Assessment Run 18 2006

Terminal deoxynucleotidyl transferase (TdT)

The slide to be stained for Terminal deoxynucleotidyl Transferase (TdT) comprised: 1. Tonsil fixed 24 h, 2. Thymoma, 3. Tonsil fixed 72 h, 4. Testis precursor B-ALL, 5. Thymus. All specimens were fixed in 10 % NBF.

Criteria for assessing a TdT staining as optimal included:

- A strong and distinct nuclear reaction of the normal subcapsular and cortical thymocytes whereas the medullar thymocytes should be negative.
- A moderate to strong distinct nuclear reaction of the majority of the neoplastic cells of the precursor B-ALL and thymoma.
- A distinct nuclear staining of few perisinusoidal cells in the interfollicular zones of the tonsils.
- No staining in other tonsillar T-cells and B-cells.

62 laboratories participated in the assessment. 35 achieved optimal marks (56 %), 23 good (37 %), 1 borderline (2 %) and 3 (5 %) poor marks.

The following Abs were used:
mAb clone **SEN28** (Novocastra, n=16; Immunomarkers, n=1; Ventana, n=1)
mAb clone N**PT26** (Novocastra, n=1)
pAb **A3524** (Dako, n=34)
pAb **ILM 004** (Immunologic/Supertechs, n=4)
pAb **760-2670** (Ventana, n=4)
pAb **18-7237** (Zymed, n=1)

Optimal staining for **TdT** in this assessment was obtained with the the mAb clone **SEN28** (13 out of 18), the pAb **A3524** (20 out of 34) and the pAb **ILM 004** (2 out of 4). All the optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

SEN28: the optimal results were based on HIER in either TRIS EDTA/EGTA pH 9 (7 out of 8), Cell Conditioning 1 (CC1 Ventana, 2 out of 3), Citrate pH 6 (1 out of 4), EDTA/EGTA pH 8 (1 out of 1), EDTA pH 9 (1 out of 1) or Target Retrieval Solution pH 6.1 (Dako S1699, 1 out of 1). The mAb SEN28 was typically used in the range of 1:20 – 1:100 depending of the total sensitivity of the protocol employed or was applied as a Ready-To-Use antibody.

A3524: an optimal staining were based on HIER in either TRIS EDTA/EGTA pH 9 (18 out of 28) or Cell Conditioning 1 (CC1 Ventana, 2 out of 4). The pAb A3524 was typically used in range of 1:10 – 1:80 depending of the total sensitivity of the protocol employed.

ILM 004: an optimal staining were based on HIER in Citrate pH 6 (2 out of 2) and diluted in the range of 1:20 – 1:40.

Grouped together, 58 out of 61 laboratories (95 %) using HIER and one of the three above mentioned markers for TdT obtained a sufficient mark.

The causes of an insufficient staining were:
- Less successful primary antibody
- Too low and too high concentration of the primary antibody

The prevalent feature of an insufficient staining was either a too high level of background staining of non-TdT expressing structures as connective tissue and the cytoplasm of normal lymphocytes and squamous epithelial cells in the tonsils, or a too weak reaction of the neoplastic cells in the thymoma and the precursor B-ALL.

Normal thymus should be the preferred control tissue in which the cortical thymocytes should show a distinct nuclear reaction with minimal cytoplasmic reaction. The medullar thymocytes should be negative.

Conclusion
mAb clone **SEN28** and the pAbs **A3524** and **ILM 004** appear to be useful and robust Abs for the demonstration

of TdT. HIER is mandatory to obtain an optimal result.

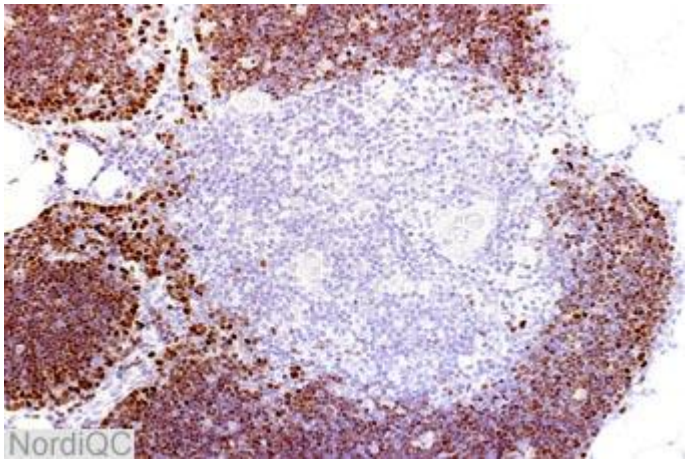

Fig. 1a
Optimal staining for TdT of the Thymus. The normal subcapsular and cortical thymocytes show a distinct nuclear reaction and the medullary cells are negative.

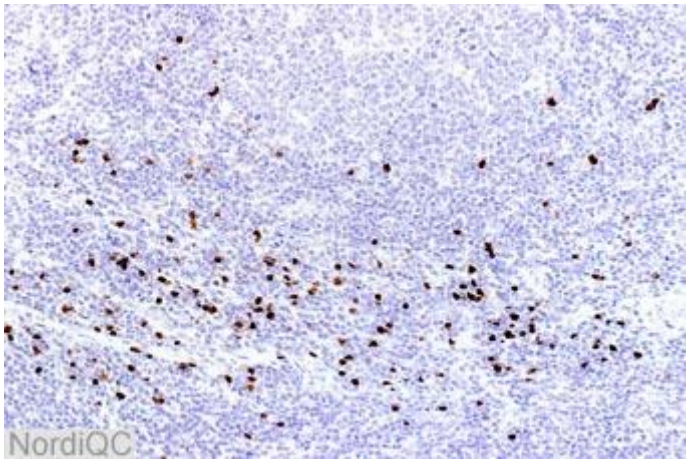

Fig. 1b
Optimal staining for TdT of the tonsil. In the interfollicular zone few perisinusoidal cells show a distinct positive nuclear reaction. All other cells in the tonsil are negative.

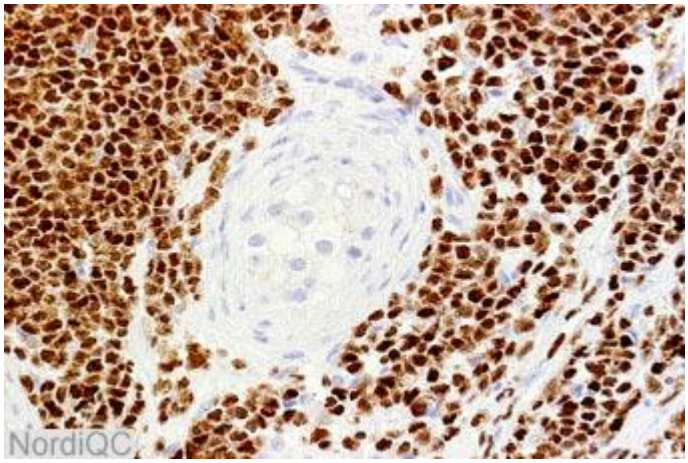

Fig. 2a
Optimal TdT staining of the Precursor B-ALL. Virtually all of the neoplastic cells show a distinct nuclear reaction, while the remnants of the normal testicular germ cells are negative.

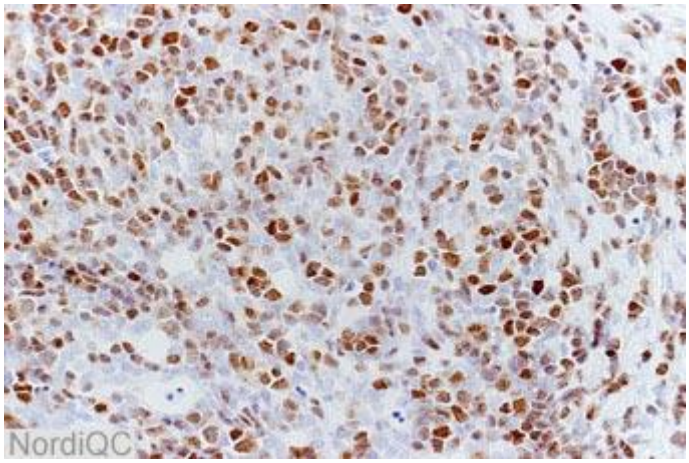

Fig. 2b
Optimal TdT staining of the thymoma. The majority of neoplastic cells show a distinct nuclear reaction.

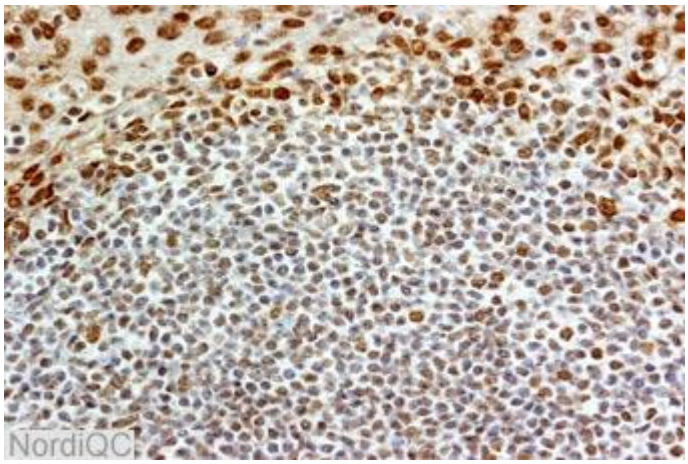

Fig. 3a
Insufficient TdT staining of the tonsil. All cells, both epithelial and lymphatic cells show a false positive nuclear staining due to a too high conc. of the primary Ab.. Compare to Fig. 1b.

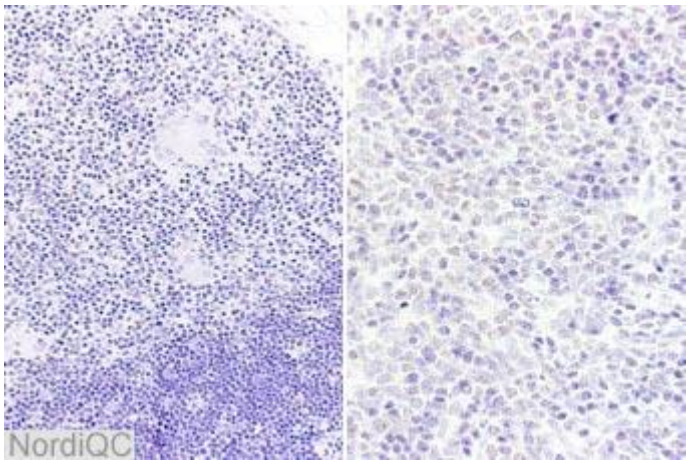

Fig. 3b
Left: Insufficient staining for TdT of the Thymus, same field as in Fig 1a. All the normal thymocytes are negative or only weakly positive.

Right: Insufficient staining for TdT of the thymoma. The majority of the neoplastic cells are negative (same protocol as in Fig. 3b left).

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