

The slide to be stained for Cyclin D1 (CyD1) comprised:

1. Tonsil fixed 48 h, 2. B-Cell chronic lymphatic leukaemia (B-CLL) 3 - 4. Mantle cell lymphoma. All specimens were fixed in 10 % NBF.



Criteria for assessing a CyD1 staining as optimal included:

- A moderate to strong, distinct nuclear staining of the suprabasal squamous epithelial cells in the tonsil.
- A moderate to strong, distinct nuclear reaction of the neoplastic cells in the two mantle cell lymphoma.
- No nuclear reaction in the neoplastic cells of the B-CLL.

In all specimens a nuclear reaction in endothelial cells and a weak cytoplasmic reaction was accepted.

87 laboratories submitted stains. At the assessment 30 achieved optimal marks (35 %), 21 good (24 %), 14 borderline (16 %) and 22 (25 %) poor marks.

The following Abs were used:

rmAb clone **SP4** (NeoMarkers, n=50; Ventana, n=2; Cell Marque, n=2; DCS, n=1)

mAb clone **P2D11F11** (Novocastra, n=12; Ventana, n=3; Vector, n=1)

mAb clone **DCS6** (Dako, n=9; Ventana, n=1; Calbiochem, n=1)

pAb **CP236** (Biocare, n=5)

Optimal staining for CyD1 in this assessment was obtained with the rmAb clone **SP4** (29 out of 54) and the pAb **CP236** (1 out of 5). All optimal protocols were based on heat induced epitope retrieval (HIER).

With clone **SP4** all protocols resulting in an optimal staining were based on **HIER** using one of the following buffers:

Tris-EDTA/EGTA pH 9 – 21/34 using this obtained an optimal mark,

CC1 (Cell Conditioning 1, Ventana) – 3/8 using this obtained an optimal mark,

TRS pH 6,1 (Dako) – 2/4 using this obtained an optimal mark,

Citrate pH 6 – 2/4 using this obtained an optimal mark,

EDTA pH 8 – 1/2 using this obtained an optimal mark.

Clone **SP4** typically was used in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed. **SP4** could also be used as an RTU Ab. The combination of the clone **SP4**, properly diluted, and HIER in Tris-EDTA/EGTA pH 9 or CC1 resulted in an optimal staining in 24 out of 43 laboratories (56 %) and an sufficient staining - optimal or good - in 40 out of 42 laboratories (95 %).

With pAb **CP236** the protocol resulting in an optimal stain was based on HIER using Tris-EDTA/EGTA pH 9 as the heating buffer, the Ab diluted 1:100. CP236 typically gave a weak cross reactivity of the membranes of epithelial cells. This was accepted, as it did not interfere with the interpretation.

The most frequent causes of insufficient staining were:

- Less successful primary antibody
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative nuclear staining of the neoplastic cells in the two mantle cell lymphoma and frequently the false negative reaction was accompanied by a moderate to strong false positive cytoplasmic reaction in both the B-CLL and mantle cell lymphoma.

Normal tonsil can be used as both positive and negative control to validate the sensitivity and specificity of the protocol for CyD1. In the squamous epithelium, the suprabasal cells should display a strong nuclear reaction with only minimal cytoplasmic reaction. In the lymphatic areas only the endothelial cells should show a nuclear reaction.

It should also be noticed that the most frequent cause for insufficient staining in this assessment seemed to be related to the primary Ab. Thus, 10 out of 11 laboratories (95 %) using the mAb clone **DCS6** obtained an insufficient mark. Likewise, 14 out of 16 laboratories (88 %) using the mAb clone **P2D11F11** were marked as insufficient. All were using HIER and comparable protocols as applied for the clone mAb **SP4** and the pAb **CP236**.

CyD1 was also assessed in Run 9 2003, in which 57 laboratories participated. Out of these 27 laboratories (47 %) had an insufficient staining. Each laboratory was given a specific recommendation how to improve their protocol. 25 laboratories, which obtained an insufficient result in Run 9 submitted a new CyD1 stain in Run 17. 17 out of these followed the recommendation, and 11 (65 %) improved their mark from insufficient to either good or optimal. 8 laboratories did not follow the recommendations and one (13 %) improved from insufficient to optimal.

The overall proportion of insufficient staining was reduced from 47 % in run 9 to 41 % in the present run. Focusing only on the laboratories participating in both runs (n=54) the proportion of insufficient staining was more significantly reduced from 48 % (n=26) to 37 % (n=20).

The results of the CyD1 assessment in Run 17 is in accordance with the results and conclusions of the study based on the NordiQC results of Run 9: **Antibody Selection in Immunohistochemical Detection of Cyclin D1 in Mantle Cell Lymphoma** by Emina Torlakovic et al., Am J Clin Pathol 2005;124:782-789: "It is apparent from the results of NordiQC testing and the results of this study that many laboratories use suboptimal protocols and suboptimal antibody selection. Both problems might be identified by participation of the laboratories in external quality control programs".

Conclusion

The rmAb clones **SP4** and the pAb **CP236** are useful Abs for CyD1. HIER, preferably in an alkaline buffer as Tris-EDTA/EGTA pH 9, is mandatory for optimal performance. Tonsil is an appropriate control for CyD1.

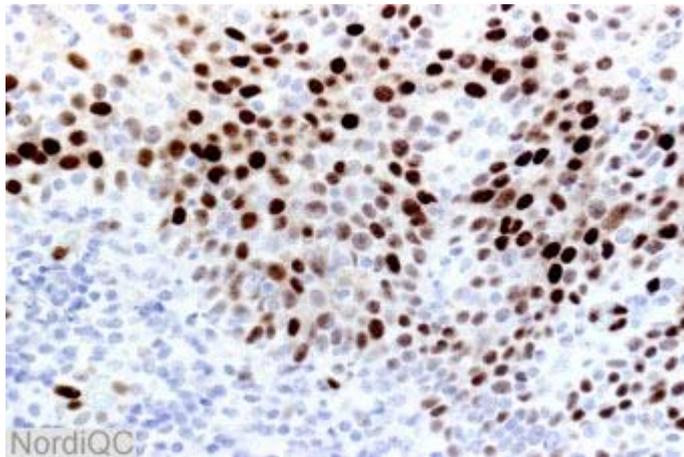


Fig. 1a
Optimal staining for CyD1 of the tonsil. The suprabasal squamous epithelial cells show a moderate to strong nuclear reaction and only a faint cytoplasmic staining. In the left lower corner a few endothelial cells are stained.

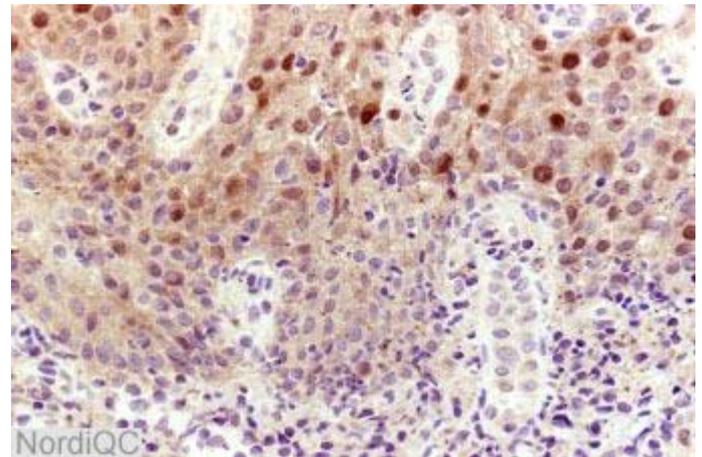


Fig. 1b
Staining for CyD1 of the tonsil using an insufficient protocol. The number of cells expected to cells are reduced compared to the result in Fig. 1a and the cytoplasmic reaction is marked. Also compare with Fig. 2b and 3b – same protocol.

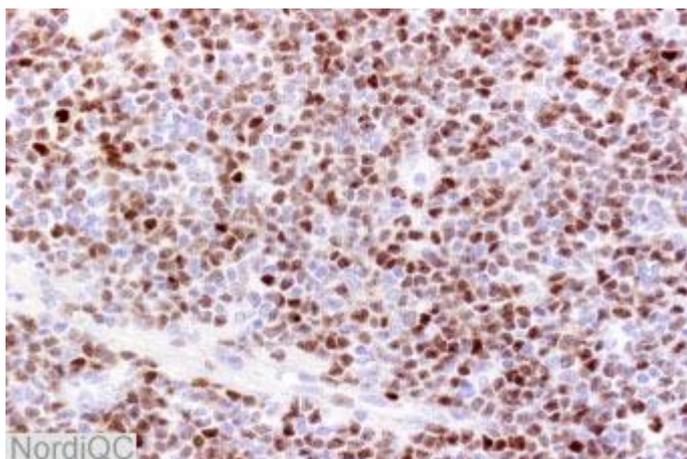


Fig. 2a
Optimal staining for Cyclin D1 in a mantle cell lymphoma (same protocol as in Fig. 1a). The majority of the neoplastic cells show a moderate to strong nuclear staining and only a faint cytoplasmic reaction.

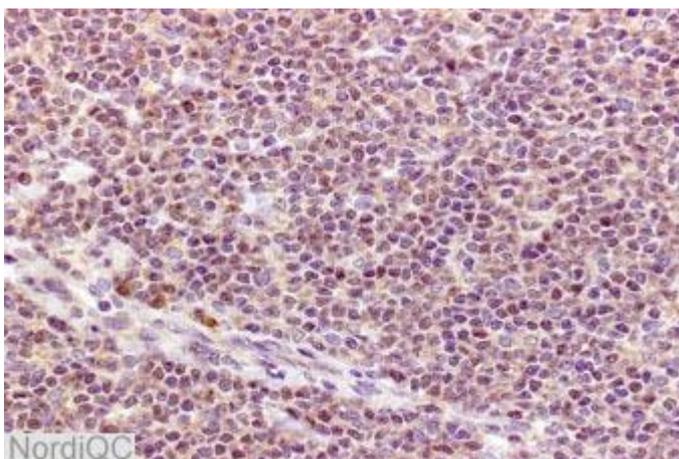


Fig. 2b
Staining for CyD1 in the mantle cell lymphoma using an insufficient protocol. The number of cells expected to cells are reduced compared to the result in Fig. 2a and the cytoplasmic reaction is marked. Also compare with Fig. 3b – same protocol.

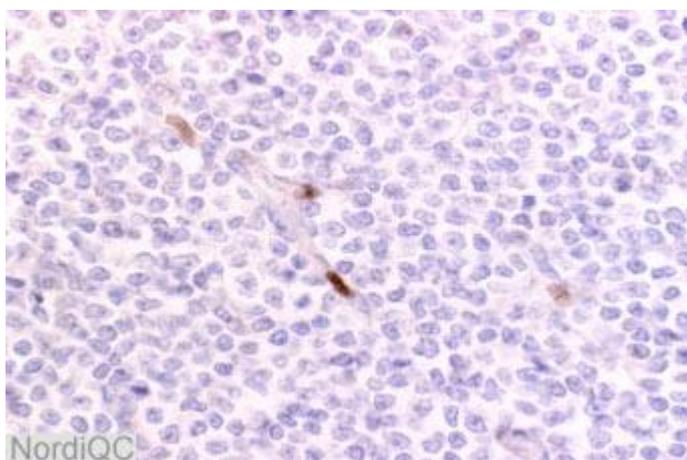


Fig. 3a
Optimal staining for CyD1 of the B-CLL (same protocol as in Fig. 1a and 2a). The neoplastic cells are negative and only the endothelial cells show a distinct nuclear staining.

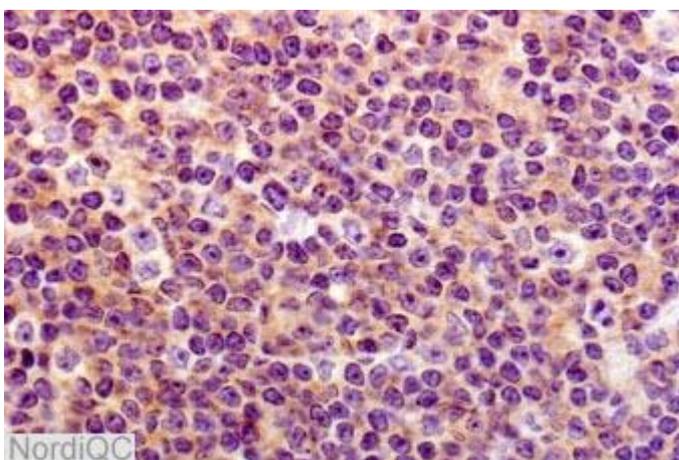


Fig. 3b
Insufficient staining for CyD1 of the B-CLL (same protocol as in Fig. 1b and 2b). The neoplastic cells show a diffuse cytoplasmic staining complicating the interpretation and differentiation between the results in Fig. 2b and 3b.

SN/MV/LE 26-6-2006