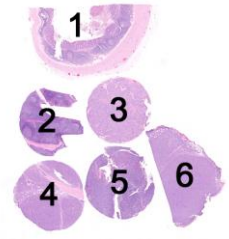
Assessment Run 36 2012
CD138

Material
The slide to be stained for CD138 comprised:



1. Appendix, 2. Tonsil, 3. Ovarian serous carcinoma, 4. Plasmacytoma, 5. & 6. Diffuse large B-cell lymphoma (DLBCL).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD138 staining as optimal included:

- A moderate to strong, distinct predominantly membranous staining reaction of activated late stage B-cells in the germinal centres and the plasma cells in the tonsil and appendix.
- A strong, distinct membranous staining reaction of the majority of the squamous epithelial cells in the tonsil.
- A moderate to strong membranous staining reaction of the majority of the neoplastic cells of the plasmacytoma and the DLBCL, core no. 6.
- An at least weak to moderate predominantly membranous staining reaction of dispersed neoplastic cells of the ovarian serous carcinoma.
- No staining in the neoplastic cells of the BLBCL, core no. 5.

181 laboratories participated in this assessment. 2 participants used an inappropriate antibody. 85 % of the remaining 179 laboratories achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for CD138, run 36**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5F7**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **B-A38**	8 6 4 2 1 1	Immunologic AbD Serotec Cell Marque Biocare Gen-Probe Zytomed	12	6	4	0	82 %	89 %
mAb clone **B-B4**	7 1	AbD Setotec IQ Products	4	4	0	0	100 %	100 %
mAb clone **CLB-1D4**	1	Biogenex	0	0	0	1	-	-
mAb **MI15**	67 5 1	Dako Thermo/NeoMarkers Genemed	23	39	9	2	84 %	88 %
rmAb **EP201**	1	Epitomics	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **B-A38 760-4248**	41	Ventana/Cell Marque	12	25	4	0	90 %	91 %
mAb clone cocktail **B-A38 PM167AA**	1	Biocare	1	0	0	0	-	-
mAb clone **B-A38 138M-17**	1	Cell Marque	0	1	0	0	-	-
mAb clone **MI15 IS/IR642**	26	Dako	9	13	4	0	85 %	85 %
mAb clone **MI15**	1	Leica	1	0	0	0	-	-

PA0088								
mAb clone **MI15 MAD-000921QD**	1	Master Diagnostica	0	1	0	0	-	-
Total	179		62	89	22	6	-	
Proportion			35 %	50 %	12 %	3 %	85 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below

Following protocol parameters were central to obtain an optimal staining:

Concentrated Abs
mAb clone **B-A38**: Protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/2)*, TRS pH 9 (Dako) (1/2), TRS low pH 6.1 (Dako) (1/1), Cell Conditioning 1 (CC1; Ventana) (3/7), Bond Epitope Retrieval Solution 2 (BERS 2; Leica) (3/4), Tris-EDTA/EGTA pH 9 (1/3) or Citrate pH6 (1/2) as the retrieval buffer.
The mAb was typically diluted in the range of 1:50-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 17 of 19 (89 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this reagent)

mAb clone **B-B4**: Protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (1/1), CC1 (Ventana) (2/4) or EDTA/EGTA pH 8 (1/1) as the retrieval buffer.
The mAb was diluted in the range of 1:50-1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 of 6 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **MI15**: Protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (3/13), TRS pH 9 (Dako) (2/5) TRS low pH 6.1 (Dako) (1/1), CC1 (Ventana) (4/21), BERS 1 (Leica) (4/5), BERS 2 (Leica) (4/8), Borg Decloaker pH 9.5 (Biocare) (1/2), Tris-EDTA/EGTA pH 9 (2/7) or Citrate pH 6 (2/4) as the retrieval buffer.
The mAb was diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 56 of 64 (88 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use antibodies
mAb clone **B-A38** (prod. no. 760-4248, Ventana/Cell Marque): Protocols giving an optimal result were typically based on HIER using mild or standard CC1, 15-48 min incubation of the primary Ab, and UltraView (760-500) +/- amplication kit or OptiView (760-700) as detection system. Using these protocol settings 30 out of 33 (91 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **MI15** (prod. no. IS/IR642 Dako): Protocols giving an optimal result were typically based on HIER in PT-Link (heating time for 10-20 min at 95-97°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, 20-35 min incubation of the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as detection system. Using these protocol settings 22 out of 26 (85 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **MI15** (product.no. PA0088, Leica/Novocastra): The protocol giving an optimal result was based on HIER using BERS 1, pH 6 (Bond, Leica), 15 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system

The most frequent causes of insufficient stainings were:
- Use of detection systems with a low to moderate sensitivity
- Insufficient HIER
- Too low concentration of primary Ab
- Less successful primary Abs

In concordance with the previous NordiQC assessment of CD138 (run 21 2007), the prominent feature of an insufficient staining was a too weak or false negative staining of structures expected to be demonstrated. The majority of laboratories were able to demonstrate CD138 in plasma cells, the squamous epithelial cells of the tonsil and the neoplastic cells of the plasmacytoma, whereas the demonstration of CD138 of the DLBCL, core no. 6 and the late stage activated germinal centre B-cells was much more challenging and required optimally calibrated protocol.

Several antibodies could produce an optimal staining result. In general, efficient HIER, typically in an alkaline buffer, and a carefully calibrated Ab titre were the main parameters for an optimal staining result. Use of 3-step polymer or multimer based detection systems gave a higher pass rate and proportion of optimal stainings compared to 2-step detection systems. If the mAb clones B-A38, B-B4 or MI15 were used as a concentrate and visualized by a 2-step polymer or multimer based detection system (e.g., EnVision Flex, Dako or UltraView, Ventana) a sufficient staining result was obtained in 52 of 66 laboratories (79)% of which 20 were optimal (30 %). If a 3-step polymer or multimer based system was applied as Bond Refine (Leica) or OptiView (Ventana) 34 of 36 laboratories obtained a sufficient staining result (94 %) out of which 16 were assessed as optimal (44 %).

All 3 protocols based on the mAb clone 5F7 were assessed as insufficient even though the protocol settings were identical to settings producing optimal staining for other clones. Normal plasma cells showed a strong cytoplasmic staining reaction, but a complete false negative staining was seen in the plasmacytoma and the DLBCL, tissue core. no. 6. Similar observation was made in run 21 and it has to be concluded that the mAb clone 5F7 cannot be used for the demonstration of CD138.

Controls
Normal tonsil is recommended as control for CD138. Plasma cells and squamous epithelial cells must show a strong predominantly membranous staining reaction, while late stage activated germinal centre B-cells must show a weak to moderate and distinct membranous staining reaction. No staining reaction must be seen in the mantle zone B-cells.

Effect of external quality assessment
This was the second NordiQC assessment of CD138 .Compared to the previous run in 2007 an increased pass rate was observed, see table 2.

Table 2. **Proportion of sufficient CD138 staining results**

	Run 21 2007	**Run 36 2012**
Participants, n=	77	179
Sufficient results	74 %	85 %

Conclusion
The mAb clones **B-A3**, **B-B4** and **MI15** can all be all recommended for the demonstration of CD138. Efficient HIER and use of sensitive 3-step detection systems gave the highest proportion of sufficient and optimal staining results.
Tonsil is an appropriate control: Late stage activated germinal centre B-cells must show an at least weak to moderate and distinct membranous reaction, while plasma cells and squamous epithelial a strong staining reaction. No staining must be seen in the mantle zone B-cells.

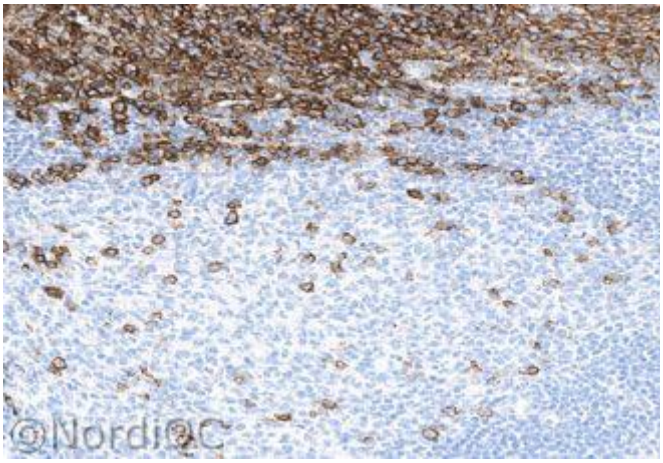


Fig 1a
Optimal staining for CD138 of the tonsil using the mAb clone B-A38 optimally calibrated, HIER in an alkaline buffer and a 3-step multimer based detection system. The activated late stage germinal centre B-cells and the plasma cells show a moderate to strong predominantly membranous staining reaction. A strong staining reaction is also seen in the squamous epithelial cells – top.
No background staining is seen.

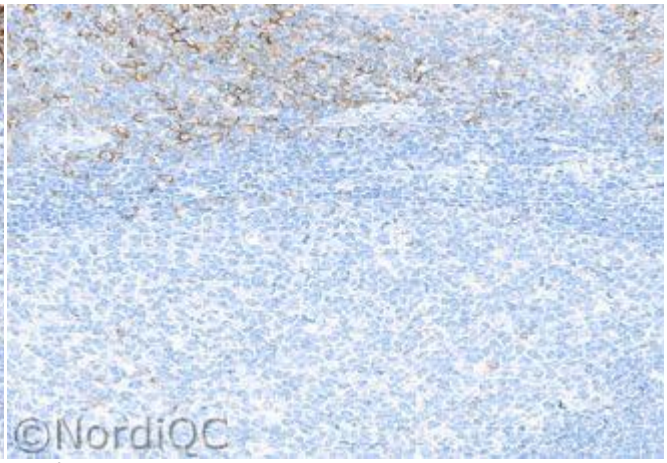


Fig 1b
Insufficient staining for CD138 of the tonsil using the mAb clone MI15 with protocol settings giving a too low sensitivity (too low conc. of the primary Ab and a 2-step polymer based detection system) - same field as in Fig. 1a.
The intensity and proportion of the cells demonstrated is significantly reduced. Also compare with Figs. 2b & 3b – same protocol.

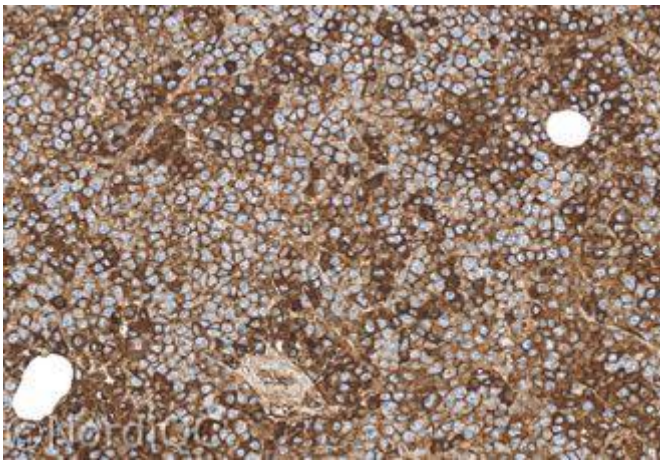


Fig 2a
Optimal staining for CD138 of the plasmacytoma using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction.

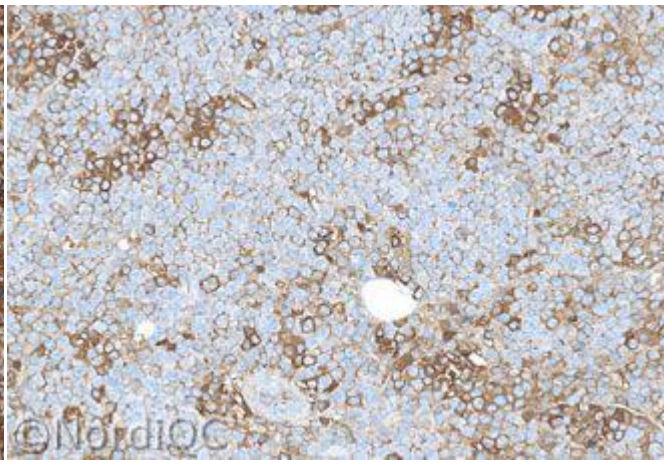


Fig 2b
Staining for CD138 of the plasmacytoma using same insufficient protocol as in Fig. 1b – same field as in Fig. 2a.
The intensity and proportion of the cells demonstrated is significantly reduced. Also compare with Fig. 3b – same protocol.

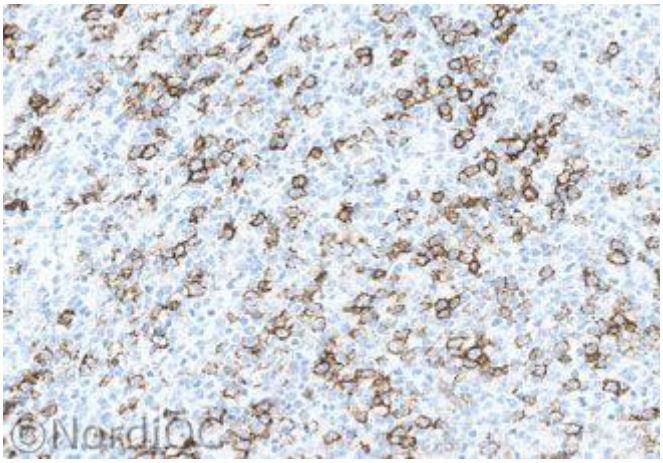


Fig 3a
Optimal staining for CD138 of the DLBCL, tissue core no. 6 in the NordiQC multi-block using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a weak to strong membranous staining reaction.

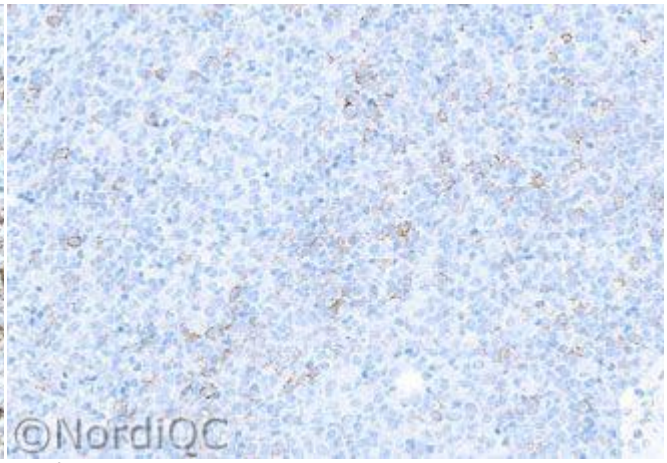


Fig 3b
Insufficient staining for CD138 of the DLBCL, tissue core no. 6 in the NordiQC multi-block using same protocol as in Figs. 1b & 2b. - same field as in Fig. 3a.
Only a weak and patchy membranous staining reaction is seen in scattered neoplastic cells.

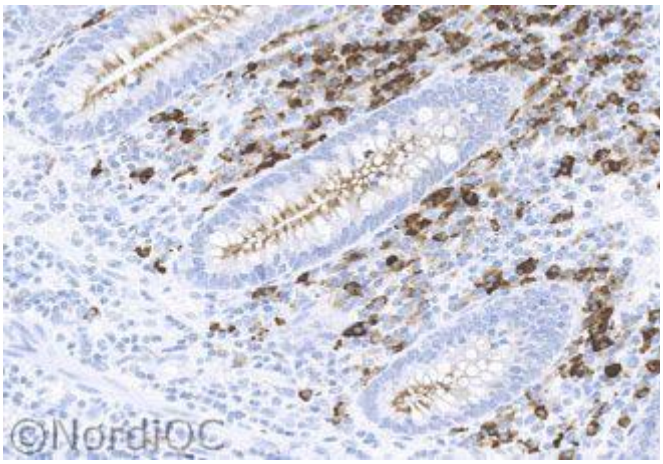


Fig 4a
Staining for CD138 of the appendix using the mAb clone 5F7. The plasma cells in lamina propria show a moderate to strong cytoplasmic staining reaction, while the appendiceal epithelial cells show a weak staining reaction. Also compare with Fig. 4b – same protocol.

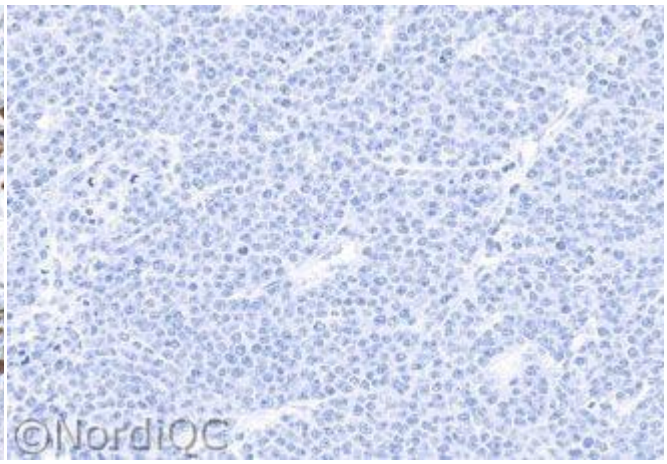


Fig 4b
Insufficient staining for CD138 of the plasmacytoma using same protocol as in Fig. 4a, based on the mAb clone 5F7. All the neoplastic plasma cells are false negative.
Both in this run and the previous assessment run 21 it has been observed, that the mAb clone 5F7 consistently has given a false negative staining in neoplastic plasma cells and cannot be recommended as marker for CD138.

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