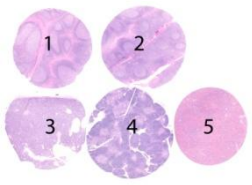
Assessment Run 40 2014

Terminal deoxynucleotidyl Transferase (TdT)

Material
The slide to be stained for TdT comprised:



1. Tonsil fixed for 24 hours, 2. Tonsil fixed for 48 hours, 3. Thymoma NOS, 4. Thymus, 5. Precursor-B-acute lymphatic leukaemia (Pre-B-ALL).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing TdT staining as optimal were:

- An at least moderate distinct nuclear staining reaction of virtually all cortical thymocytes of the normal thymus.
- A moderate to strong distinct nuclear staining reaction of the vast majority of neoplastic cells of the Pre-B-ALL and thymoma.
- A distinct nuclear staining reaction of dispersed perisinusoidal cells in the interfollicular zones of the two tonsils.
- No nuclear staining reaction of T- and B-cells in the tonsils and the vast majority of medulary thymocytes of the normal thymus.

Results
185 laboratories participated in this assessment. 144 (78 %) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (see page 2).

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary Ab
- Less successful primary Abs

Performance history
This was the 2nd NordiQC assessment of TdT and a decrease in pass rate was seen as shown in table 2.

Table 2. **Proportion of sufficient results for TdT in two NordiQC runs performed**

	Run 18 2006	Run 40 2014
Participants, n=	62	185
Sufficient results	93%	78%

The reduced pass rate (78%) in this run may in part be explained by a large proportion of new participants and new and more challenging tissue material circulated. However, also the less successful performance of the pAbs in this assessment seemed to have a significant impact. In run 18, 2006 97% of the laboratories (33 of 34) using the most widely applied antibody for TdT, pAb A03514 (Dako) obtained a sufficient mark (good or optimal), whereas a pass rate of 67% (24 of 36) for the laboratories using the same pAbs in this run was seen.

Conclusion
The mAb clone **SEN28** was in this assessment the most robust and successful marker for TdT.
Efficient HIER using an alkaline buffer in combination with a sensitive and specific 3-step polymer/multimer based detection system gave the highest proportion of optimal results.
The concentrated format of the mAb clone **SEN28** provided an optimal result on the 3 main IHC systems (Dako, Leica and Ventana).
Thymus is recommended as positive tissue control for TdT. Virtually all cortical thymocytes must show an at least moderate and distinct nuclear staining reaction.
Tonsil can be used as negative tissue control as no staining reaction should be seen in the mantle zone and germinal centre B-cells.

Table 1. **Antibodies and assessment marks for TdT, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **SEN28**	44 4 1 1 1	Leica/Novocastra Thermo/NeoMarkers Diagnostic Biosystems Gentech Vector	30	12	5	4	82%	84%
rmAb clone **EP266**	1	Abcam/Epitomics	0	1	0	0	-	-
pAb **A3524**	36	Dako	10	14	10	2	67%	81%
pAb **ILP 0049**	7	Immunologic	2	4	1	0	86%	100%
pAb **18-7237**	1	Life Tech/Invitrogen	0	1	0	0	-	-
pAb **61-0155-2**	1	Genemed	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **SEN28 PA0339**	6	Leica/Novocastra	3	1	1	1	67%	80%
mAb clone **SEN28 PDM 096**	1	Diagnostics Biosystems	0	0	1	0	-	-
mAb clone **SEN28 MAD-00909QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **SEN28 ZM-0358**	1	Zhonggshan	0	1	0	0	-	-
pAb **338A-78**	2	Cell Marque	0	1	1	0	-	-
pAb **760-2670**	37	Ventana/Cell Marque	1	24	10	2	68%	50%
pAb **IS001/IR001**	39	Dako	11	25	3	0	92%	92%
pAb **PP134**	1	Biocare	0	1	0	0	-	-
Total	185		58	86	32	9	-	
Proportion			32%	46%	17%	5%	78%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of TdT, Run 40

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies

mAb clone **SEN28**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/5)*, TRS pH 9 (Dako) (3/3), Cell Conditioning 1 (CC1; Ventana) (9/19), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (12/14), Tris-EDTA/EGTA pH 9 (2/4) or Citrate pH 6 (2/5) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 42 of 50 (95%) laboratories produced a sufficient staining reaction (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

pAb **A3524**: Protocols with optimal results were all based on HIER using TRS pH 9, 3-in-1 (Dako) (3/7), TRS pH 9 (Dako) (2/4), TRS pH 6.1 (Dako) (1/1), CC1 (Ventana) (3/13) or Tris-EDTA/EGTA pH 9 (1/3) as retrieval buffer. The pAb was typically diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 21 of 26 (81%) laboratories produced a sufficient staining reaction.

pAb **ILP 0049**: Protocols with optimal results were all based on HIER using CC1 (Ventana) (1/3) or Tris-EDTA/EGTA pH 9 (1/3) as retrieval buffer. The pAb was diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 of 6 (100%) laboratories produced a sufficient staining reaction.

Table 3**. Proportion of optimal results for TdT using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **SEN28**	5/8** (63%)	0/1	9/19 (47%)	-	11/13 (85%)	-
pAb **A3524**	4/10 (40%)	1/1	3/12 (25%)	0/1	0/4	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems

mAb clone **SEN28**, product no. PA0339, Leica, Bond-max/Bond-III:
Protocols with optimal results were all based on HIER using BERS2 pH 9 (Bond, Leica) (efficient heating time 20 min.at 97-100°C), 20-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 4 of 5 (80%) laboratories produced a sufficient staining reaction (optimal or good).

pAb product no. **760-2670**, Ventana/Cell Marque, BenchMark XT/Ultra:
The protocol with an optimal result was based on HIER using mild Cell Conditioning 1, 32 min incubation of the primary Ab and iVIEW (760-091) as detection system. Using these protocol settings 1 of 2 (50%) laboratories produced a sufficient staining reaction.

pAb product no. **IS001/IR001**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min at 95-97°C) and 20-30 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 34 of 37 (92%) laboratories produced a sufficient staining reaction).

In this assessment, the prevalent features of an insufficient staining were false positive staining reaction, poor signal-to-noise ratio or an overall too weak staining reaction.
False positive staining reaction and a poor signal-to-noise was seen in 56% of the insufficient results (23 of 41).
The false positive staining reaction was typically seen as a moderate to strong aberrant cytoplasmic staining reaction of germinal centre B-cells and squamous epithelial cells of the tonsils compromising the interpretation of nuclear TdT expression. Occasionally, also a false positive nuclear staining reaction in these cells or a general background staining was seen.
In particular the concentrated format of the pAb A3524 (Dako) gave a high proportion of insufficient results due to a false positive staining result and/or poor signal-to-noise ratio. The same pattern was also observed for the Ready-To-Use formats based on a pAb e.g. prod. no. IR/IS001 (Dako) and 760-2670 (Ventana/Cell Marque). The latter giving a false positive staining result in 10 of 12 insufficient results. Only 1 of 37 laboratories using the pAb prod. no 760-2670 produced an optimal result (see table 1). In this context it has to be stressed, that a weak cytoplasmic staining reaction did not affect the evaluation when the aberrant staining did not compromise the interpretation.
No single protocol parameter, as concentration of the primary Ab, HIER conditions, detection system etc., could be identified as the central source for the insufficient staining results of the three above mentioned pAb formats for TdT. Use of protocols with high sensitivity, typically based on HIER in an alkaline buffer and a 3-step polymer/multimer based detection systems, seemed to amplify both the specific signal for TdT but also the aberrant cytoplasmic staining reaction. Lot-to-lot variations of the pAbs might be a cofactor as using the concentrated format of the pAb A03524, lot no. 10072158 (titre of 1:25, HIER in TRS High pH and a 3-step polymer detection system, EnVision Flex+, Dako) an optimal result without aberrant staining reaction was obtained. Using the lot no. 1004890 and similar protocol settings, excessive aberrant cytoplasmic staining in virtually all cells compromising the interpretation was seen. However no conclusive data on lot-to-lot variations of the pAbs for TdT are available and NordiQC will contact the respective vendors for more data and their observations. At this point Dako has informed NordiQC, that the present rabbit pAb A3524 as concentrate and the corresponding RTU formats IR001/IS001 will be discontinued april, 2014 and replaced by a new antibody.
The pAb ILP 0049, Immunologic, did not give any aberrant cytoplasmic staining reaction despite that similar protocol settings were applied. Same lot no. 1021, was used by all the participants using this product (n=7).
A too weak staining result was typically characterized by a reduced staining reaction both in regard to the intensity and proportion of the structures expected to be demonstrated. This was in particular observed in the neoplastic cells of the Pre-B-ALL. A too weak staining was most frequently caused by a too low titre of the primary antibody and/or insufficient HIER.

The mAb clone SEN28 gave the highest proportion of sufficient and optimal results, as seen in table 2.
Optimal result could be obtained on the 3 most widely used IHC systems, as shown in table 3.
Efficient HIER in an alkaline buffer in combination with a 3-step polymer/multimer based detection system were the main protocol prerequisites for an optimal staining result. In general, protocols based on the mAb clone SEN28 displayed a high signal-to-noise ratio.

Controls
Normal thymus was found to be the most reliable and recommendable positive tissue control for TdT. In thymus virtually all cortical thymocytes must demonstrate an at least moderate distinct nuclear staining reaction, while the vast majority of medular thymocytes must be negative. Scattered thymocytes in the medular zone will be distinctively positive. Tonsil was less reliable as positive tissue control for TdT, as a false negative staining reaction of Pre-B-ALL and Thymoma could be seen, despite the perisinusoidal cells showed a strong and distinct nuclear staining reaction. The recommendation given by NordiQC in run 18, 2006 to use tonsil as single positive tissue control for TdT was thus unfortunately not correct.
Tonsil can be used as negative tissue control for TdT - no staining reaction should be seen in mantle zone and germinal centre B-cells.

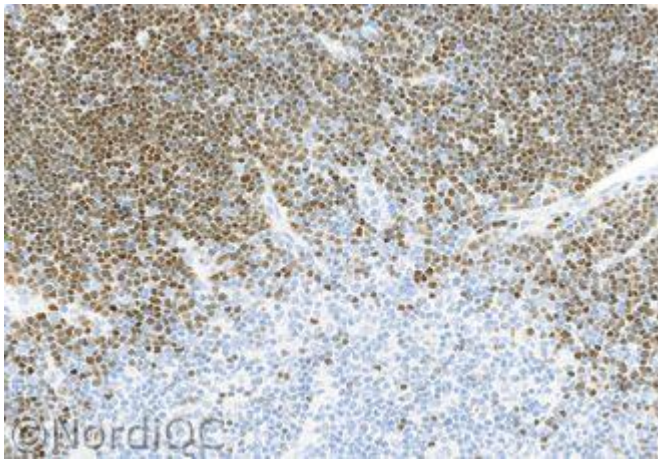


Fig. 1a
Optimal TdT staining of the thymus using the mAb clone SEN28 optimally calibrated, HIER in an alkaline buffer and a 3-step multimer based detection system (OptiView, Ventana). Virtually all the cortical T-cells show a moderate distinct nuclear staining reaction, while the vast majority of the medulary T-cells are unstained. Also compare with Figs. 2a and 3a, same protocol.

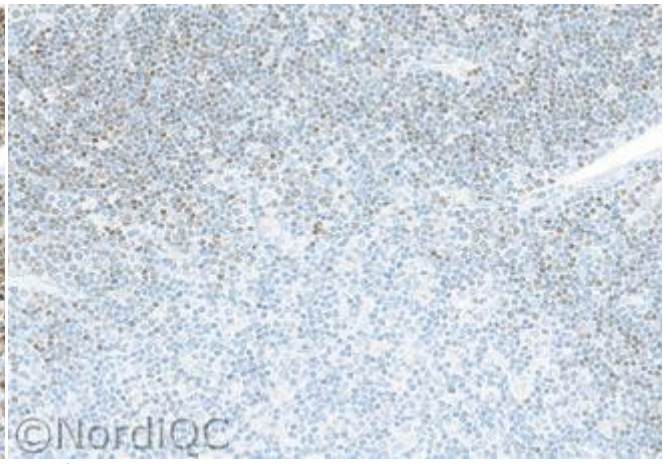


Fig. 1b
Insufficient TdT staining of the thymus using the mAb clone SEN28 with a protocol based on a 2-step multimer based detection system and/or a too low. concentration of the primary Ab providing a low sensitivity - same field as in Fig. 1a. Only a weak and equivocal nuclear staining reaction in the cortical T-cells is seen. Also compare with Fig. 2b, same protocol.

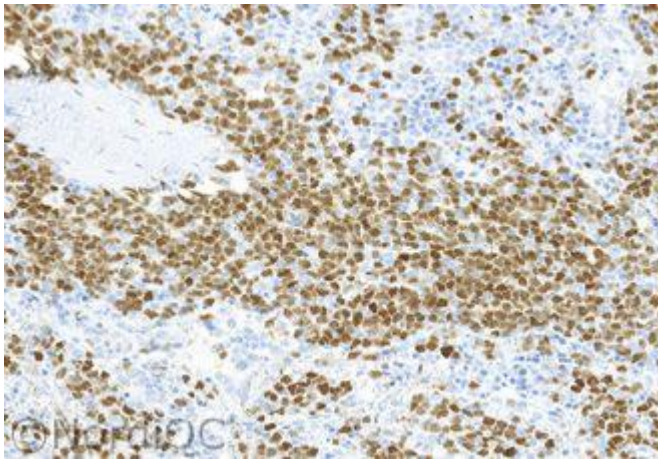


Fig. 2a
Optimal TdT staining of the Pre-B-ALL using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a distinct moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained and no background staining is seen.

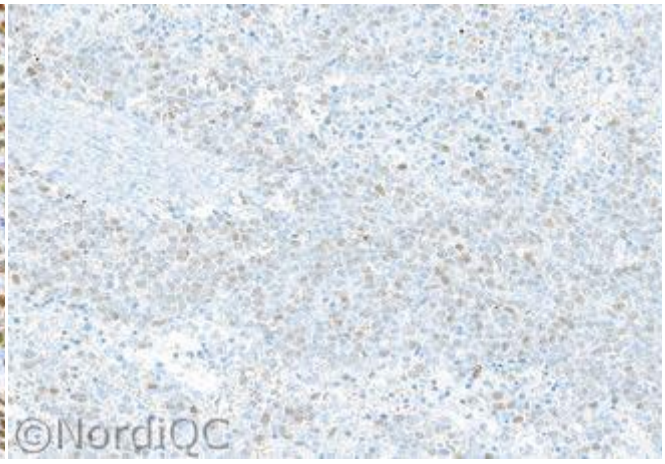


Fig. 2b
Insufficient TdT staining of the Pre-B-ALL using same protocol as in Fig. 1b - same field as in Fig. 2a.
The proportion and intensity of the staining reaction of the neoplastic cells is significantly reduced compared to result expected and obtained in Fig. 2a.

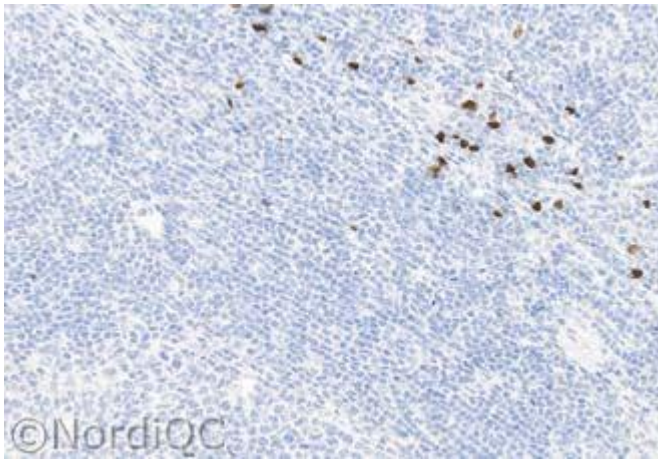


Fig. 3a
TdT staining in the tonsil using same optimal protocol as in Figs. 1a and 2a. A distinct nuclear staining reaction of dispersed perisinusoidal cells in the interfollicular zone is seen. However, as these cells express high levels of TdT, tonsil cannot be recommended to be used as single positive tissue control to evaluate if the protocol applied provides a high or low sensitivity – see Figs. 1b – 3b. For this purpose, thymus seems to be more reliable.

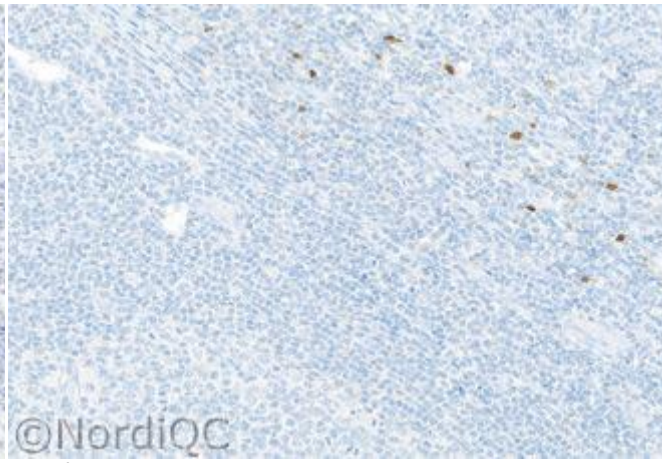


Fig. 3b
TdT staining in the tonsil using same insufficient protocol as in Figs. 1b and 2b. Scattered perisinuoidal cells in the interfollicular zone are distinctively demonstrated. As an insufficient and too weak staining reaction using this protocol was seen in the other tissues included in the material circulated, tonsil cannot be recommended as single positive tissue control for TdT.

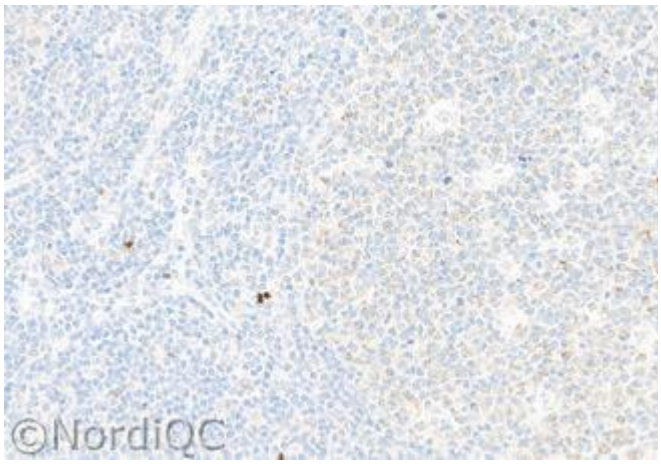


Fig. 4a
TdT staining in the tonsil using the pAb A3524, Dako. A weak, granular cytoplasmic staining reaction in the germinal centre B-cells is seen. Interpretation for presence of the specific nuclear expression of TdT can still be performed. The overall result was evaluated as "Good". Also compare with Fig. 4b, same antibody.

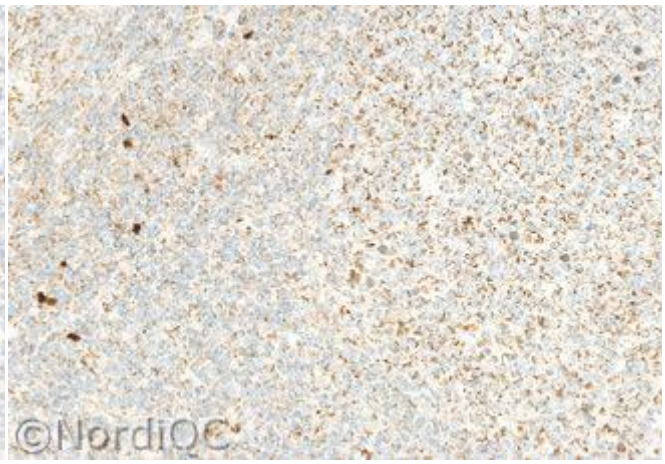


Fig. 4b
Insufficient TdT staining of the tonsil using the pAb A3524, Dako. A moderate and diffuse granular cytoplasmic staining reaction in virtually all cells is seen. The interpretation of TdT is significantly compromised. The aberrant cytoplasmic staining reaction was typically seen for the pAb as concentrate A3524, Dako but also as Ready-To-Use format IR001/IS001, Dako and 760-2670 Ventana. Application of high sensitive protocol settings amplifies both the specific nuclear and unspecific cytoplasmic staining reaction.

SN/RR/MV/LE 26-03-2014