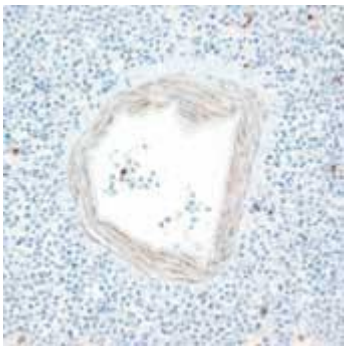
Assessment Run 8 2003
CD5

The slide to be stained for CD5 comprised:
1: Mantle cell lymphoma, 2-3: small lymphocytic lymphoma/CLL, 4-5: tonsil (fixed for 24 and 96 hours, respectively, in 10 % neutral buffered formalin), 6: Burkitt lymphoma.
Criteria for assessing a CD5 staining as optimal included: A strong and distinct membranous staining of the normal T-cells in the two tonsil specimens, the neoplastic B-cells of the mantle cell lymphoma and of one of the CLL's. The other CLL should give a weak but distinct staining of the neoplastic cells. In Burkitt's lymphoma, only the normal T-cells should react. With clone 4C7 a faint cross reaction with smooth muscle cells in larger vessels was accepted.

65 laboratories submitted stainings. At the assessment 25 achieved optimal staining (39 %), 17 good (26 %), 12 borderline (18 %) and 11 (17%) poor staining.



50 laboratories used mAb clone 4C7, obtained from Novocastra (42 labs.), Ventana (5 labs.) and NeoMarkers (3 labs.). 15 used mAb clone CD5/54/F6 from DakoCytomation. Optimal staining could be obtained with both clones.

All laboratories achieving an optimal staining used HIER, most frequently (22/25) with Tris-EDTA/EGTA pH 9 as the heating buffer. There was a slight difference in the reaction patterns of the two clones used. Both labelled very intensively the normal T-cells in the two tonsil specimens, but the staining of the mantle cell lymphoma was more intense and constant using mAb clone 4C7. In the two CLL's the reaction patterns with the two clones was identical.
The optimal dilution of clone 4C7 was 1:25 – 1:200, of clone CD5/54/F6 1:20 – 1:50.
Almost all laboratories were able to detect CD5 in the normal T-cells, but the identification of CD5 in the CLL and the mantle cell lymphoma could only be obtained in protocols with a high sensitivity.

The most frequent causes of insufficient stainings were:
-Insufficient HIER – too short efficient heating time (< 15 min.) and/or use of citrate pH 6
-Too dilute concentration of the primary antibody
-Too high concentration of the primary antibody.

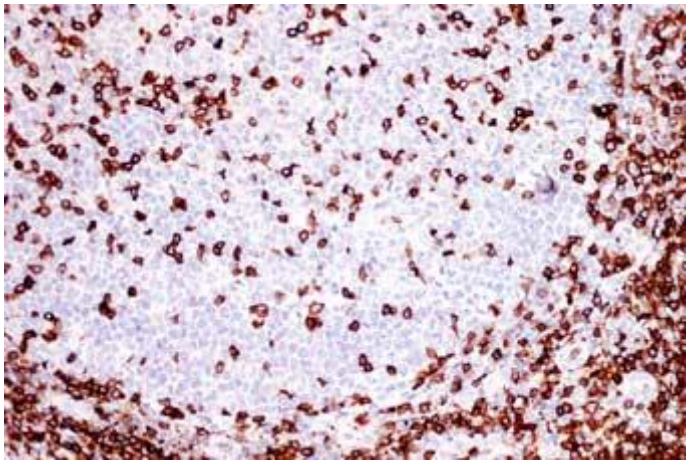


Fig. 1a
An optimal CD5 staining of the tonsil using the mAb clone 4C7. All T-cells in the T-zone and scattered inside the germinal centre are strongly stained without any reaction of the B-cells in the germinal centre.

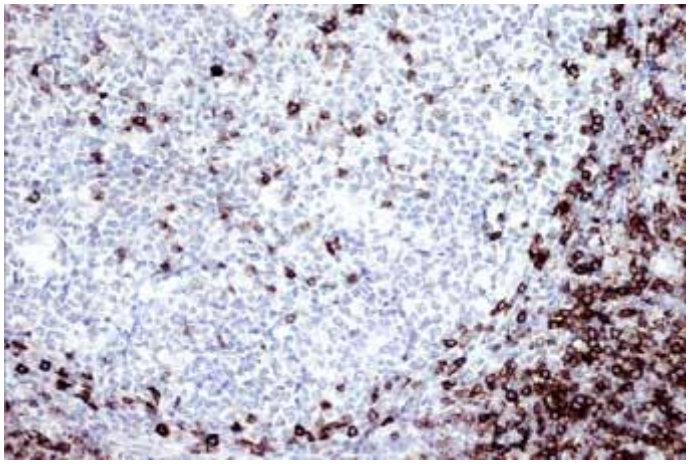


Fig. 1b
An optimal CD5 staining of the tonsil using the mAb clone CD5/54/F6 with the same reaction pattern as obtained with mAb clone 4C7 (fig. 1a).

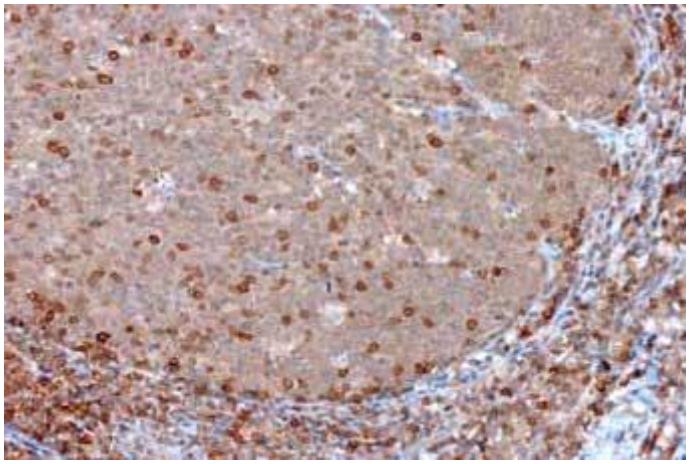


Fig. 1c
An insufficient CD5 staining of the tonsil (too high concentration of the primary Ab combined with an insufficient HIER procedure). The normal T-cells are stained, but a strong, unspecific reaction is seen in the B-cells of the germinal centre, resulting in a staining with a poor signal-to-noise ratio. Furthermore, using this protocol, an insufficient staining of the mantle cell lymphoma (fig. 2c) and the CLL (fig. 3c) was seen.

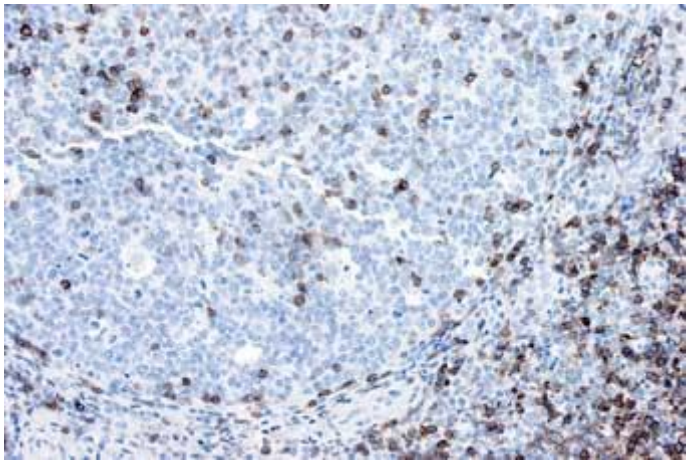


Fig. 1d
CD5 staining of the tonsil using an insufficient protocol (too low concentration of the primary Ab). The majority of the T-cells are moderately stained but the number of T-cells is low compared to fig. 1a. Furthermore, using this protocol, an insufficient staining of the mantle cell lymphoma (fig. 2d) and the CLL (fig. 3d) was seen.

Fig. 2a
An optimal CD5 staining of the mantle cell lymphoma using the mAb clone 4C7. All the neoplastic cells are strongly stained. The scattered normal T-cells are darker than the neoplastic cells.

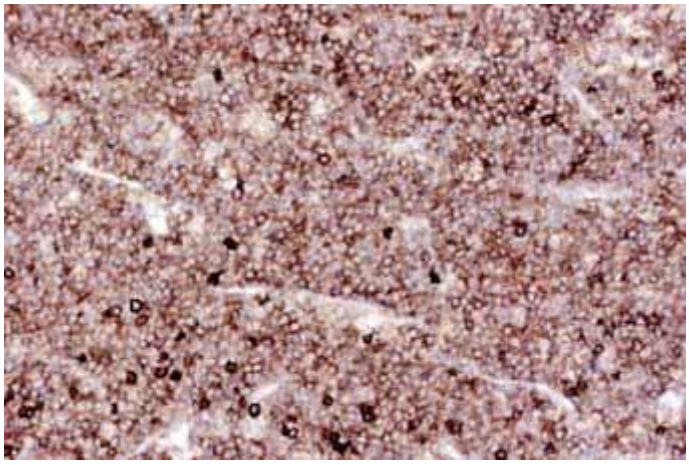


Fig. 2b
An optimal CD5 staining of the mantle cell lymphoma using the mAb clone CD5/54/F6. The reaction pattern is similar to the MaB. clone 4C7 (fig. 2a). However, the difference between the staining intensity of the neoplastic B-cells and the normal T-cells appear more pronounced than in the other staining.

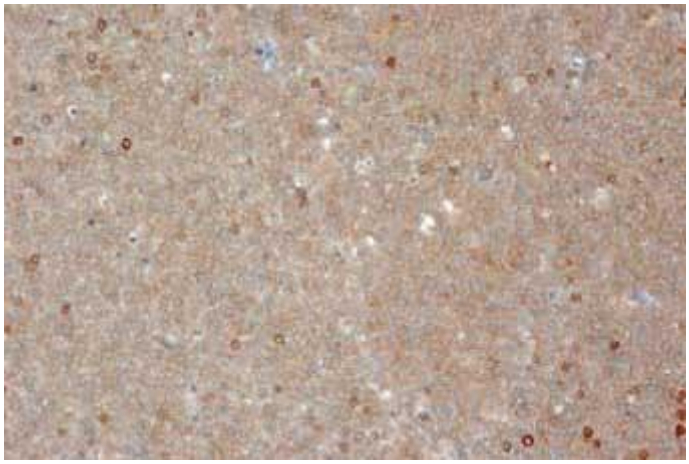


Fig. 2c
An insufficient CD5 staining of the mantle cell lymphoma due to a too high conc. of the primary ab. combined with an insufficient HIER procedure. The normal T-cells are stained, but the neoplastic cells of the mantle cell lymphoma are only weakly stained with the same apperance as the unspecific reaction seen in fig 1c.

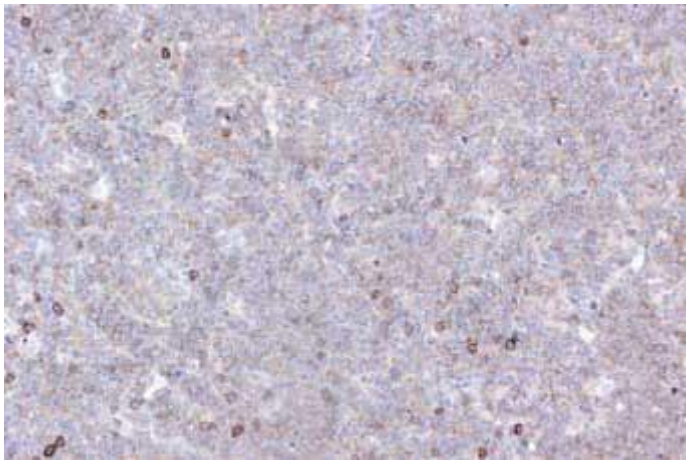


Fig. 2d
An insufficient CD5 staining of the mantle cell lymphoma due to a too low conc. of the primary ab. The neoplastic cells of the mantle cell lymphoma are allmost unstained.

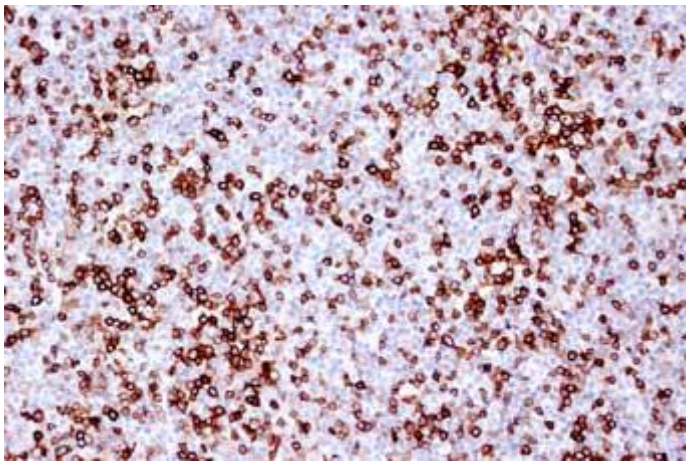


Fig. 3a
An optimal CD5 staining of the CLL with the weak CD5 expression usingthemAb clone 4C7. Two populations of cells can be discerned. The neoplastic cellsare moderately stainedand the normal T-cells strongly stained.

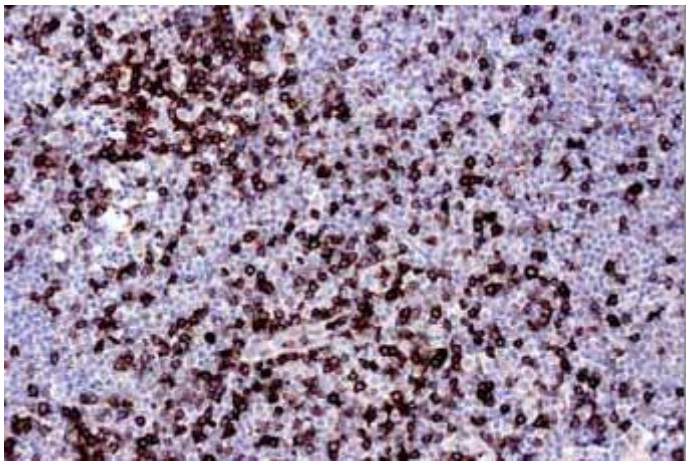


Fig. 3b
An optimal CD5 staining of the CLL with the weak CD5 expresion using the mAb clone CD5/54/F6. A staining pattern as in Fig. 3a. is seen.

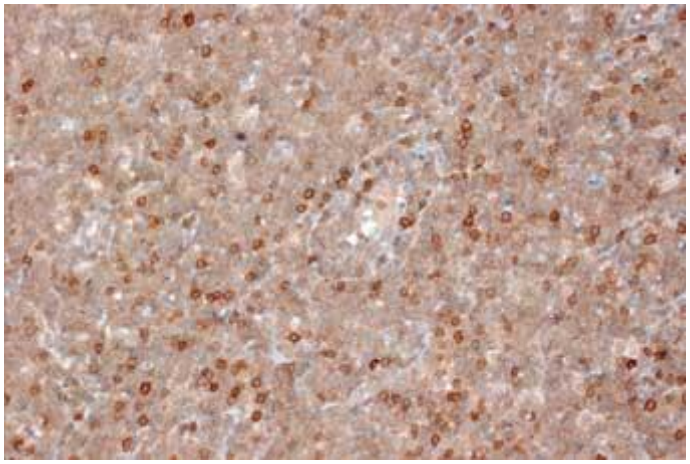


Fig. 3c
An insufficient CD5 staining of the CLL with the weak CD5 expression. Same protocol as in fig.1c and 2c resulting in a staining with a poor signal-to-noise ratio.

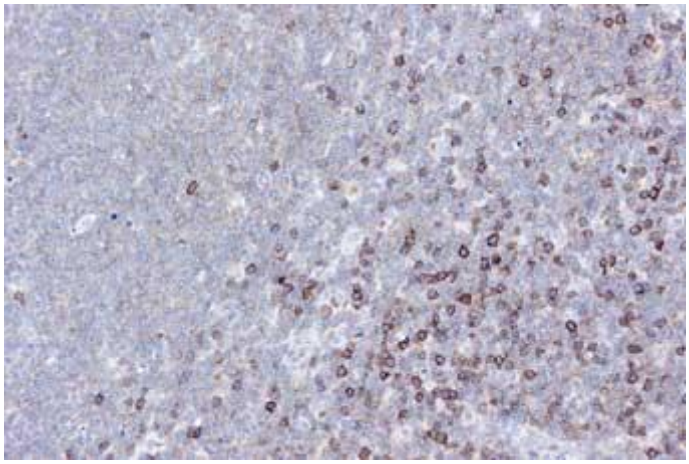


Fig. 3d
An insufficient CD5 staining of the CLL with the weak CD5 expression. A too low concentration of the primary Ab has been used. The neoplastic cells of the CLL are almost unstained.

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