Assessment Run 9 2003

Cyclin D1 (CyD1)

The slide to be stained for Cyclin D1 comprised:
1: tonsil, 2: placenta, 3: B-CLL, 4-5: mantle cell lymphoma.

Criteria for assessing a Cyclin D1 staining as optimal included: A moderate to strong distinct nuclear staining of the the proliferating squamous epithelium of the tonsil and sub-trophoblastic cells in the placenta, as well as the two mantle cell lymphomas, whereas the nuclei of the large majority of lymphocytes and the B-CLL should be negative. A slight cytoplasmatic reaction was accepted both in the cells expected to react but also in other cell types. However the interpretation of the specific nuclear reaction should not be affected.

57 laboratories submitted stainings. At the assessment 13 achieved an optimal score (23 %), 17 good (30 %), 14 borderline (25 %) and 13 (22%) poor.

25 laboratories used mAb clone DCS6 (DakoCytomation, n=(21, Novocastra, n=2, or NeoMarkers, n=2), 24 used mAb clone P2D11F11 (NovoCastra, n=20, or Ventana, n=3), 3 laboratories used mAb (rabbit) clone SP4 (NeoMarkers/LabVision), 4 laboratories used a pAb (BioCare, n=3, Upstate, n=1).

In this assessment optimal staining could only be obtained with the mAb P2D11F11 (obtained in 10/23) and SP4 (obtained in 3/3).

All laboratories achieving an optimal staining used HIER, most frequently (11/13) with Tris-EDTA/EGTA pH 9 as the heating buffer. MWO, pressure cooker and water bath could be used as the heating device.
The optimal dilution of mAb clone P2D11F11 was in the range of 1:10 – 100, and that of the rabbit mAb SP4 was in the range of 1:50 - 500.

27/57 laboratories (47 %) submitted a staining marked sub-optimal. The most frequent causes of insufficient stainings (often in combination) were:
- Insufficient HIER: too short efficient heating time (MWO< 15 min., water bath <40 min.), use of citrate pH 6 as the heating buffer
- Inappropriate calibration of primary Ab dilution (too high or too low concentration).
- Inappropriate choice of primary Ab

The insufficient epitope retrieval combined with an inappropriate calibration of the primary Ab caused either a false positive reaction in the B-CLL and a generaly too high background staining, or a false negative reaction in the two mantle cell lymphomas.
A very important element in setting up an immunohistochemical protocol for Cyclin D1 is the choice of control tissue.

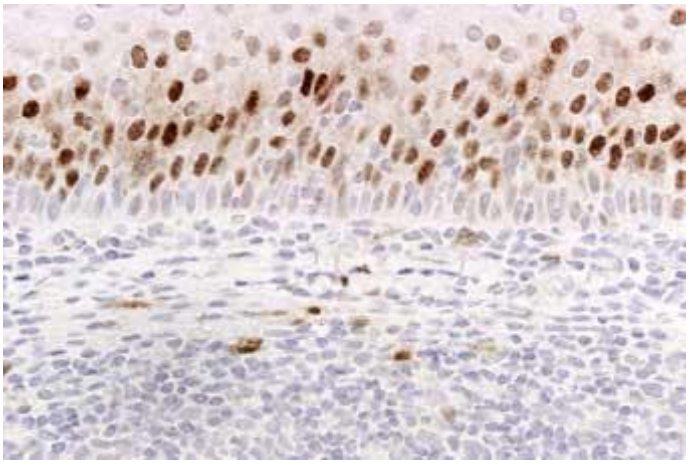

Fig 1a
Optimal staining for Cyclin D1 in a normal tonsil using mAb PD2D11F11. Most nuceli of the suprabasal cells of the squamous epithelium are strongly stained.

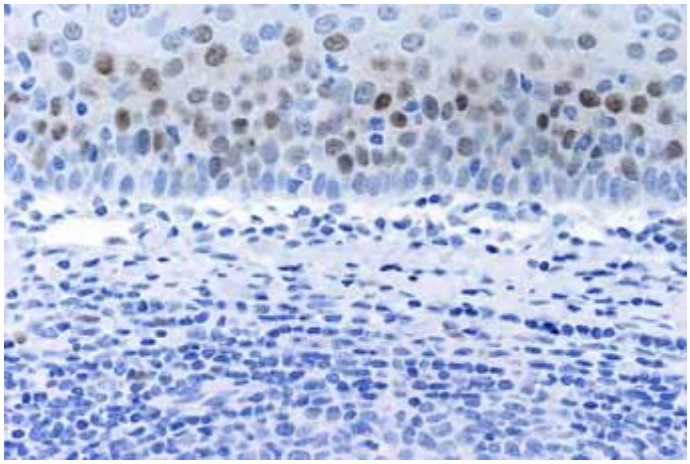

Fig 1b
Good staining for Cyclin D1 in a normal tonsil using mAb PD2D11F11. Most nuceli of the suprabasal cells of the squamous epithelium are moderately stained.

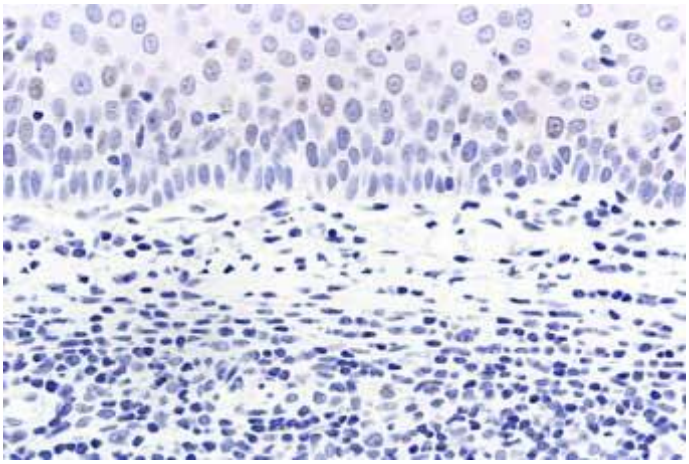

Fig. 1c
Insufficient staining for Cyclin D1 in a normal tonsil using mAb PD2D11F11. The nuclei of the suprabasal epithelial cells are almost unstained.

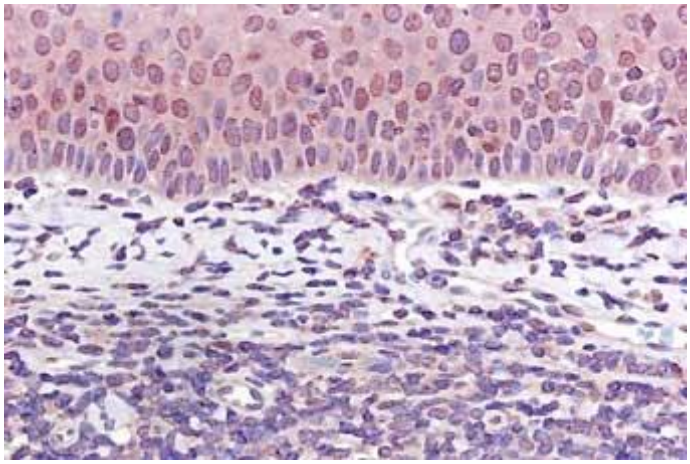

Fig. 1d
Insufficient staining for Cyclin D1 in a normal tonsil using mAb DCS6. The nuclei of the suprabasal epithelial cells are weakly stained and a false positive staining of the cytoplasm in all cells is seen.

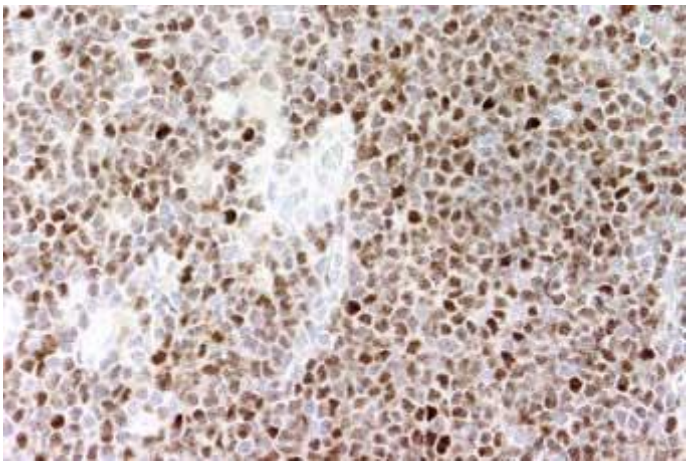

Fig. 2a
Optimal staining for Cyclin D1 in a mantle cell lymphoma using mAb PD2D11F11. Moderate to strong nuclear staining of almost all neoplastic cells with faint cytoplasmic reaction.

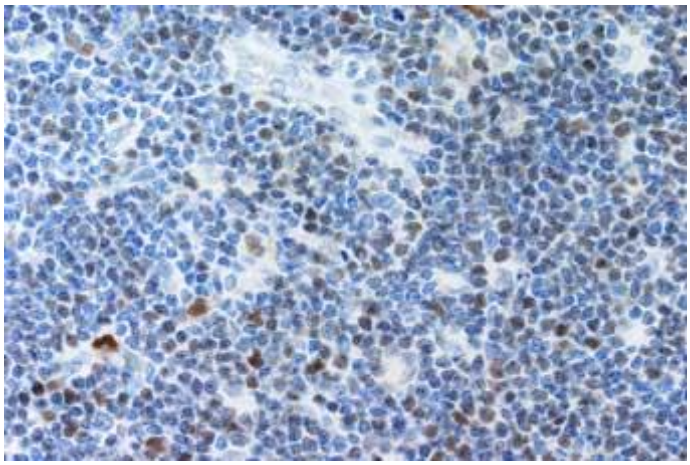

Fig. 2b
Good staining for Cyclin D1 in a mantle cell lymphoma using mAb PD2D11F11 (same field as in Fig. 2a). Weak to moderate nuclear staining of most neoplastic cells.

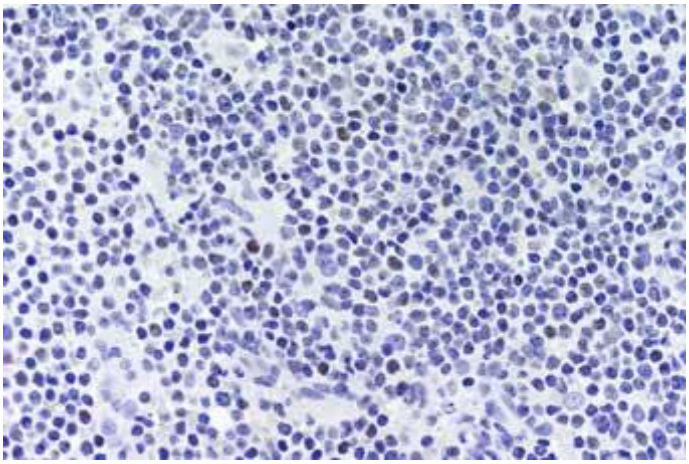

Fig. 2c
Insufficient staining for Cyclin D1 in a mantle cell lymphoma using mAb PD2D11F11 (same field as in Fig. 2a). Almost no staining is revealed.

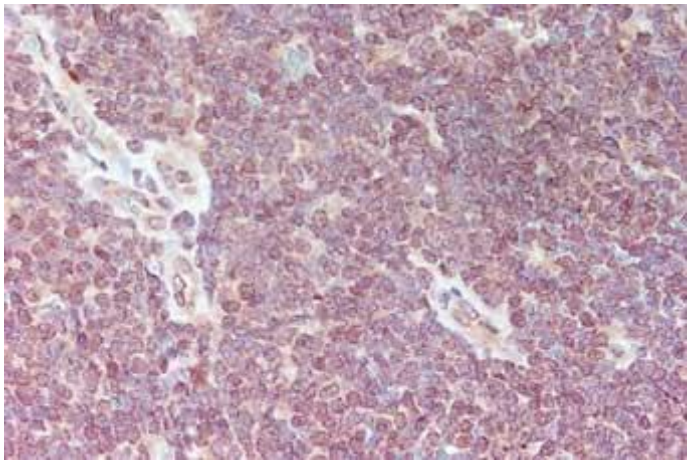

Fig. 2d
Insufficient staining for Cyclin D1 in a mantle cell lymphoma using mAb PD2D11F11 (same field as in Fig. 2a). A moderate staining of most nuclei is revealed but also the cytoplasm is stained compromising the interpretation.

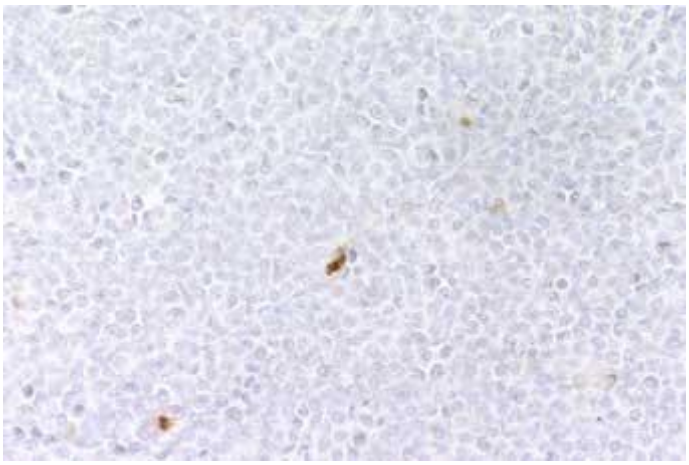

Fig. 3a
Optimal staining for Cyclin D1 in B-CLL using mAb PD2D11F11. No staining of the neoplastic cells.

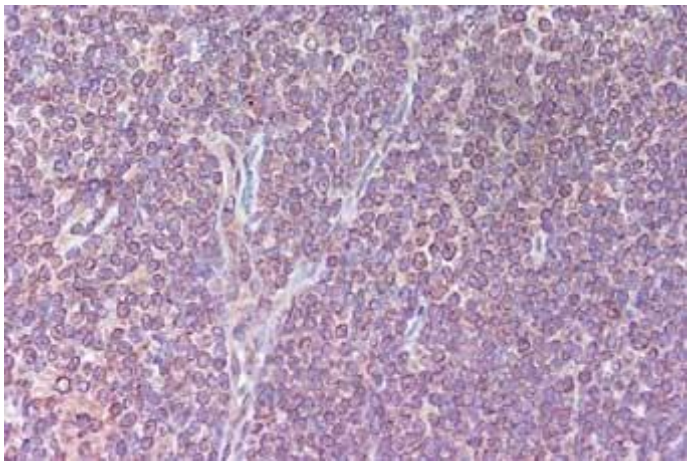

Fig. 3b
Insufficient staining for Cyclin D1 in B-CLL using mAb PD2D11F11. A moderate false postive staining of the cytoplasm of the neoplastic cells compromises the interpretation.

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