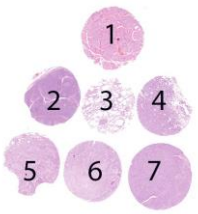
Assessment Run 58 2020
Thyroid transcription factor-1 (TTF1)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for TTF1 performed by the NordiQC participants, identifying lung as origin for adenocarcinomas of unknown origin and differentiation between lung adenocarcinoma and lung squamous cell carcinoma. Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of TTF1 antigen densities (see below).

Material
The slide to be stained for comprised:



1. Thyroid gland, 2. Tonsil, 3. Normal lung, 4-5. Lung adenocarcinoma, 6. Lung squamous cell carcinoma, 7. Colon adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a TTF1 staining as optimal included:

- A strong, distinct nuclear staining reaction of all type II pneumocytes and basal cells of the terminal bronchioles in the lung.
- An at least weak to moderate, distinct nuclear staining reaction of the vast majority of luminal epithelial cells of the terminal bronchioles in the normal lung.
- A strong, distinct nuclear staining reaction of all follicular epithelial cells in the thyroid gland.
- A moderate to strong nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma, tissue core no. 4, and at least weak to moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the lung adenocarcinoma, tissue core no. 5.
- No staining reaction of neoplastic cells in the lung squamous cell carcinoma (a strong staining reaction is expected in remnants of normal pneumocytes) and colon adenocarcinoma.
- No staining reaction in the tonsil*.

*A weak nuclear staining reaction of scattered lymphocytes (<1%) mainly in germinal centres was accepted[1].

[1] Van Bockstal M, Camboni A, De Vlieghere E, et al. Some diffuse large B cell lymphomas (DLBCLs) present with clone-dependent TTF-1 positivity. Histopathology. 2018;72(7):1228–1230.

Participation

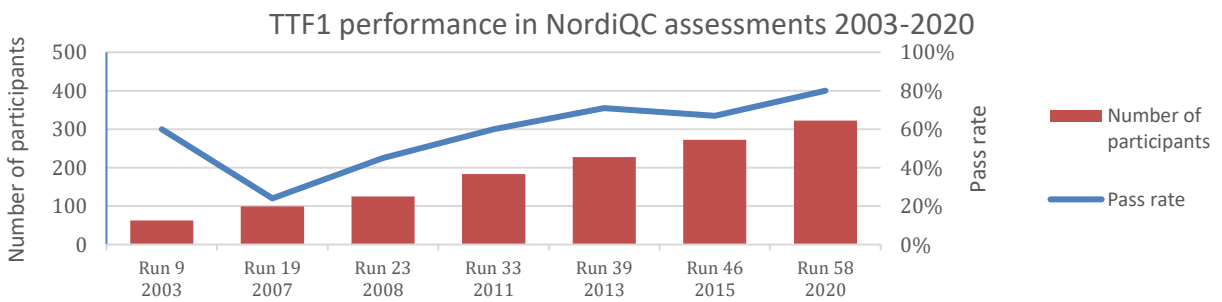
Number of laboratories registered for TTF1, run 58	340
Number of laboratories returning slides	322 (95%)

Results
322 laboratories participated in this assessment. 80% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab (the mAb clone 8G7G3/1).
- Use of less sensitive detection systems.

Performance history
This was the seventh NordiQC assessment of TTF1. A pass rate of 80% was seen, which is the highest level obtained in all NordiQC assessments of TTF1 (see Graph 1).

Graph 1: **Proportion of sufficient results for TTF1 in the NordiQC runs performed**



Conclusion

In this run, and in concordance with previous NordiQC assessments for TTF1, the mAb clone SPT24 and the rmAb clone SP141 were most successful for the demonstration of TTF1 providing pass rates of 87% and 99%, respectively. In contrast, mAb clone 8G7G3/1 was less successful and provided a significantly lower pass rate of 20%

The overall pass rate for TTF1 run 58 was 80%. Compared to previous assessments, increased use of the successful clones at the expense of 8G7G3/1 and extended use of sensitive 3-step detections systems , seemed to have a positive impact on the pass rate in this assessment.

Lung tissue is recommendable as positive tissue control: Virtually all columnar epithelial cells of the terminal bronchi must show an at least weak to moderate, distinct nuclear staining and type II pneumocytes and basal epithelial cells a strong, distinct nuclear staining. Tonsil can serve as negative tissue control in which no staining should be seen. However scattered lymphocytes (<1%), mainly in germinal centres, may show a weak nuclear staining reaction which is acceptable.

Table 1. **Antibodies and assessment marks for TTF1, run 58**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **8G7G3/1**	2 6 1 8 1 1 1	Biocare Medical Cell Marque CliniSciences Dako/Agilent Diagnostic BioSystems Zytomed Thermo Scientific	0	3	11	6	15%	0%
rmAb clone **BSR40**	1	Nordic Biosite	0	1	0	0	-	-
mAb clone **SPT24**	8 1 2 107 9 1 1	Biocare Medical DCS Diagnostics Immunologic Leica/Novocastra Monosan Zytomed Immunologic	84	27	13	5	86%	65%
rmAb clone **EP229**	3	Cell Marque	2	1	0	0	-	-
Ready-To-Use Antibodies								OR[2]
mAb clone **8G7G3/1 790-4398 (VRPS)[3]**	1	Ventana/Roche	0	0	0	1	-	-
mAb clone **8G7G3/1 790-4398 (LMPS)[4]**	11	Ventana/Roche	0	0	7	4	0%	0%
mAb clone **8G7G3/1 IR056 (VRPS)[3]**	9	Dako/Agilent	0	4	5	0	44%	0%
mAb clone **8G7G3/1 IR056 (LMPS)[4]**	14	Dako/Agilent	0	4	5	5	29%	0%
rmAb **EP229 343R-17/18**	1	Cell Marque	0	0	1	0	-	-
rmAb **EP229 8224-C010**	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone **SP141 790-4756 (VRPS)[3]**	30	Ventana/Roche	25	5	0	0	100%	83%
rmAb clone **SP141 790-4756 (LMPS)[4]**	75	Ventana/Roche	54	20	1	0	99%	72%
mAb clone **SPT24 PA0364 (VRPS)[3]**	6	Leica/Novocastra	5	1	0	0	100%	83%
mAb clone **SPT24 PA0364 (LMPS)[4]**	16	Leica/Novocastra	10	4	1	1	88%	63%
rmAb clone **SP141 AN887**	1	Biogenex	0	1	0	0	-	-
mAb clone **SPT24 MAD-000486QD**	1	Master Diagnostica SL	1	0	0	0	-	-
mAb clone **SPT24 API 3126**	3	BioCare	0	3	0	0	-	-
Total	322		182	74	44	22	-	
Proportion			56%	23%	14%	7%	80%	

1) Proportion of sufficient stains (optimal or good). For Laboratory Developed (LD) assays (≥5 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

Detailed analysis of TTF1, Run 58
The following protocol parameters were central to obtain optimal staining:

Concentrated Antibodies
mAb clone **SPT24**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using either Cell Conditioning 1 (CC1) (Ventana) (38/53)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (9/14), TRS pH 9 (Dako) (19/32), TRS pH 6.1 (Dako) (1/1) TRS pH 6.1 (3-in-1) (Dako) (1/2), Bond Epitope Retrieval Solution 2 (BERS2) (Leica) (14/17) or unknown (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 106 of 121 (88%) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for TTF1 for the mAb clone SPT24 as concentrate on the main IHC systems***

Concentrated antibodies	**Dako Autostainer**		**Dako Omnis**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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 **SPT24**	9/14** (64%)	1/2	19/32 (59%)	1/1	38/52 (73%)	-	14/17 (82%)	-

* 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 (RTU) antibodies and corresponding systems
rmAb clone **SP141** product no. **790-4756**, Ventana, BenchMark GX, XT and Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-92 min.), 8-48 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 88 of 89 (99 %) laboratories produced a sufficient staining result. 12 laboratories applied amplification kits to above mentioned protocol settings. All obtained sufficient results.

mAb clone **SPT24** product.no. **PA0364**, Leica/Novocastra, Bond III and Max:
Protocols with optimal results were typically based on HIER using Bond Epitope Retrieval Solution 1 or BERS2 (efficient heating time 15-30 min.), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 14 of 14 (100 %) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 assessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included (in Table 1 Laboratory Modified Protocol Settings (LMPS) also includes off label use on deviant IHC stainers).

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb 8G7G3/1 **790-4398**	0/1	0/1	0/11 (0%)	0/11 (0%)
Dako AS Link 48+ mAb 8G7G3/1 **IR056**	4/9 (44%)	0/9 (0%)	3/5 (60%)	0/5 (0%)
VMS Ultra/XT rmAb SP141 **790-4756**	30/30 (100%)	25/30 (83%)	70/71 (99%)	53/71 (75%)
Leica BOND III/Max mAb SPT24 **PA0364**	6/6 (100%)	5/6 (83%)	8/8 (100%)	7/8 (88%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments
In this assessment and in concordance with previous assessments for TTF1, the prevalent feature of an insufficient result was a general too weak or false negative staining of cells/structures expected to be demonstrated. Too weak or false negative staining reaction was seen in 99% of the insufficient results (65 of 66)

Virtually all the participating laboratories were able to demonstrate TTF1 in structures with a high-level antigen expression (thyroid epithelial cells and type II pneumocytes of the lung). Cells with low levels of antigen expression, as columnar epithelial cells of terminal bronchioles of the lung and in particular neoplastic cells of the lung adenocarcinoma, tissue core no. 5, were more challenging and could only be demonstrated when using a correctly calibrated protocol.

48% (153 of 322) of the participants used Abs as concentrated format within laboratory developed (LD) assays for TTF1, with an overall pass rate of 77% (118 of 153), 56% optimal. 13% (20 of 153) of the LD assays was based on the less successful mAb 8G7G3/1, with a pass rate of 15% (3 of 20), none optimal (see Table 1). 84% (129 of 153) of LD assays was based on mAb SPT24, with a significant higher pass rate of 86% (111 of 129), 65% optimal.

mAb clone SPT24 was the most widely used antibody for demonstration of TTF1. Used as a concentrate within a LD assay, it provided an optimal staining result on all the three main IHC platforms from Dako, Leica and Ventana (see Table 2). 66% (85 of 129) of the laboratories used SPT24 with a sensitive 3-step polymer/multimer based detection system with a pass rate of 98% (83 of 85), 85% optimal, compared to a significant lower pass rate of 64% (28 of 44), 27% optimal, when using a 2-layer technique.

52% (169 of 322) of the participants used Abs in RTU formats, with an overall pass rate of 82% (138 of 169), 56% optimal. 21% (35 of 169) of the RTU systems was based on mAb 8G7G3/1 with a pass rate of 23% (8 of 35), none optimal. When using an RTU based on another clone, a significant higher pass rate of 97% (130 of 134) was obtained, 72% optimal.

The Ventana RTU format, 790-4756 based on rmAb SP141 obtained a high pass rate both when applying vendor recommended protocol settings (100%) and when modifying the protocol settings (99%), see Table 3. The most common modifications seen were shorter HIER time and prolonged incubation time of primary Ab.

mAb SPT24 PA0364, RTU format from Leica, obtained a pass rate of 100% both when applying the protocol settings as recommended by Leica or when modifying the protocol settings on the Bond platform, see Table 3.

Most important for optimal and consistent result for TTF1 was the choice of the primary Ab: The mAb clone SPT24 and the rmAb SP141 had significantly higher pass rate compared to the mAb clone 8G7G3/1. In this run, pass rates of 87% and 99% were seen for mAb clone SPT24 and rmAb SP141, respectively. In comparison, a pass rate of 20% was seen when the mAb clone 8G7G3/1 was used. This pattern was also observed in the previous NordiQC assessments for TTF1.
In the last five TTF1 assessments, none of 314 submitted protocols based on the mAb clone 8G7G3/1 produced an optimal result despite protocol settings were similar to those used for the mAb clone SPT24 or rmAb clone SP141. The mAb clone 8G7G3/1 has thus shown to have a significantly lower affinity/analytical sensitivity for TTF1 compared to the mAb clone SPT24 and the rmAb clone SP141. In this and all previous NordiQC assessments for TTF1, the reduced affinity/analytical sensitivity have induced false negative results in both lung adenocarcinomas and lung carcinoids with the risk of misclassification of carcinoma of unknown primary origin. Cumulated data and pass rates for the last five runs (Run 23, 33, 39, 46 and 58) are shown in Table 4.

Table 4. **The overall pass rate in the last five runs for the mAb clones SPT24, 8G7G3/1 and the rmAb clone SP141**

	SPT24 All protocol settings		**SP141* All protocol settings**		**8G7G3/1 All protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal	Sufficient	Optimal
Participants	89% (564/635)	64% (408/635)	97% (164/169)	71% (120/169)	9% (28/314)	0% (0/314)

* Because rmAb clone SP141 is only recently introduced, data represents Run 39, 46 and 58 only

Controls
Normal lung is recommendable as positive tissue control for TTF1. The columnar epithelial cells of the terminal bronchioles serve as a "low expressor" (LE) positive tissue control and must show an at least weak to moderate and distinct nuclear staining reaction. The type II pneumocytes and the basal cells of the terminal bronchioles all serve as "high expressors" (HE), in which a strong nuclear staining reaction

must be seen. The nuclear staining in the HE should be as strong as possible without significant cytoplasmic reaction. Thyroid is less reliable as positive tissue control for TTF1, as the thyroid epithelial cells express very high levels of TTF1, making it difficult to evaluate the level of analytical sensitivity of the protocol used. Tonsil can serve as negative tissue control, in which no staining should be seen. However scattered lymphocytes (<1%), mainly in germinal centres, may show a weak nuclear staining reaction, which is accepted. The recommendations of the tissue controls for IHC mentioned above are concordant to the guidelines published by the International Ad Hoc Expert Committee[1].

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

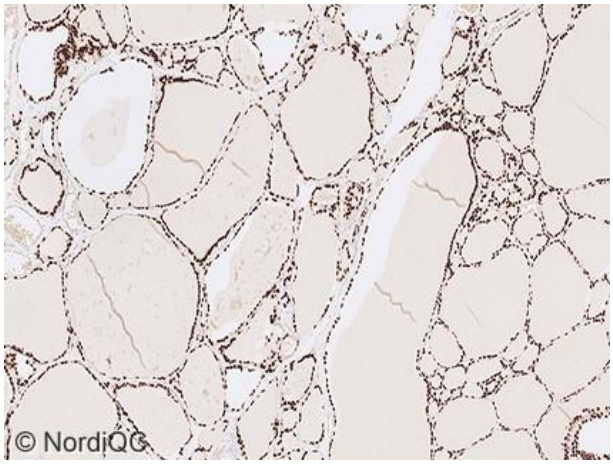


Fig. 1a
Optimal staining of TTF1 in thyroid using the mAb clone SPT24 diluted 1:100 with an incubation time of 32 min., HIER in CC1 for 48 min. and performed on the BenchMark Ultra, using the OptiView detection system.
A strong nuclear staining reaction is seen in virtually all follicular epithelial cells.
Also compare with Figs. 2a - 4a – same protocol.

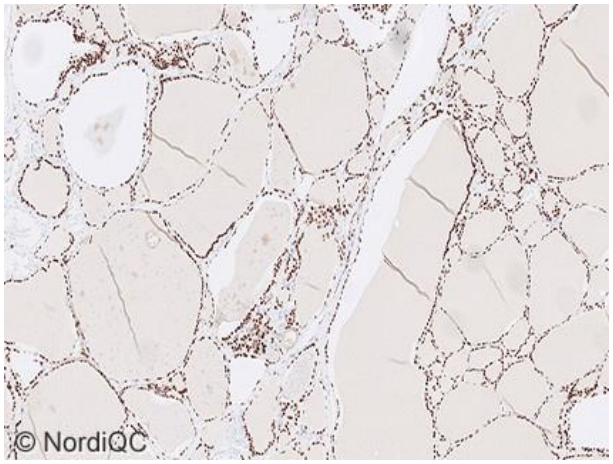


Fig. 1b
TTF1 staining of the thyroid using the mAb clone SPT24 diluted 1:1200 with an incubation time of 32 min. - HIER in CC1 for 24 min., OptiView as detection kit and performed on the BenchMark Ultra, Ventana.
Only a moderate nuclear staining reaction is seen in the majority of follicular epithelial cells – same field as in Fig. 1a. Also compare with Figs. 2b - 4b – same protocol. Overall an insufficient result was provided.

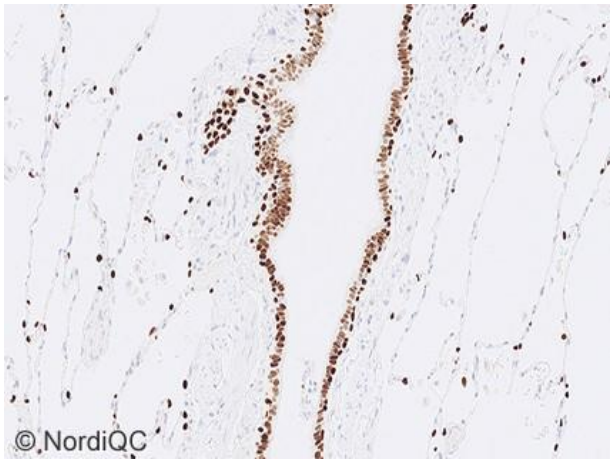


Fig. 2a
Optimal staining of TTF1 in the normal lung using same protocol as in Fig. 1a.
The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a weak to moderate nuclear staining reaction.

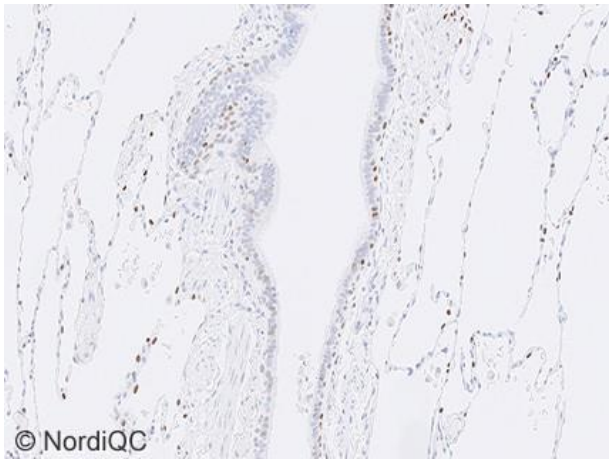


Fig. 2b
Insufficient staining of TTF1 in the normal lung using same protocol as in Fig. 1b.
Only type II pneumocytes and dispersed basal epithelial cells lining the terminal bronchioles are demonstrated, while no staining reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

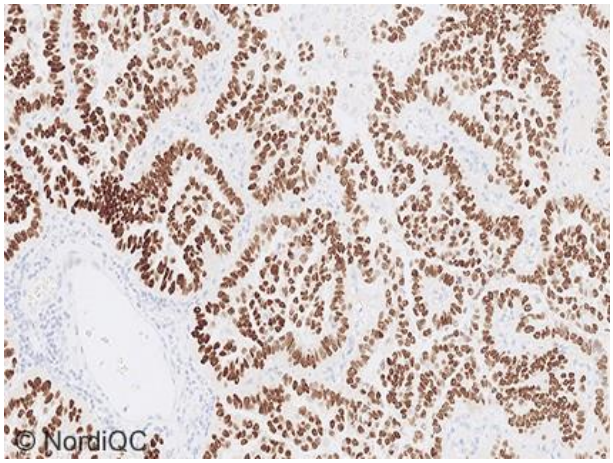


Fig. 3a
Optimal staining of TTF1 in the lung adenocarcinoma, tissue core no. 4 (high expression of TTF1), using same protocol as in Figs. 1a - 2a.
Virtually all the neoplastic cells show a moderate to strong and distinct nuclear staining reaction.

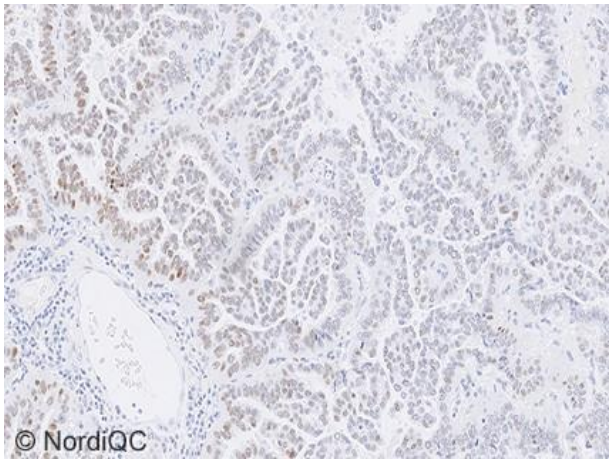


Fig. 3b
Insufficient staining of TTF1 in the lung adenocarcinoma, tissue core no. 4, using same insufficient protocol as in Figs. 1b - 2b.
Despite being a tumour with high expression level of TTF1, both the proportion of positive neoplastic cells and staining intensity is significant reduced compared to the level expected and obtained by optimal protocol settings as seen in Fig. 3a – same field.

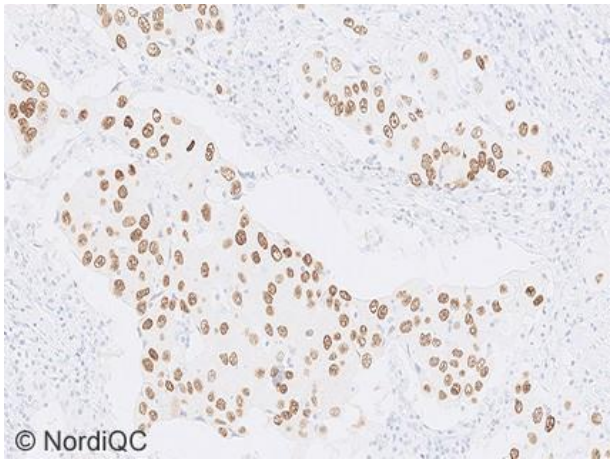


Fig. 4a
Optimal staining of TTF1 in the lung adenocarcinoma, tissue core no. 5, with low level TTF1 expression, using same protocol as in Figs. 1a - 3a.
Virtually all the neoplastic cells show a weak to moderate nuclear staining reaction.

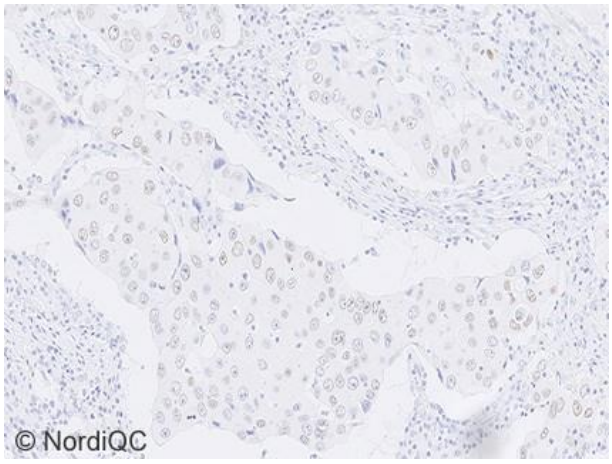


Fig. 4b
Insufficient staining of TTF1 in the lung adenocarcinoma, tissue core no. 5, with low level TTF1 expression, using same protocol as in Figs. 1b - 3b.
Virtually all neoplastic cells are negative – same field as in Fig. 4a.

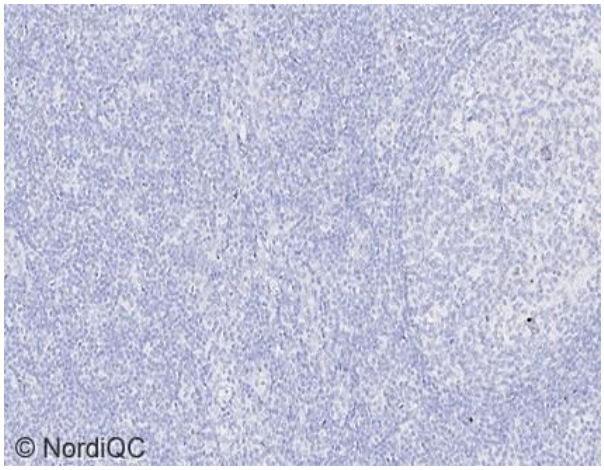


Fig. 5a
Optimal staining of TTF1 in tonsil using the RTU rmAb clone SP141, 790-4756 (Ventana) with incubation time of 24 min, HIER in CC1 for 64 min. and performed at the BechMark Ultra instrument (Ventana), using the OptiView detection system. Only scattered lymphocytes (<1%) display a weak staining reaction. All other cells are negative. Compare with Fig 5b.
Also compare with Figs. 6a - 7a – same protocol.

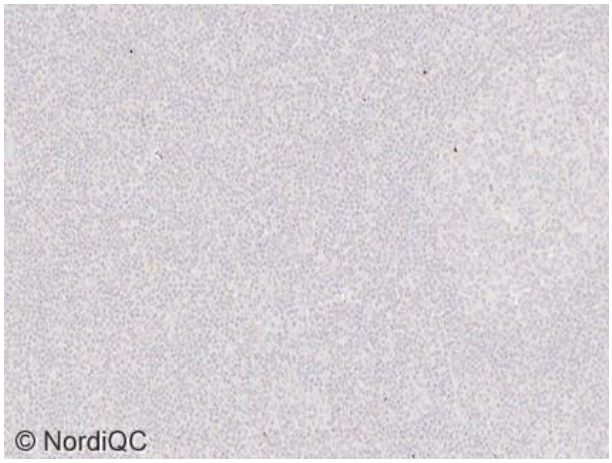


Fig. 5b
Insufficient staining of TTF1 in tonsil using the RTU mAb 8G7G3/1, IR056 (Dako) on the BenchMark Ultra (Ventana) with incubation time of 36 min., HIER in CC1 for 24 min., and OptiView as detection system giving a poor signal-to-noise ratio – same field as in Fig. 5a.
Off label use cannot be recommended, as it requires meticulous calibration and validation of the assay.

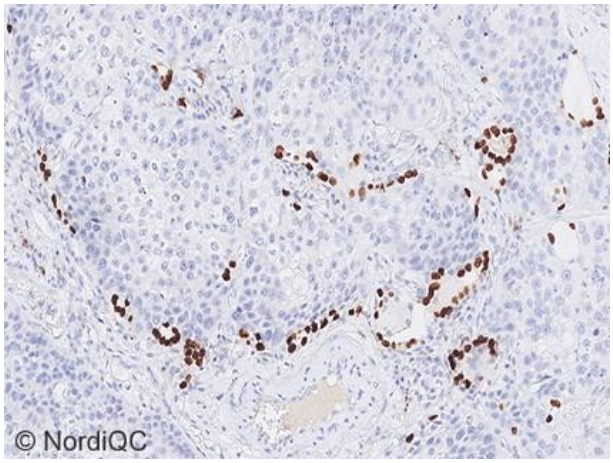


Fig. 6a
Optimal staining of TTF1 in the lung squamous cell carcinoma using same protocol as in Fig. 5a.
No staining reaction is seen in the neoplastic cells.
Compare with Fig. 6b.

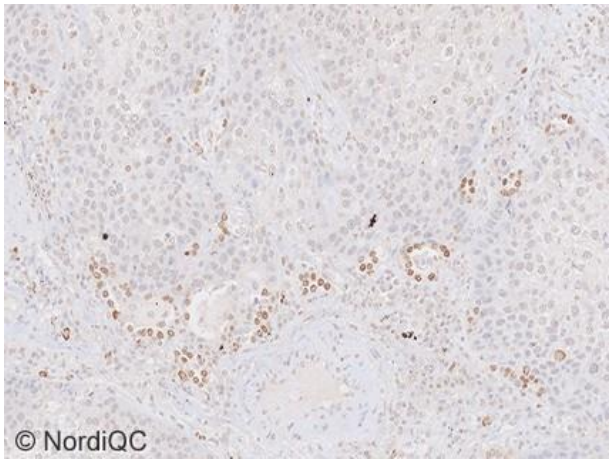


Fig. 6b
Insufficient staining of TTF1 in the lung squamous cell carcinoma using same protocol as in Fig. 5b giving a poor signal-to-noise ratio, interfering the interpretation - same field as in Fig. 6a.

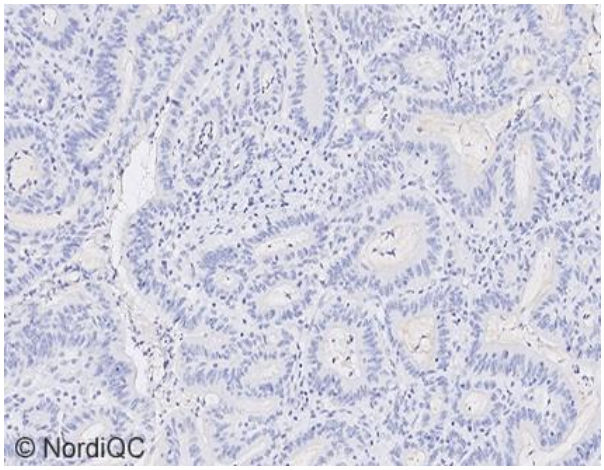


Fig. 7a
Optimal staining of TTF1 in the colon adonocarcinoma using same protocol as in Figs. 5a - 6a.
No staining reaction is seen in the neoplastic cells.
Compare with Fig. 7b.

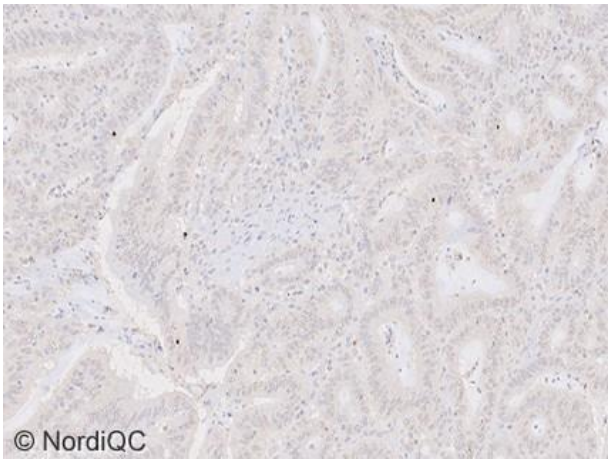


Fig. 7b
Insufficient staining of TTF1 in the colon adenocarcinoma using same protocol as in Figs. 5b - 6b giving a poor signal-to-noise ratio, interfering the interpretation - same field as in Fig. 7a.

HLK/RR/LE/SN 30.03.2020