Assessment Run 19 2007

Cyclin D1 (CyD1)

The slide to be stained for Cyclin D1 (CyD1) comprised:
1-3. Tonsils fixed 4h, 72h, and 168h, resp., 4. B-chronic lymphatic leukaemia (B-CLL),
5. + 6. Mantle cell lymphoma (MCL).
All tissues were fixed in 10% neutral buffered formalin (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 tonsils.
- A moderate to strong, distinct nuclear reaction of the two MCLs.
- No nuclear reaction of the B-CLL.

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

92 laboratories submitted stains. At the assessment 30 achieved optimal marks (33 %), 39 good (42 %), 14 borderline (15 %) and 9 (10 %) poor marks.

The following Abs were used:
rmAb clone **SP4** (NeoMarkers, n=67; Ventana, n=7; Cell Marque, n=4; DCS, n=2)(concentrated, n=67; ready-to-use [RTU], 12))
mAb clone **DCS6** (Dako, n=4; Novocastra, n=1)
mAb clone **P2D11F11** (Novocastra, n=3)
pAb **CP236** (Biocare, n=4)

Optimal staining for CyD1 in this assessment was obtained with the rmAb clone **SP4** (29 out of 81) and the mAb **P2D11F11** (1 out of 3). All optimal protocols were based on heat induced epitope retrieval (HIER).

Clone **SP4**: All protocols resulting in an optimal staining were based on HIER using either Tris-EDTA/EGTA pH 9, CC1 (Cell Conditioning 1, Ventana), EDTA pH 8, TRS pH 6,1 (Dako) or Citrate pH 6. The dilution of the concentrated Ab was typically in the range of 1:25 – 1:100 depending on the total sensitivity of the protocol employed. With the concentrated Ab and the protocol settings above, 57/67 laboratories obtained a sufficient result (85 %). With RTU, it was 9/12 laboratories (75 %).

Clone **P2D11F11**; The optimal protocol based on HIER in Tris-EDTA/EGTA pH 9, a dilution of 1:70 with Powervision+ (Immunovision) as the detection system.

The most frequent causes of insufficient staining were:
- Less successful primary Ab
- Too low concentration of the primary Ab
- Inappropriate epitope retrieval (HIER combined with proteolytic pre-treatment)
- Insufficient HIER.

In this as well as previous assessments of CyD1 the prevalent feature of an insufficient staining was a too weak or completely false negative nuclear staining of the neoplastic cells in the MCLs.

Normal tonsil is a reliable control: In the squamous epithelium, the suprabasal cells should display a strong nuclear reaction with only minimal cytoplasmic staining. In the lymphatic areas only the endothelial cells should show a nuclear reaction. No difference in the reaction pattern of the tonsils fixed for 4 h, 72h and 168 h in 10 % NBF was seen.

This is the 3rd assessment of CyD1 staining in NordiQC. The proportion of sufficient stains has increased from 55% through 59% to 75% (Table 1). The proportion of sufficient results increases in parallel with the expanding part of the laboratories stocking clone SP4.

	Run 9 2003		Run 17 2006		Run 19 2007	
	Labs.	Sufficient	Labs.	Sufficient	Labs.	Sufficient
mAb clone DCS6	25	6	11	0	5	0
mAb clone P2D11F11	24	18	16	4	3	2
mAb clone SP4	3	3	55	44	80	64
pAb CB236	3	3	5	3	4	3
Total	55	30 (55%)	87	51 (59%)	92	69 (75%)

Table 1. **Number of laboratories participating during three Cyclin D1 assessments, the number of sufficient results, and Abs used.**

To summarize: Clone SP4 is the Ab giving the best performance. pAb CB326 gave good staining in most cases and has previously shown optimal results. Clone P2D11F11 gave sufficient results in 2/3 cases in this run but only 4/16 in the previous. Clone DCS6 have given no sufficient stains in two runs.

The three main recommendations given to the laboratories in run 9 and 17 achieving an insufficient staining were:
1) Consider change of primary antibodies
2) Increase the primary Ab concentration
3) Optimize HIER, i.e., prolong heating time and/or substitute Citrate pH 6 with an alkaline buffer (Tris/EDTA pH 9 or equivalent).
Among laboratories participating in all three runs, 57 recommendations have been given. The results are indicated in Table 2. The identification of tonsil as a robust control for Cyclin D1 and specific recommendations (including change of Ab, see Table 1) tailored to the individual laboratories seem to be major reasons for improvement.

	Followed recommendations	
	Yes	No
Number of laboratories advised	43	14
Number of laboratories improved	31 (72%)	2 (14%)

Table 2. **Improvement of results from insufficient to sufficient as consequence of recommendations given to 57 laboratories participating in all three Cyclin D1 assessments.**

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: the suprabasal layer of squamous epithelium should express strong staining while the lymphoid tissue should be unstained.

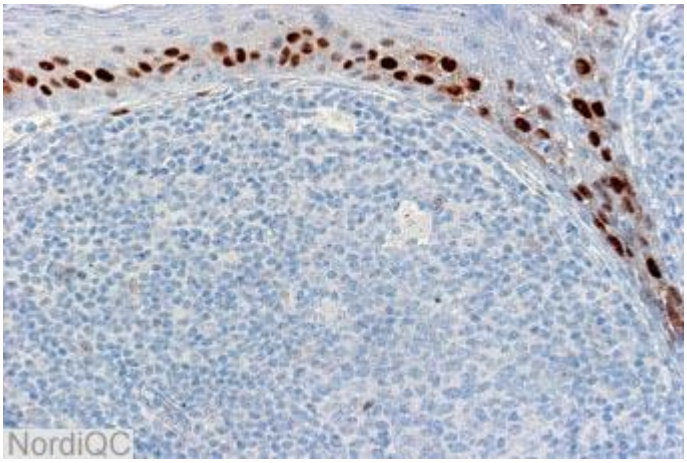

Fig. 1a
Optimal staining for CyD1 of the tonsil. The suprabasal squamous epithelial cells show a moderate to strong nuclear reaction and only a faint intra-cytoplasmic staining. In the germinal centre a few endothelial cells are stained.

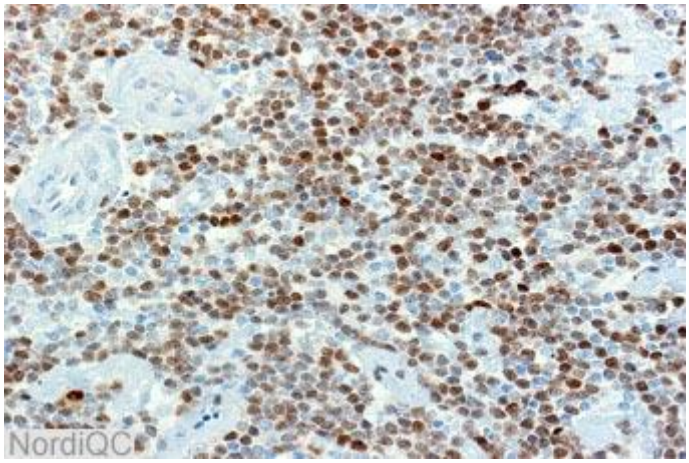

Fig. 1b
Optimal staining for Cyclin D1 in the mantle cell lymphoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a moderate to strong distinct nuclear staining and a well preserved morphology.

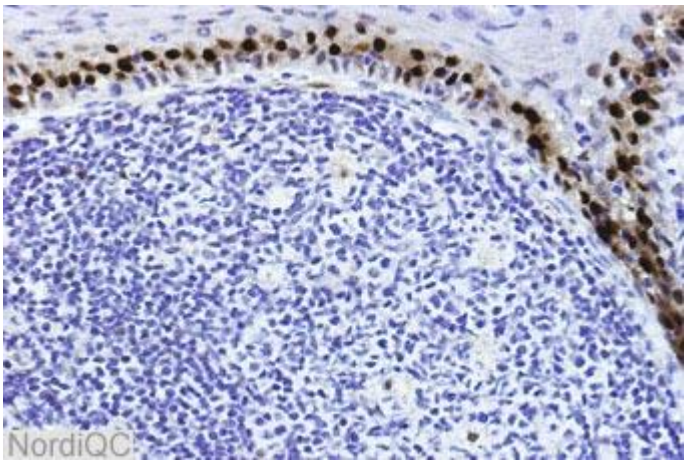

Fig. 2a
Staining for CyD1 of the tonsil assessed as good. Same field as Fig. 1a. The number and intensity of the demonstrated suprabasal squamous epithelial cells is similar to the optimal staining in Fig. 1a, but the morphology is impaired as the nuclei of the germinal centre cells show an extensive wrinkled appearance, probably due to excessive HIER. Also compare with Fig. 2b – same protocol.

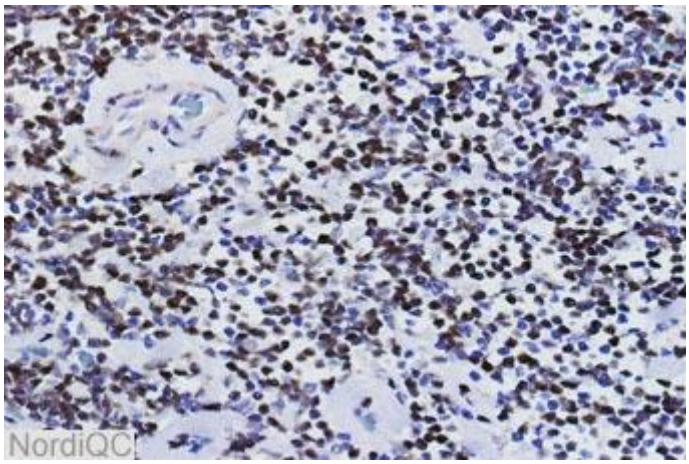

Fig. 2b
Staining for CyD1 of the mantle cell lymphoma assessed as good. Same field as Fig. 1b and same protocol as in Fig. 2a. The neoplastic cells show a positive nuclear reaction, but show an impaired morphology. HIER is mandatory for the demonstration of CyD1, but has to be controlled. The extensive wrinkled appearance is a typical feature with excessive HIER.

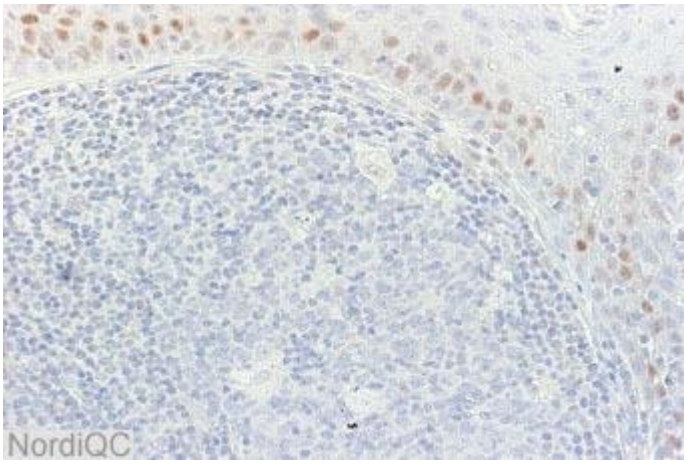

Fig. 3a
Insufficient staining for CyD1 of the tonsil. Same field as Fig. 1a. The suprabasal squamous epithelial cells only show a weak to moderate staining. Also compare with Fig. 3b – same protocol.

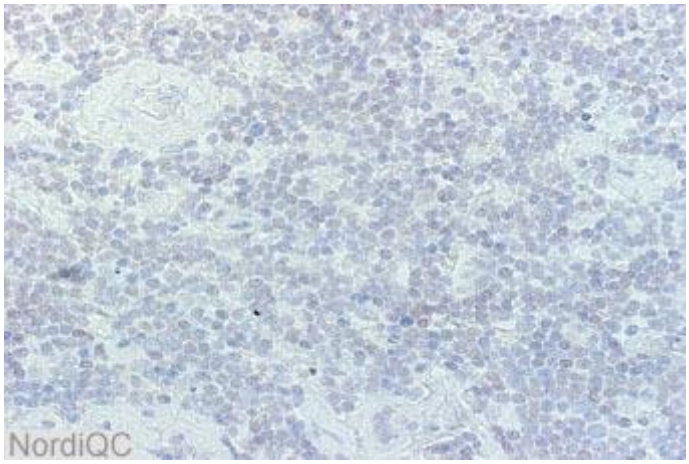

Fig. 3b
Insufficient staining for CyD1 of the mantle cell lymphoma. Same field as Fig. 1 and 2b and same protocol as in Fig. 3a. The neoplastic cells are virtually negative.

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