760-6082 products as a LD assay, mostly increasing incubation times of either primary Ab or HIER providing pass rates of 89% (UltraView) and 96% (OptiView).
The assay seems very robust and was able to demonstrate all cells expected to be positive for MUM1, however it was not possible with the submitted protocols to make changes to lower the cytoplasmic reaction and maintain a distinct nuclear reaction.

The RTU system **PA0129** based on mAb clone **EAU32** from Leica Biosystems provided a high pass rate of 95% when used by recommended protocol settings, but no optimal results was observed. This is in contrast to the previous run 48 (2016) in which all protocols were assessed as sufficient (6/6) and 83% (5/6) were optimal. Using VRPS in this run, the pass rate maintained at a high level of 95% (20/21) but as mentioned, no optimal results. All protocols displayed a cytoplasmic staining reaction as described previously. In addition, 22% also provided a false positive reaction in the resting B-cells of the mantle zones and/or an increased number of positive cells than expected to be demonstrated (see Figs 7a-b). As a concentrated format of the mAb clone EAU32, the product was submitted on both Ventana BenchMark and Leica BOND platforms in the dilution range of 1:50-400. All protocols did produce a cytoplasmic reaction even with an overall reduced analytical sensitivity.

Performance history
This was the fourth NordiQC assessment of MUM1. The pass rate increased to 91% in this assessment compared with the previous assessment in run 58 as shown in Graph 1.

Graph 1**. Proportion of sufficient results for MUM1 in the four NordiQC runs performed.**



Summary
This was the fourth assessment of MUM1 in NordiQC and a pass rate of 91 % was obtained, which is a significant increase compared to the result obtained in run 58, 2020 (see graph 1). However, the number of optimal results has declined from 46% to 41%. The main reason for an increase in pass rate is primarily due to the use of more robust assays with the level of analytical and diagnostic sensitivity expected for the MUM1 assay. However, many of these assays seem to struggle with the analytical specificity. **MUM1p** was the only widely used clone that consistently produced a distinct nuclear staining reaction without substantial cytoplasmic interference. In run 58, it was already observed that the mAb **EAU32** and rmAb clone **EP190** produced an extended cytoplasmic staining reaction compared to the level seen for e.g. clone MUMp1. In this run 77, the TMAs used for MUM1 assessment contained tissue samples from different patients compared to the previous TMAs, thereby changing their composition, which rendered the cytoplasmic staining reaction issue more apparent. This clearly displays a problem for the interpretation in situations where cytoplasm is stained as strongly as the nuclear compartment.

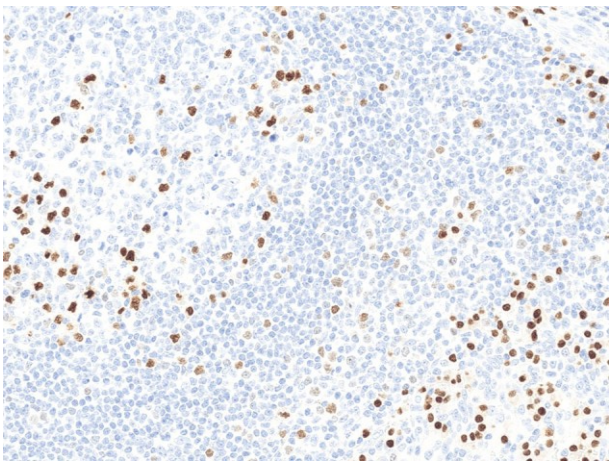


Fig. 1a
Optimal staining result for MUM1 of the tonsil using the mAb clone MUM1p as a concentrate, HIER in an alkaline buffer (CC1) and a 3-step multimer based detection

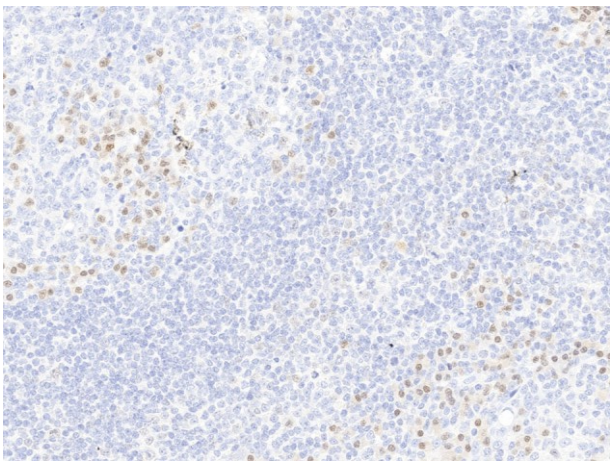


Fig. 1b
Insufficient staining result for MUM1 of the tonsil using the rmAb clone EP190 as RTU, inefficient HIER in CC1 (too short time) and UltraView as the detection system

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.

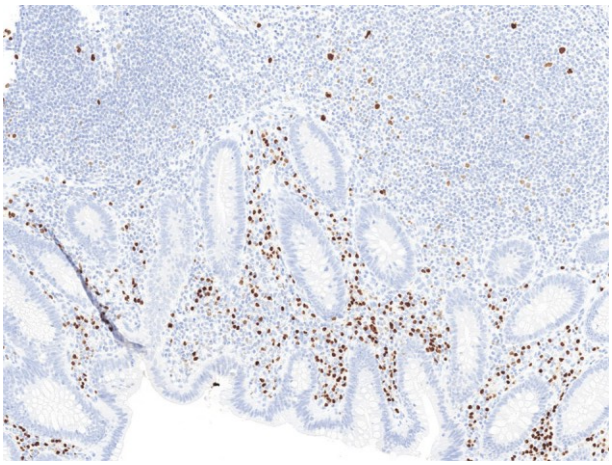


Fig. 2a
Optimal staining result for MUM1 in the appendix using the same protocol as in Fig. 1a. Plasma cells show a distinct and strong nuclear staining reaction, while all other structures are negative.

- 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 weakly demonstrated - compare with Fig. 1a (same field).

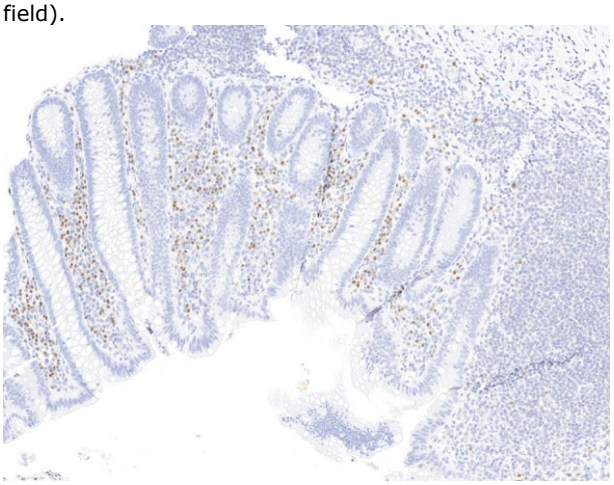


Fig. 2b
Insufficient staining result for MUM1 in the appendix using the same protocol as in Fig 1b. The number and staining intensity of plasma cells is significantly reduced - compare with Fig. 2a.

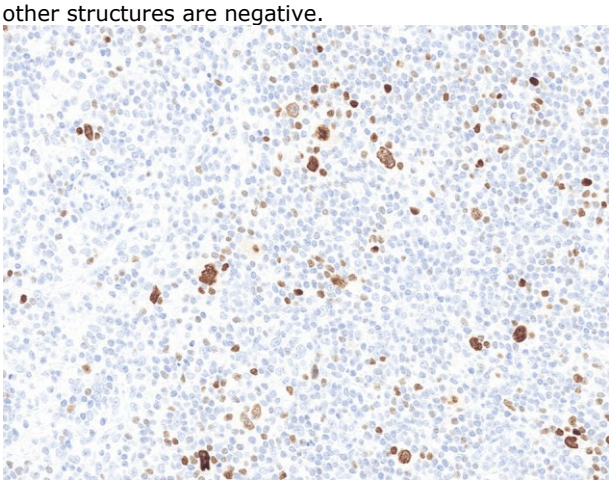


Fig. 3a
Optimal staining result for MUM1 of the Hodgkin Lymphoma using the same protocol as in Figs. 1a and 2a. Virtually all the neoplastic cells (Reed Sternberg cells) display a moderate-strong nuclear staining intensity, whereas scattered activated lymphocytes/normal plasma cells show a weak to strong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

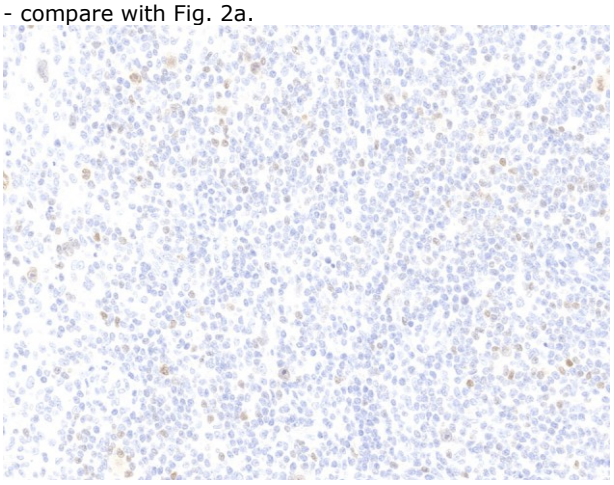


Fig. 3b
Insufficient staining result for MUM1 of the Hodgkin Lymphoma using the same protocol as in Figs. 1b and 2b. The Reed Sternberg cells can hardly be identified and only display a faint to weak nuclear staining intensity. Intermingling activated lymphocytes/normal plasma cells are virtually all negative - compare with Fig. 3a (same field).

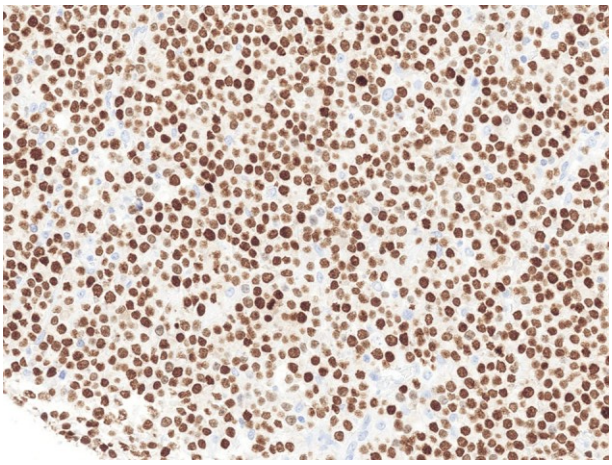


Fig. 4a
Optimal staining result for MUM1 of the DLBCL, non-GCB subtype using the same protocol as in Figs. 1a - 3a. The vast majority of neoplastic B-cells show a moderate to strong nuclear staining reaction.

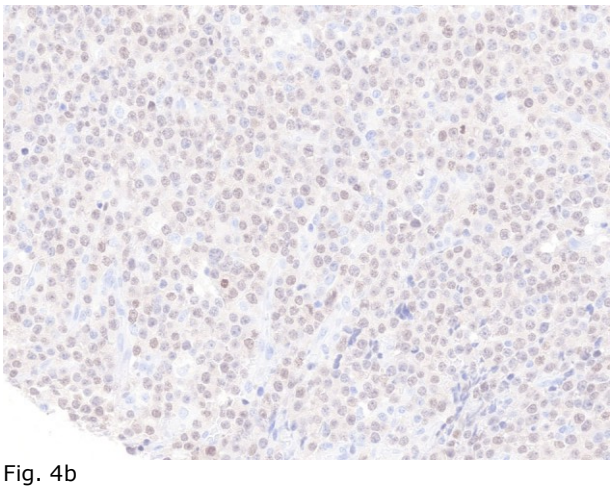


Fig. 4b
Insufficient staining result for MUM1 of the DLBCL, non-GCB subtype using the same protocol as in Figs. 1b -3b. The proportion and intensity of positive neoplastic B-cells is significantly reduced - compare with Fig. 4a (same field).

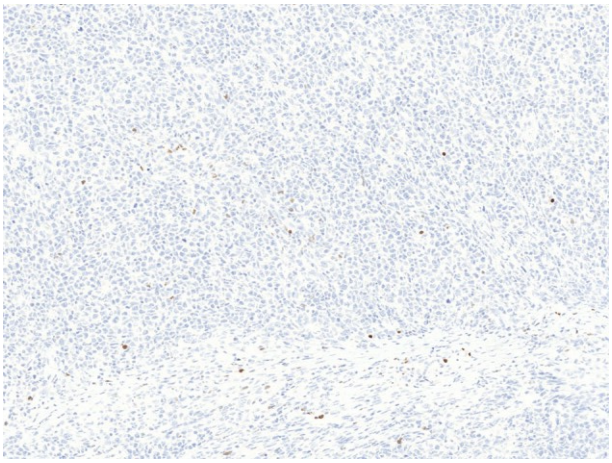


Fig. 5a
Optimal staining result for MUM1 of the DLBCL, GCB subtype using the same protocol as in Figs. 1a - 4a. Virtually all neoplastic cells are negative. Together with the corresponding CD10 and BCL6 results, this supported classification as DLBCL, GCB subtype according to the Hans algorithm.
Only scattered activated lymphocytes and plasma cells display weak to strong but distinct nuclear staining reaction.

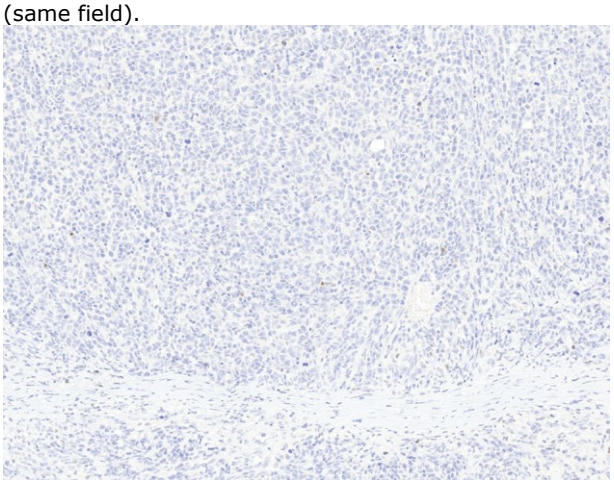


Fig. 5b
Staining for MUM1 of the DLBCL, GCB subtype using the same insufficient protocol as in Figs.1b - 4b. Although this DLBCL can be classified correctly using Hans algorithm, the protocol provides a general reduced analytical sensitivity and false negative staining result of the DLBCL, non-GCB subtype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

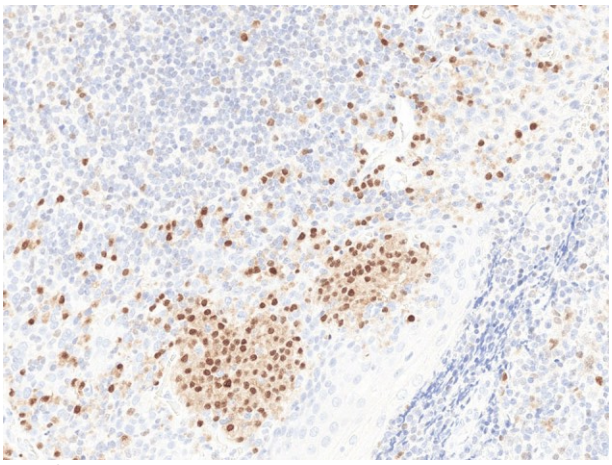


Fig. 6a
Sufficient staining result for MUM1 of the tonsil using rmAb clone EP190 as RTU from Ventana/Roche with vendor recommended protocol settings for OptiView. Same protocol used in Fig. 6b.
Even though the cells are stained with the expected staining intensity almost all cells express an aberrant cytoplasmic staining reaction, down grading the assessment score from optimal to good. Also compare with Fig 1a.

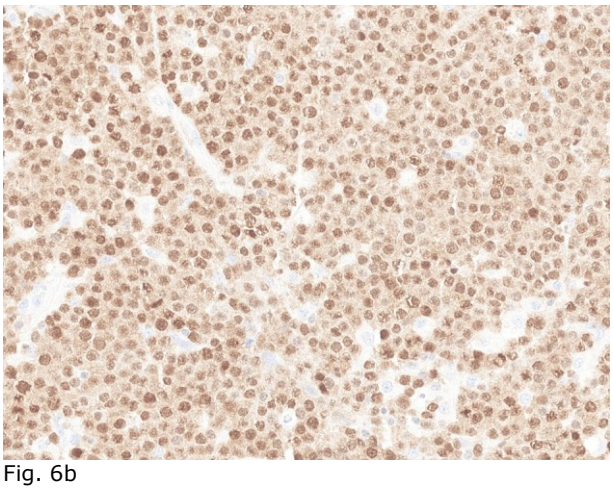


Fig. 6b
Sufficient MUM1 staining result of the DLBCL, non-GCB subtype, using the same protocol as in Fig. 6a. The neoplastic cells lack a distinct nuclear staining reaction, and the cytoplasmic staining intensity is almost comparable to the nuclear intensity in many cells. Despite this cytoplasmic interference, the tumour would not be misclassified according to Hans algorithm. Also compare with Fig. 4a.

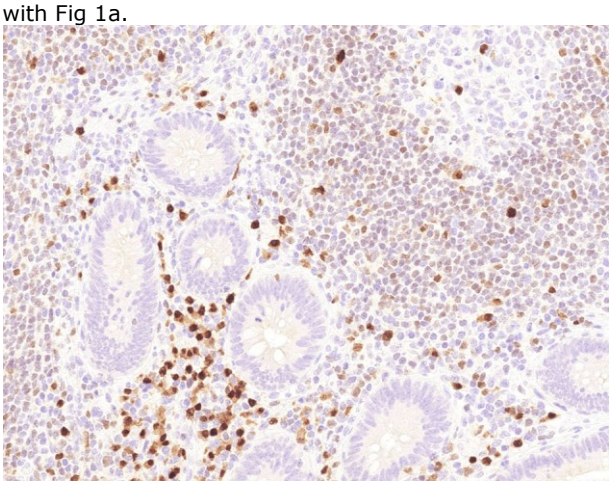


Fig. 7a
Insufficient staining result for MUM1 of the tonsil using the mAb clone EAU32 with a protocol providing a too high level of analytical sensitivity (too long HIER in BERS2 and BOND Refine (Leica Biosystems) as the detection system). Same protocol used in Fig 7b.
The late-stage germinal centre 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. 7b - also compare with optimal protocol in Fig. 1a.

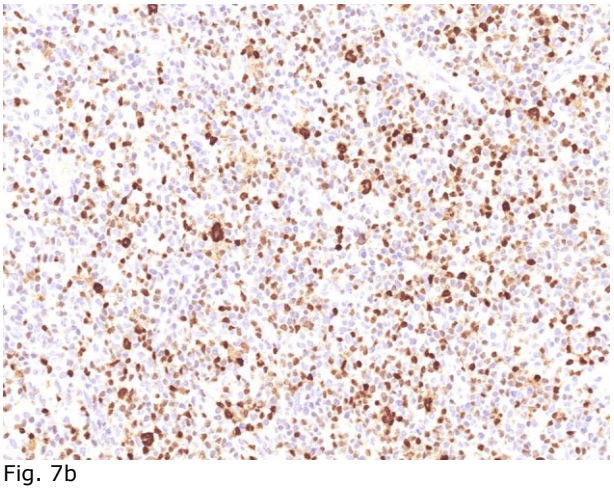


Fig. 7b
Insufficient staining result for MUM1 of the Hodgkin Lymphoma, using the same protocol settings as in Fig. 7a. The significantly increased number of demonstrated cells compromise the selective demonstration of Reed-Sternberg cells – compare with optimal protocol in Fig 3a. In addition, a general cytoplasmic staining reaction was seen compromising the interpretation especially in the non-GCB subtype tissue core no. 5.

TJ/SN/KBA/RR 29.06.2026

[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.