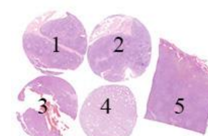
NordiQC

Assessment Run 69 2023
CD138

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD138, typically used to identify and classify plasma cell disorders, e.g., multiple myeloma in the diagnostic work-up of hematological neoplasms. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD138 (see below).

Material
The slide to be stained for CD138 comprised:

1-2. Tonsils, 3. Plasmacytoma, 4. Diffuse large B-cell lymphoma (DLBCL), 5. B-cell chronic lymphatic leukemia (B-CLL).



All tissues were fixed in 10% neutral buffered formalin.
Criteria for assessing a CD138 staining as optimal included:

- An at least moderate, distinct membranous staining reaction of activated late-stage B-cells in the germinal centres of the tonsils.
- A strong, distinct membranous staining reaction of virtually all plasma cells (all specimens), squamous epithelial cells of the tonsils and of all the neoplastic cells in the plasmacytoma.
- No staining reaction of the neoplastic cells in the DLBCL and in the B-CLL.

Participation

Number of laboratories registered for CD138, run 69	425
Number of laboratories returning slides	387 (91%)

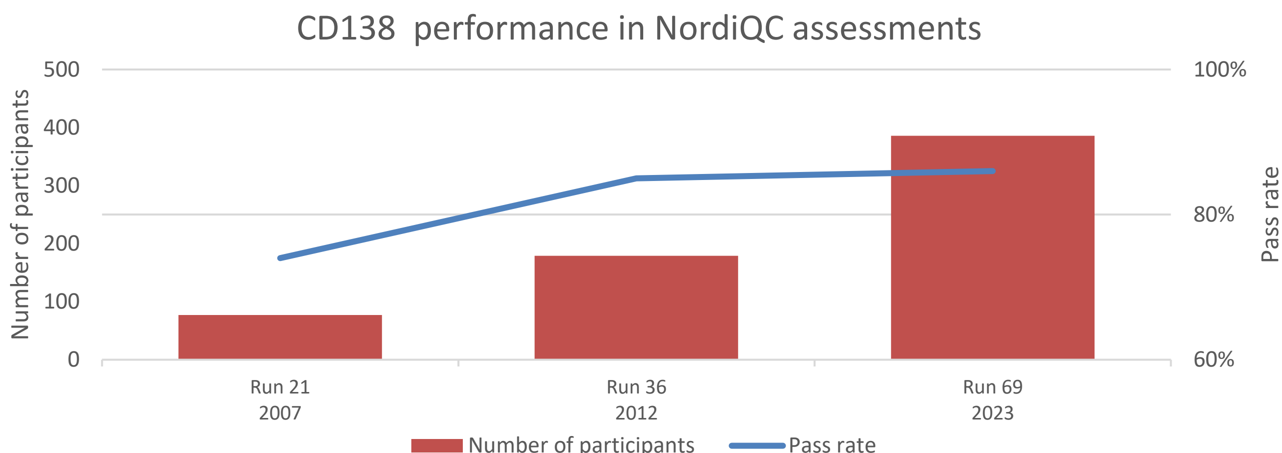
Results
At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

387 laboratories participated in this assessment. One laboratory used an inappropriate antibody and of the remaining participants (386), 332 (86%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Inefficient Heat Induced Epitope Retrieval (HIER) e.g., too short HIER time and/or use of citric based HIER buffer
- Too diluted and too short incubation time in primary antibody
- Less successful performance of rmAb clones EP201, QR102 and 505B7H1 giving false positive results
- OptiView with amplification giving unspecific granular staining reaction of cells expected to be negative
- Less sensitive detection systems

Performance history
This was the third NordiQC assessment of CD138. The pass rate was similar to the results obtained in run 36, 2012 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD138 in the three NordiQC runs performed**



Conclusion
The mAb clones **MI15, B-A38, IHC138 MX135, C5G6, DA013, BY083** and the rmAb clones **EP201, ZR251, GR106,** could all be used to obtain an optimal staining result for CD138. Irrespective of the clone applied, efficient HIER, careful calibration of the primary antibody and the use of a sensitive detection system were the most important requirements for an optimal performance. In this assessment, and using vendor recommended protocol settings, the Ready-To-Use (RTU) systems IR/GA642 (Dako/Agilent) and PA0088 (Leica Biosystems), all based on the mAb clone MI15, gave superior results with a pass rate of 100% (68/68). In addition, and for the RTU systems IR642 and GA642, a high proportion of optimal results was also seen, 86% and 84%, respectively.
Insufficient results were characterized by giving too weak/false negative results due to use of protocol settings providing too low analytical sensitivity or false positive staining reactions.
Tonsil is recommended as positive and negative tissue controls: Late stage activated germinal centre B-cells must show an at least moderate and distinct membranous staining reaction, while plasma cells and squamous epithelial cells should display a strong staining reaction. No staining must be seen in the mantle zone B-cells or other lymphocytes e.g., T-cells.

Table 1. **Antibodies and assessment marks for CD138, run 69**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **MI15**	48	Dako/Agilent	34	9	5	0	90%	71%
mAb clone **B-A38**	14 4 3 3 2 2 1 1	Cell Marque Immunologic ZytoMed Systems Bio SB Biocare Medical Bio-Rad/Serotec Geneprobe IQ-Products	13	11	6	0	80%	43%
mAb clone **IHC138**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **EP201**	2 1 1	Cell Marque Abcam PathnSitu	1	1	1	1	-	-
rmAb clone **ZR251**	1	Zeta Corporation	1	0	0	0	-	-
rmAb clone **QR102**	1	Quartett	0	0	1	0	-	-
Conc total	85		50	21	13	1	84%	59%
Ready-To-Use antibodies								
mAb clone **MI15 PA0088**[3]	17	Leica Biosystems	9	8	0	0	100%	53%
mAb clone **MI15 PA0088**[4]	12	Leica Biosystems	8	3	1	0	92%	67%
mAb clone **MI15 IR642**[3]	7	Dako/Agilent	6	1	0	0	100%	86%
mAb clone **MI15 IR642**[4]	20	Dako/Agilent	13	3	4	0	80%	65%
mAb clone **MI15 GA642**[3]	44	Dako/Agilent	37	7	0	0	100%	84%
mAb clone **MI15 GA642**[4]	25	Dako/Agilent	18	5	2	0	92%	72%
mAb clone **B-A38 760-4248**[3]	13	Ventana/Roche	1	5	7	0	46%	8%
mAb clone **B-A38 760-4248**[4]	120	Ventana/Roche	71	34	14	1	88%	59%
mAb clone **B-A38 138M-10/17/18/19**	28	Cell Marque	10	12	6	0	79%	36%
mAb clone **B-A38 8241-C010**	3	Sakura FineTek	2	1	0	0	-	-
mAb clone **MX135 MAB-1118**	1	Fuzhou Maixin	1	0	0	0	-	-
mAb clone **C5G6 CCM-0782**	1	Celvnovte	1	0	0	0	-	-
mAb clone **DA013 RMB1A054**	1	Shenzhen Dartmon Biotechnology	1	0	0	0	-	-
mAb clone **BY083 BFM-0397**	1	Bioin Biotechnology	1	0	0	0	-	-
rmAb clone **EP201 MAD-000735QD**	3	Master Diagnostica	0	0	2	1	-	-
rmAb clone **EP201 AN837**	1	BioGenex	0	1	0	0	-	-
rmAb clone **GR106 GT245102**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **DGR040 DGR040**	1	Shanghai DG Diag. Tec	0	1	0	0	-	-
rmAb clone **BP6065 I10182E-05**	1	Biolynx Biotechnology	0	0	1	0	-	-

rmAb clone **505B7H1 PA117**	1	Abcarta	0	0	1	0	-	-
RTU total	301		180	81	38	2	87%	60%
Total	386		230	102	51	3	-	
Proportion			60%	26%	13%	1%	86%	

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 CD138, Run 69
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **MI15**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (5/8)*, Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (7/10), Cell Conditioning 1 (CC1; Ventana/Roche) (19/24) and Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 34 of 37 (92%) laboratories produced a sufficient staining result. Two laboratories obtained an optimal result performing no antigen retrieval at all.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **B-A38**: Protocols with optimal results were all based on HIER using CC1 (Ventana/Roche) (6/14), TRS pH 9 (3-in-1) (Dako/Agilent) (4/6), BERS2 (Leica Biosystems) (1/5), TRIS-EDTA/EGTA pH 9 (1/2) and Target Retrieval Solution (TRS) pH 6 (3-in-1) (Dako/Agilent) (1/2) as 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, 21 of 27 (78%) laboratories produced a sufficient staining result.
rmAb clone **EP201**: One protocol with an optimal result was based on HIER using CC1 as retrieval buffer. The rmAb was diluted 1:300 and OptiView (Ventana/Roche, 760-700) was used as detection system.

Table 2. **Proportion of optimal results for CD138 for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark Ultra/XT/GX**		**Leica Biosystems Bond III**	
	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 **MI15**	2/5** (40%)	0/1	3/3	-	15/19 (79%)	-	7/9 (78%)	1/1
mAb clone **B-A38**	3/4	-	1/2	1/2	6/14 (43%)	-	1/3	-

* 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 **MI15**, product no. **PA0088**, Leica Biosystems, Bond-III/PRIME:
Protocols with optimal results were based on HIER using BERS1 or BERS2 (efficient heating time 20-40 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 25 of 25 (100%) laboratories produced a sufficient staining result.

mAb clone **MI15**, product no. **IR642**, Dako/Agilent, Autostainer:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002+K8021) as detection systems. Using these protocol settings, 12 of 12 (100%) laboratories produced a sufficient staining result.

mAb clone **MI15**, product no. **GA642**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/823+GV821) as detection systems. Using these protocol settings, 44 of 44 (100%) laboratories produced a sufficient staining result.

mAb clone **B-A38**, product no. **760-4248**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/XT/GX:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab. and UltraView with amplification (760-500+760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 56 of 57 (98%) laboratories produced a sufficient staining result.

mAb clone **B-A38**, product no. **8241-C010**, Sakura Finetek, Tissue-Tek Genie Advanced stainer:
Protocols with optimal results were based on HIER using Tissue-Tek Genie High pH Antigen Retrieval (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit, DAB (8826-K250) as detection system. Using these protocol settings, 2 of 3 protocols were assessed as optimal.

Table 3 summarizes 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 changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CD138 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb MI15 **IR642**	100% (7/7)	86% (6/7)	92% (11/12)	67% (8/12)
Dako Omnis mAb MI15 **GA642**	100% (44/44)	84% (37/44)	92% (23/25)	72% (18/25)
Leica BOND III/PRIME mAb MI15 **PA0088**	100% (17/17)	53% (9/17)	100% (10/10)	70% (7/10)
VMS Ultra/PLUS/XT/GX mAb B-A38 **760-4248**	46% (6/13)	8% (1/13)	88% (105/120)	59% (71/120)

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

Comments

In this third NordiQC assessment for CD138, the prevalent features of insufficient staining results were characterized by a generally too weak/false negative staining reaction of the cells expected to be demonstrated and were observed in 64% of the insufficient results (43/54). Virtually all laboratories were able to detect CD138 in high-level antigen expressing cells as the neoplastic cells of the plasmacytoma, whereas demonstration of CD138 in low-level antigen expressing cells as late-stage activated B-cells of the germinal centres in the tonsils, but also plasma cells (both intensity and proportion) intermingling between the neoplastic cells of the B-CLL, was more challenging and could only be obtained with optimally calibrated protocols. In the remaining insufficient results, the most common observations were an unspecific granular staining reaction in cellular structures expected to be negative using OptiView with amplification (Ventana/Roche) as detection system, compromising interpretation of the specific signal for CD138 (see Fig. 5a) and a false positive staining reaction of vascular structures, primarily related to the use of the rmAb clones EP201, QR102 and 505B7H1 (see Fig. 5b).

Used within laboratory developed assays (LD) or Ready-to-Use formats (RTU), the mAb clones MI15 and B-A38 were the most widely used antibodies for demonstration of CD138 being applied by 95% (368/386) of the laboratories (see Table 1). Used as concentrated format within LD-assays, the mAb clone MI15 provided the highest proportion of sufficient and optimal results, 90% (43/48) and 71% (34/48), respectively. As shown in Table 2, the mAb clone MI15 gave optimal results on all main semi- and fully automated platforms. Virtually all protocols assessed as optimal were based on efficient HIER in an alkaline buffer (31/34), the primary antibody was carefully calibrated in the dilution range of 1:10 -1:100 (30/34) and a sensitive 3-step detection system was applied (26/34) e.g., EnVision Flex+ (Dako/Agilent) or OptiView (Ventana/Roche). The main causes for the insufficient staining results using the mAb clone MI15 within a LD-assay, were primarily related to use of too diluted antibody in combination with less efficient HIER time in an acidic buffer BERS1 (Leica Biosystems) or in combination with less sensitive detection system as e.g., EnVision Flex (Dako/Agilent). For one protocol assed as insufficient, no protocol parameters could be identified unraveling the central sources for the less successful performance.

Compared to the use of the mAb clone MI15 within a LD-assay, protocols based on the mAb clone B-A38 as concentrate, provided a lower proportion of sufficient and optimal results, 80% (24/30) and 43% (13/30), respectively. This antibody could also give optimal results on all main platforms (see Table 2). The protocol settings providing optimal results for mAb clone B-A38 were similar to the concentrated format of the mAb clone MI15 (see above) – efficient HIER in in an alkaline buffer (12/13), careful calibration of the primary Ab in the dilution range 1:25-400 (13/13) and the use of a sensitive detection system e.g., Bond Refine (Leica Biosystems), OptiView (Ventana/Roche) and EnVision Flex+ (Dako/Agilent) (11/13). For the six protocols assed as insufficient, the main problems was related to the use of less sensitive detections systems (4/6) and the use of the detection systems OptiView with amplification (2/6) – giving an unspecific granular staining reaction of cellular structures expected to be negative (e.g., the neoplastic cells of the B-CLL and germinal centre B-cells of the tonsils), compromising interpretation of the specific CD138 reactions.

78% (301/386) of the laboratories used a RTU format for demonstration of CD138. This is a significant increase compared to the former run 36 in which only 40% (71/179) of the participants applied a RTU format. In this assessment, and using vendor recommended protocol settings, the RTU systems IR/GA642 (Dako/Agilent) and PA0088 (Leica Biosystems), all based on the mAb clone MI15, provided a superior performance as 100% (68/68) of the results were assessed as sufficient. The Dako/Agilent RTU systems were most successful as 84% (43/51) of the sufficient results were optimal (see Table 3). For the Leica Biosystems RTU system PA0088 developed for the Bond platforms, the proportion of optimal results was increased from 53% (9/17) to 70% (7/10) when the basic vendor recommended protocol settings were modified. Especially the substitution of the vendor recommended HIER acidic buffer BERS1 with the alkaline buffer BERS2 was successful providing 100% (5/5) sufficient results - 80% (4/5) being optimal. The one protocol based on HIER in BERS2 not giving an optimal mark (assessed as good), was based on a reduced HIER time in BERS2 and incubation time in primary Ab.

In contrast to the RTU systems described above, and applying vendor recommended protocol settings, the RTU system from Ventana/Roche 760-4248 based on the mAb clone B-A38 and developed for the Benchmark platforms provided a low pass rate of 46% (6/13) of which 8% (1/13) were assessed as optimal (see Table 3). For this RTU system, the choice of detection system had a high impact on performance and using HIER in CC1 for 32-64 min at 95-100°C, incubation time in primary Ab for 16-32 min. and UltraView as the detection system, the proportion of sufficient results were 65% (28/43) of which only 12% (5/43) were optimal. In comparison, and using the same protocol settings, except for substituting the detection system with UltraView with amplification or OptiView, the proportion of sufficient results increased to 100% (6/6) - 83% (5/6) being optimal or 98% (50/51) - 80% (41/51) being optimal, respectively. Thus, the vendor is encouraged to change and update the package insert, replacing the less sensitive detection system UltraView with e.g., OptiView. However, data from the assessment analysis indicated that protocols based on UltraView as the detection system, required a prolonged incubation time in both primary antibody and HIER time in CC1 for optimal performance with a median incubation time in primary antibody of 36 min. (range 16-48 min.) and a median HIER time in CC1 of 64 min. (range 36-92 min.). In comparison and using the same detection system with a negative outcome (borderline), the median incubation time in primary antibody and HIER time in CC1 was lower, 19 min. (range 16-32 min.) and 28 min. (range 8-52 min.), respectively.
Six laboratories used the RTU format with OptiView in combination with Tyramide amplification and as noted for the concentrated formats using the same detection system, 50% (3/6) were assessed as insufficient due to unspecific granular staining reactions in cells/structures not expressing CD138.

Twenty-eight laboratories used the RTU format 138M-10/17/18/19 (Cell marque) also based on the mAb clone B-A38. The majority of laboratories (26/28) used this format on the Ventana Benchmark platform, providing a pass rate of 77% (20/26) of which 35% (9/26) were giving an optimal mark. Using all protocol settings e.g., variable HIER times, temperatures and incubation times in the primary Ab, all insufficient results (6/6) were based on protocol settings applying UltraView as the detection system. Using similar protocol settings, but applying OptiView instead of UltraView, the pass rate was 100% (9/9) of which 67% (6/9) were optimal. These results indicate, and as seen with the Ventana RTU format 760-4248 also based on the mAb clone B-A38, that a sensitive detection system is required for optimal performance using these RTU formats of mAb clone B-A38 on the Benchmark platforms.

This was the third assessment of CD138 in NordiQC (see Graph 1). The pass rate was similar to results obtained in the previous run 36 (2012) for CD138. In this assessment, the RTU systems IR/GA642 (Dako/Agilent) and PA0088 (Leica systems), all based on the mAb clone MI15, provided the highest proportion of sufficient results. In this assessment, the most common cause for an insufficient staining result were related to protocols providing too low analytical sensitivity, and in particular use of low

sensitive detection systems which were applied in 44% (24/54) of the inadequate protocols (see descriptions above).
Importantly, the primary Abs must be careful calibrated according to the expected antigen level of the recommended control material (see below).

Controls
Tonsil is recommended as positive and negative tissue controls for CD138. Late stage activated germinal centre B-cells must show an at least moderate and distinct membranous staining reaction, while plasma cells and squamous epithelium should display a strong staining reaction. No staining must be seen in the mantle zone B-cells or other lymphocytes e.g., T-cells.
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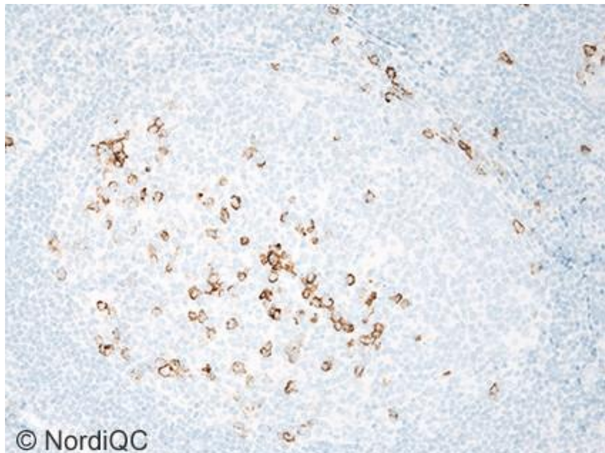

Fig. 1a (x200)
Optimal CD138 staining of the tonsil (tissue core no 2) using the RTU format 760-4248 (Benchmark, Ventana/Roche) based on the mAb clone B-A38, applying laboratory modified protocol settings with HIER in CC1 (40 min. at 100°C), incubation time in primary Ab for 44 min and OptiView as detection system.
The activated germinal centre B-cells show a moderate to strong and distinct membranous staining reaction. No staining reaction was observed in mantle zone B-cells. Same protocol used in Figs. 2a - 4a.

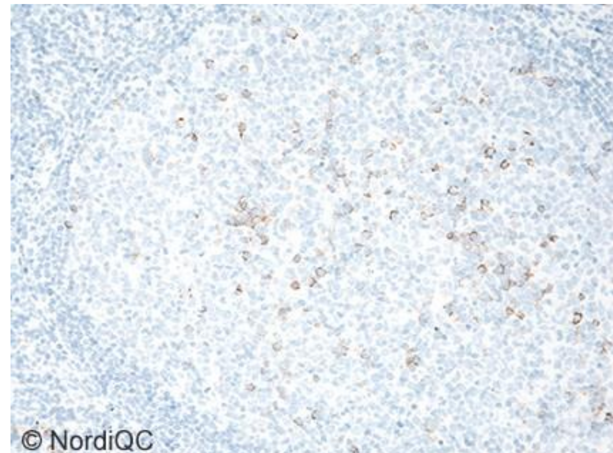

Fig. 1b (x200)
Insufficient CD138 staining of the tonsil (tissue core no 2) using the RTU system 760-4248, following vendor recommended protocol settings based on HIER in CC1 (mild), incubation time in primary Ab for 16 min. and UltraView as the detection system - same protocol used in Figs. 2b - 4b.
Staining intensity and proportion of cells demonstrated is significantly reduced in the activated germinal centre B-cells. In general, this "RTU system" provides too low analytical sensitivity (see description in comments), risking misinterpretation in plasma cell disorders.

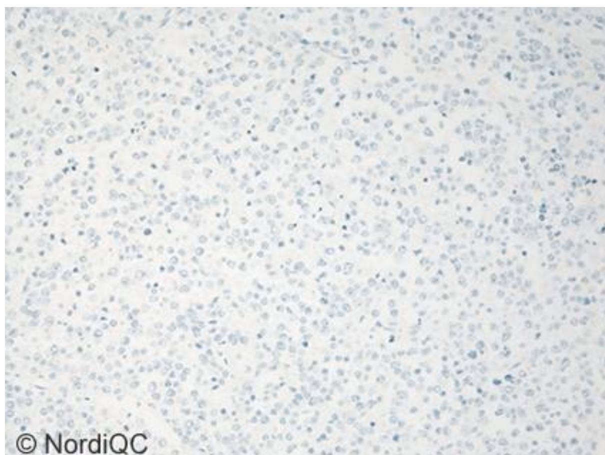

Fig. 2a (x200)
Optimal staining for CD138 of the DLBCL using same protocol as in Fig. 1a. All cells, both normal and neoplastic, display the expected negative reaction pattern.

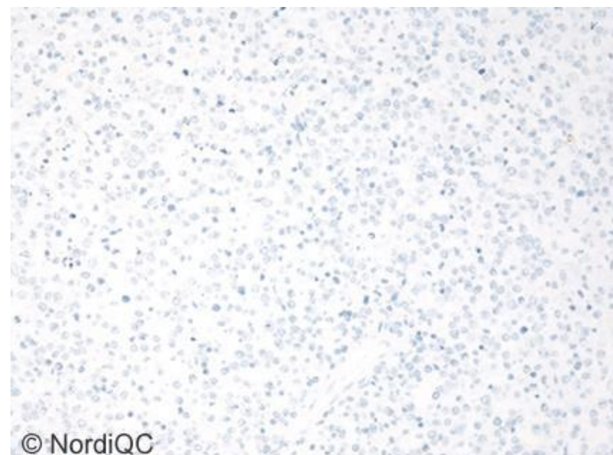

Fig. 2b (x200)
CD138 staining of the DLBCL using same insuffcient protocol as in Fig. 1b. Although the reaction pattern is similar to result obtained in Fig. 2a, the protocol overall was challenged, displaying the correct level of analytical sensitivity, and failed to stain e.g., plasma cells and the neoplastic cells of the plasmacytoma with the expected level of intensity and proportion of cells to be demonstrated – compare with Figs. 3a-4b.

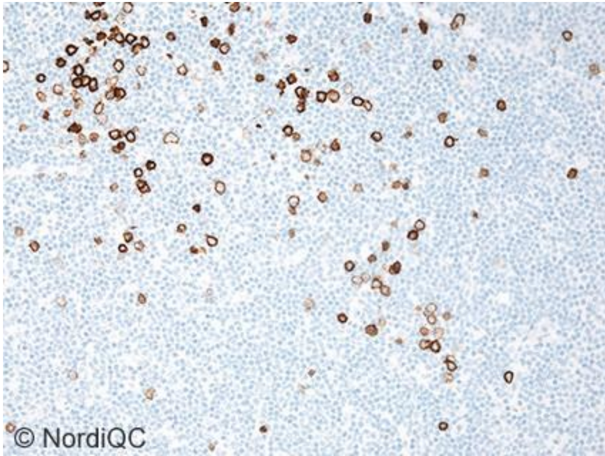

Fig. 3a (x200)
Optimal CD138 staining of the B-CLL using same protocol as in Figs. 1a and 2a. All the neoplastic B-cells are negative as expected. Plasma cells are distinctively demonstrated intermingling between the neoplastic B-cells.

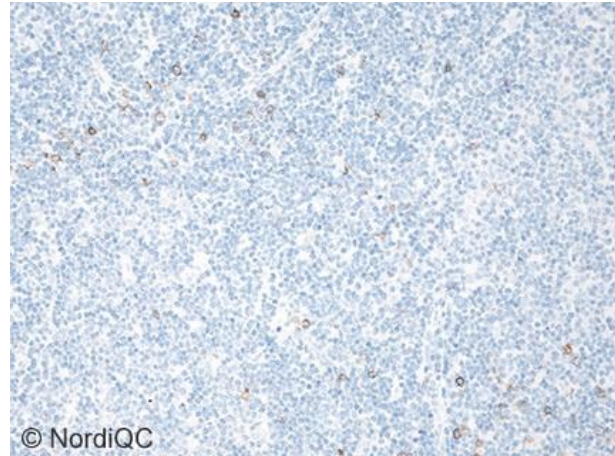

Fig. 3b (x200)
Insufficient CD138 staining of the B-CLL using same protocol as in Figs. 1b and 2b. The protocol provided too weak staining intensity and virtually all plasma cells, intermingling between the negative neoplastic B-cells, showed reduced or false negative staining reactions – compare with Fig. 3a.

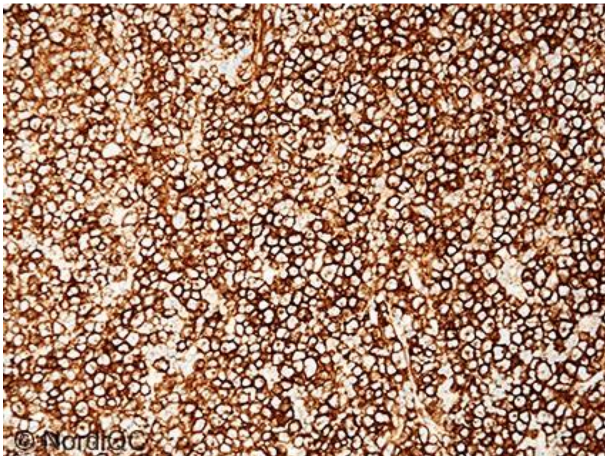

Fig. 4a (x200)
Optimal CD138 staining of the plasmacytoma, using same protocol as in Figs. 1a – 3a. All neoplastic plasma cells display a strong and distinct membranous staining reaction.

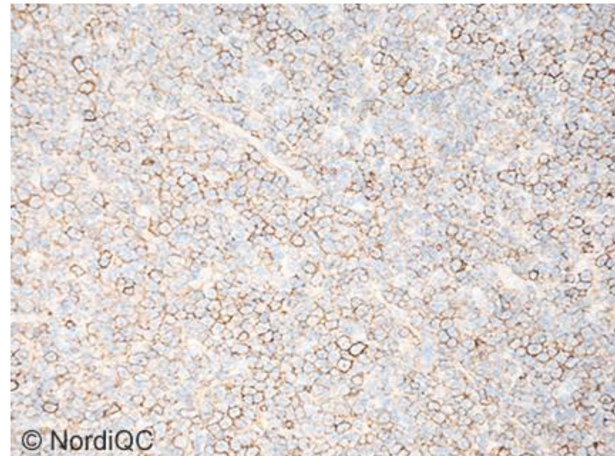

Fig. 4b (x200)
Insufficient CD138 staining of the plasmacytoma, using same protocol as in Figs. 1b – 3b. Staining intensity and proportion of neoplastic cells expected to be demonstrated is significantly reduced – compare with Fig. 4a.

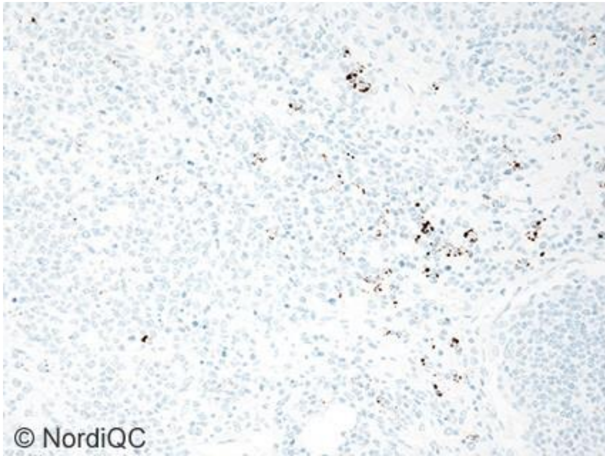

Fig. 5a (x200)
Insufficient CD138 staining of the DLBCL using a protocol based on OptiView with tyramide amplification (Ventana/Roche). Protocols based on this detection system often gave an aberrant granular staining reaction e.g, neoplastic cells of the DLBCL and the B-CLL, but also in normal B-cells, and thus, hampering interpretation of the specific signal for CD138. This insufficient and unspecific granular staining result was observed in 57% (4/7) of the protocols using this detection system - compare with optimal result in Fig. 2a.

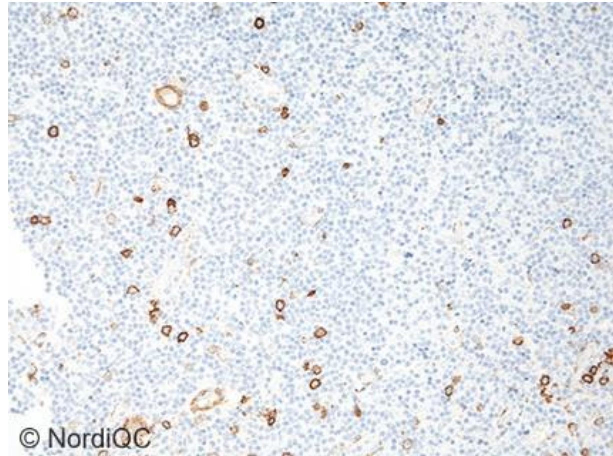

Fig. 5b (x200)
Insufficient CD138 staining of the B-CLL using a protocol based on the rmAb clone QR102. The protocol gave a false positive staining reaction of vascular structures, and consequently, risking misclassification of tumors on unknown origin. Similar unspecific reaction pattern was also be seen with the rmAb clones EP201 and 505B7H1.

MB/LE/SN 15.11.2023