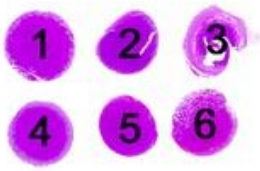
Assessment Run 21 2007

CD138

The slide to be stained for CD138 comprised:
1. Ovarian serous carcinoma, 2. Tonsil, 3. Appendix, 4. Diffuse large B-cell lymphoma, germinal cell like (GCL) type, 5. Diffuse large B-cell lymphoma, activated B-cell like (ABCL) type, 6. Plasmacytoma.
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 of the activated late stage B-cells in the germinal centres and the plasma cells in the tonsil and in the appendix.
- A strong, distinct membranous staining of the columnar epithelial cells of the appendix and the squamous epithelial cells in the tonsil.
- A moderate to strong membranous staining of the majority of the neoplastic cells of the ABCL diffuse large B-cell lymphoma and the ovarian serous carcinoma, and a distinct reaction in the stromal cells in both tumour types.
- No staining in the neoplastic cells and the stromal component of the GCL diffuse large B-cell lymphoma.

79 laboratories submitted stains. One laboratory used an inappropriate Ab, clone VS38c, and in one case, the CD38 Ab was not stated and could not be identified. At the assessment of 77 laboratories, 30 achieved optimal marks (39 %), 27 good (35 %), 9 borderline (12 %) and 11 poor marks (14 %).

The following Abs were used:
mAb clone **MI15** (Dako, n=36; NeoMarkers, n=2)
mAb clone **B-B4** (Serotec, n=8; NeoMarkers, n=3; BeckmanCoulter, n=1; IQ Products, n=1; Ventana, n=1)
mAb clone **B-A38** (Ventana, n=5; Serotec, n=6; IQ Products, n=1; Cell Marque, n=1)
mAb clone **5F7** (Novocastra, n=7; NeoMarkers, n=1)
mAb clone **BC/B-B4** (BioCare, n=2; Abcam, n=1)
pAb **RB-9422** (NeoMarkers, n=1)

Optimal staining for CD138 in this assessment was obtained with the mAb clone **MI15** (15 out of 37), mAb clone **B-B4** (6 out of 14), the mAb clone **B-A38** (7 out of 13) and the mAb **BC/B-B4** (2 out of 3).

MI15: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (9/18)*, Citrate pH 6 or pH 7 (3/5), Cell Conditioning 1 (BenchMark, Ventana) (2/4), Cell Conditioning 2 (BenchMark, Ventana) (1/1) or Target Retrieval Solution pH 6,1 (TRS, Dako) (2/4) as the heating buffer. The mAb was typically diluted in the range of 1:25 – 1:100 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) antibody. Using these protocol settings 28 out of 32 (88 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

B-B4: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (3/5), Cell Conditioning 1 (BenchMark, Ventana) (2/4) or Target Retrieval Solution pH6,1 (TRS, Dako) (1/1) as the heating buffer. The Ab was diluted in the range of 1:100 – 1:750 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 6 (100 %) laboratories produced a sufficient staining, all marked as optimal.

B-A38: The protocols giving an optimal result were all based HIER using Tris-EDTA/EGTA pH 9 (4/4), Cell Conditioning 1 (BenchMark, Ventana) (1/6), EDTA/EGTA pH8 (1/1) or Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (1/1) as the heating buffer. The Ab was diluted in the range of 1:100 – 1:3,000 depending on the total sensitivity of the protocol employed or as a RTU antibody. Using these protocol settings 9 out of 12 (75%) laboratories produced a sufficient staining (optimal or good).

BC/B-B4: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (1/2) or 1mM EDTA pH 9 (1/1) as the heating buffer. The Ab was diluted 1:200. Using these protocol settings both of 2 laboratories produced a sufficient staining, both marked as optimal.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody

In this assessment the prevalent feature of an insufficient staining was either a too weak general staining or a complete false negative reaction of the structures supposed to be demonstrated. A general weak staining was characterized by a diffuse staining of both the ABCL diffuse large B-cell lymphoma and the ovarian serous carcinoma, whereas the plasmacytoma was demonstrated. This staining pattern was typically seen when using one of the clones otherwise capable to give a sufficient result, but was used in a too dilute titter or with a too low sensitivity in the protocol employed.
A complete false negative reaction was seen when using the clone 5F7 (all 8 out of 8 laboratories achieved poor marks). Neither the plasmacytoma nor the ovarian serous carcinoma were stained in spite of the 5F7 protocols otherwise being similar to protocols giving optimal results with other clones. The staining pattern of the clone 5F7 was entirely different, as 5F7 demonstrated a cytoplasmic component in plasma cells and the epithelial cells showed a faint predominantly cytoplasmic granular reaction.

Tonsil is an appropriate control: The late stage activated B-cells in the germinal centres and the plasma cells should show a distinct membranous staining, whereas other lymphocytes should be negative. The squamous epithelial cells should show a strong membranous reaction.

Conclusion
The mAb clones **MI15**, **B-B4**, **B-A38** and **BC/B-B4** were all useful for the demonstration of CD138.
HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated.
Tonsil is an appropriate control: The late stage activated germinal centre B-cells, plasma cells and squamous epithelial cells must show a distinct membranous reaction.

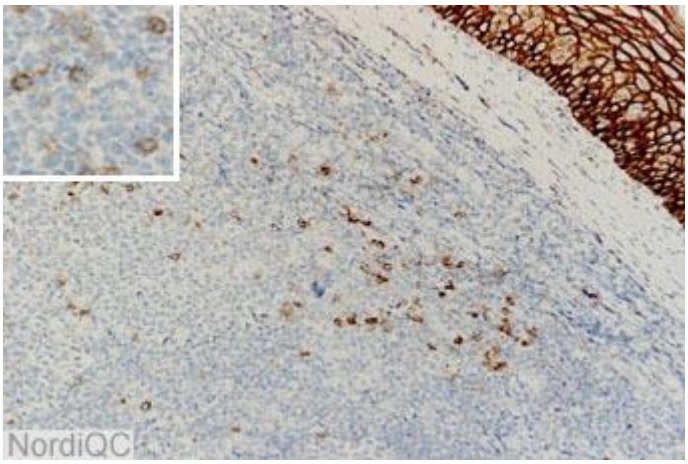


Fig. 1a
Optimal CD138 staining of the tonsil using the mAb clone MI15. The activated late stage B-cells in the germinal centres and the plasma cells show a moderate to strong distinct staining, while the squamous epithelial cells are strongly stained. Insert: high magnification of the germinal centre showing the positive late stage B-cells.

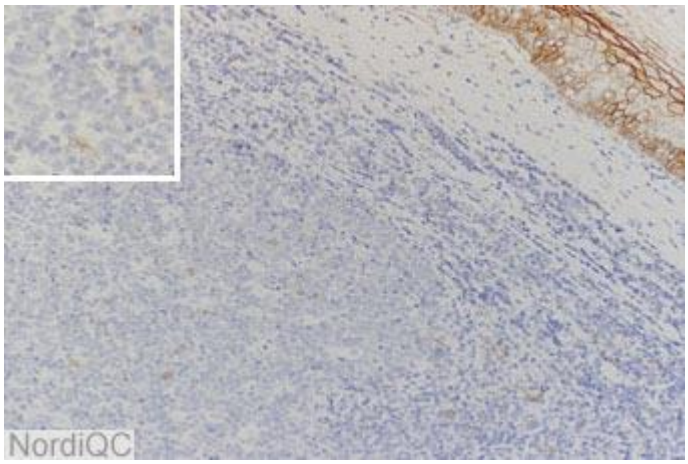


Fig. 1b
Insufficient CD138 staining of the tonsil – same field as in Fig. 1a – using a protocol based on the mAb clone MI15 in a too low concentration. Only the squamous epithelial cells show a distinct staining.
Insert: at high magnification of the germinal centre the late stage B-cells are very weakly demonstrated. Also compare with Fig. 2b & 3b – same protocol.

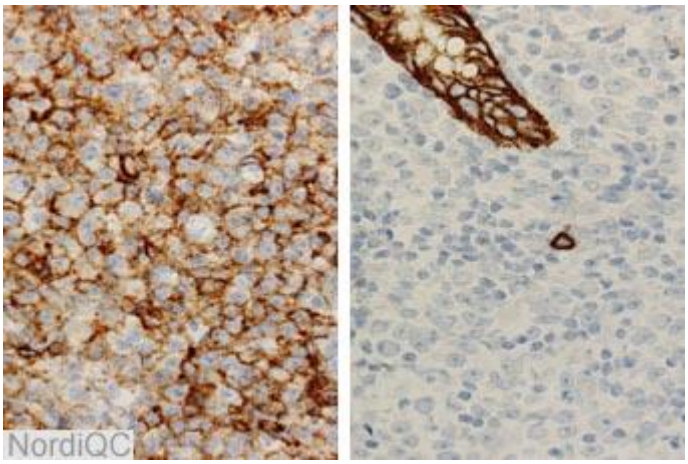


Fig. 2a
Optimal CD138 staining of the two diffuse large B-cell lymphomas using same protocol as in Fig. 1a. Left: The majority of the neoplastic cells of the ABCL lymphoma show a distinct membraneous reaction.
Right: The neoplastic cells of the GCL lymphoma are negative and only the epithelial cells and plasma cells are stained.

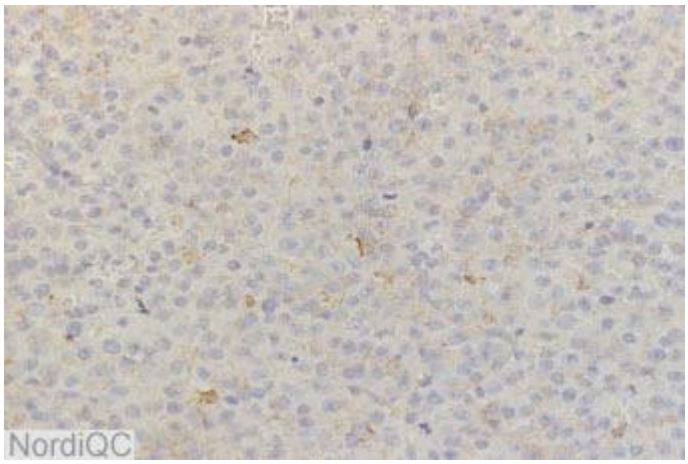


Fig. 2b
Insufficient CD138 staining of the ABCL diffuse large B-cell lymphoma using same protocol as in Fig. 1b. The majority of the neoplastic cells are negative and only scattered cells show a weak reaction.

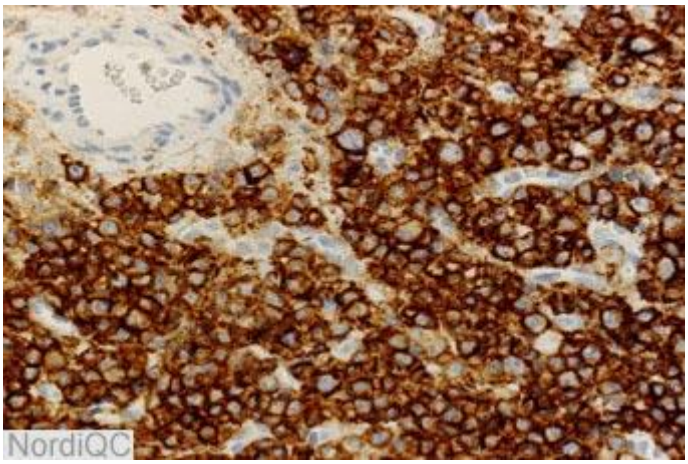


Fig. 3a
Optimal CD138 of the plasmacytoma using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong reaction with no background reaction.

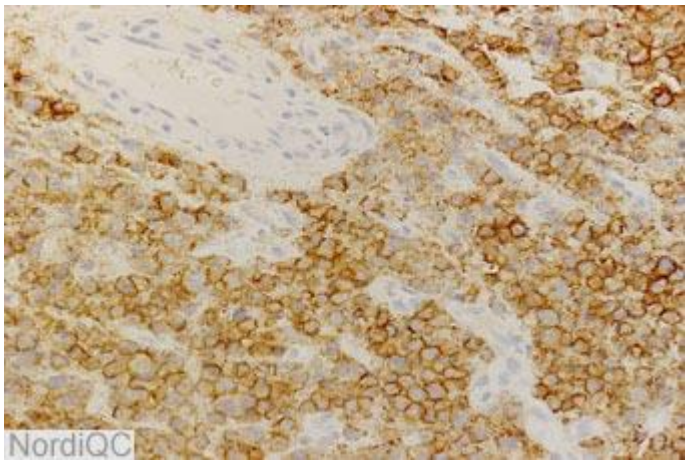


Fig. 3b
CD138 of the plasmacytoma using the same insufficient protocol as in Fig. 1b & 2b. The neoplastic cells show a diffuse staining compared to Fig. 3a – same field. However also compare with Fig. 1b and 2b.

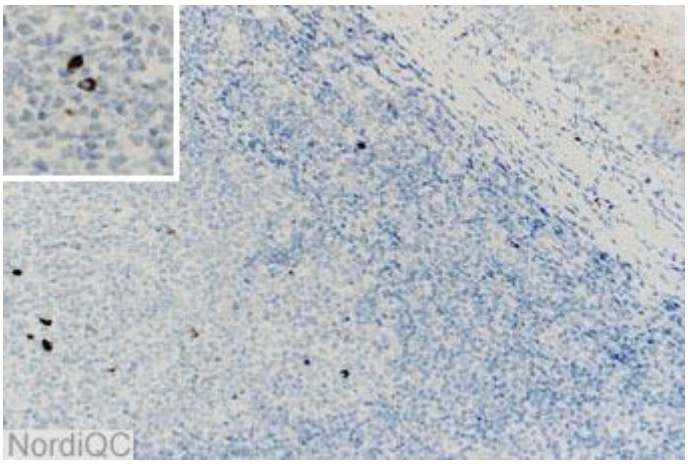


Fig. 4a
Insufficient staining of the tonsil using the mAb clone 5F7. Only the plasma cells show a strong staining. Compare with Fig. 1a – same field.
Insert: at high magnification the plasma cells show a cytoplasmic reaction in contrast to the predominantly membranous pattern obtained with e.g., the mAb clone MI15.

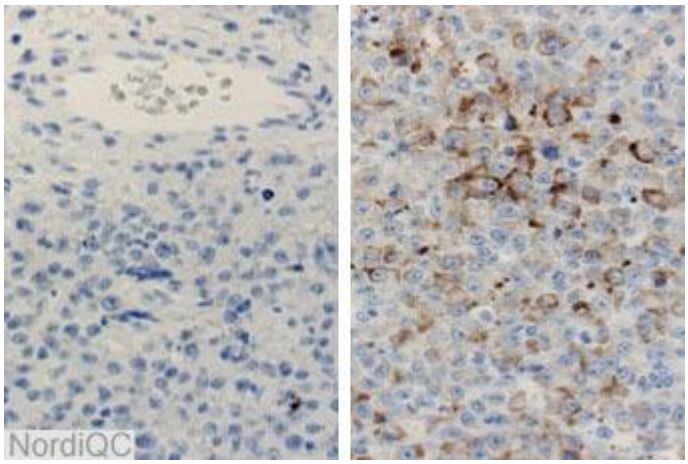


Fig. 4b
Left: Insufficient staining of the plasmacytoma using same protocol as in Fig. 4a. All the neoplastic cells are negative.
Right: Focally the neoplastic cells of the ABCL lymphoma show a cytoplasmic reaction in contrast to the predominantly membranous pattern obtained with e.g., the mAb clone MI15.

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