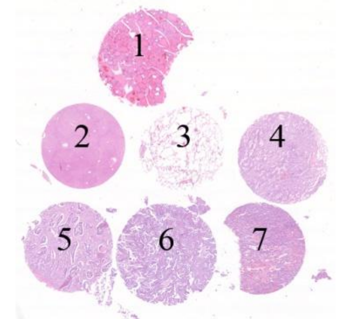
Thyroid transcription factor-1 (TTF1)
Assessment run 39 2013



The slide to be stained for TTF1 comprised:

1. Thyroid gland, 2. Liver, 3. Normal lung, 4. Lung adenocarcinoma 5. Colon adenocarcinoma, 6 & 7. Lung adenocarcinomas.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a TTF1 staining as optimal were:

- A strong, distinct nuclear staining reaction of type II pneumocytes, Clara cells and basal cells of the terminal bronchioles in the lung.
- A moderate to strong, distinct nuclear staining reaction of the columnar epithelial cells of the terminal bronchioles in the lung.
- A strong, distinct nuclear staining reaction of all follicular epithelial cells in the thyroid gland.
- A strong nuclear staining reaction of the majority of neoplastic cells in two of the lung adenocarcinomas (no. 6 & 7) and at least weak to moderate, distinct nuclear staining reaction of the majority of neoplastic cells of the lung adenocarcinoma no. 4.
- A negative staining reaction of the colon adenocarcinoma.

Cytoplasmic staining in hepatocytes was accepted when using the mAb clone 8G7G3/1.

227 laboratories participated in this assessment. 71 % achieved a sufficient mark. Antibodies (Abs) used and marks are summarized in table 1.

Table 1: **Antibodies and assessment marks for TTF1 run 39**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **8G7G3/1**	12 3 2	Dako Thermo/NeoMarkers Cell Marque	0	2	11	4	12 %	-
mAb clone **SPT24**	125 11 1 2 1 1	Leica/Novocastra Monosan Ortomedic Immunologic Menarini Genemed	95	37	9	0	94 %	94 %
rmAb clone **EP1584Y**	1	Abcam	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **8G7G3/1 790-4398**	23	Ventana	0	0	18	5	0 %	-
mAb clone **8G7G3/1 IR056**	20	Dako	0	6	13	1	30 %	-
mAb clone **8G7G3/1 343M-96/97**	1	Cell Marque	0	0	1	0	-	-
mAb clone **8G7G3/1 PM087**	1	Biocare	0	0	1	0	-	-
mAb clone **8G7G3/1 GM357502**	1	GeneTech	0	0	0	1	-	-
rmAb clone **SP141 790-4756**	12	Ventana	11	0	0	1	92 %	92 %

mAb clone **SPT24** **PA0364**	8	Leica/Novocastra	8	0	0	0	100 %	100 %
mAb clone **SPT24** **MAD-000486QD**	1	Master Diagnostica SL	1	0	0	0	-	-
mAb clone **8G7G3/1** **MS-699-R7**	1	Thermo/NeoMarkers	0	0	1	0	-	-
Total	227		115	46	54	12	-	
Proportion			51 %	20 %	24 %	5 %	71 %	

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

Detailed analysis of TTF1, Run 39

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 (Ventana) (CC1) (36/62)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (17/21), TRS pH 9 (Dako) (17/21), TRS pH 6.1 (Dako) (1/1), Bond Epitope Retrieval Solution 2 (Leica) (11/14), Bond Epitope Retrieval Solution 1 (Leica) (3/4), Diva Decloaker pH 6.2 (Biocare) (1/3), Tris-EDTA/EGTA pH 9 (9/14), or Citrate pH 6,7 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:30-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 132 of 141 (94 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

Ready-To-Use (RTU) Antibodies
rmAb clone **SP141** (product no. 790-4756, Ventana) Protocols with optimal results were typically based on 36-64 min. HIER using Cell Conditioning 1 (Ventana), 12-56 min. incubation of the primary Ab and UltraView (Ventana 760-500) or OptiView (Ventana 760-700) as detection system. Using these protocol settings 11 of 12 (92 %) laboratories produced an optimal staining.

mAb clone **SPT24** (product.no. PA0364, Leica/Novocastra): Protocols with optimal results were typically based on 15-30 min. HIER using Bond Epitope Retrieval Solution 1 (Leica) or Bond Epitope Retrieval Solution 2 (Leica), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (Leica DS9800) as detection system. Using these protocol settings 8 of 8 (100 %) laboratories produced an optimal staining.

mAb clone **SPT24** (product.no. MAD-000486QD, Master Diagnostica SL): The protocol with an optimal result was based on 20 min. HIER using Tris-EDTA/EGTA pH 8, 10 min incubation of the primary Ab and Quanto (Master Diagnostica product no. MAD-021881QK) as detection system.

Table 2 summarizes the overall proportion of optimal staining results, using the mAb SPT24 concentrated Ab on the three most commonly used IHC stainer platforms.

Table 2: **Optimal results for TTF1 for the mAb clone SPT24 as concentrate on the 3 main IHC systems***

Concentrated antibodies	Dako Autostainer Link / Classic		Ventana BenchMark XT / Ultra		Leica Bond III / Max	
HIER buffer	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	80 % 36/45**	100 % 1/1	58 % 36/62	- -	71 % 10/14	75 % 3/4

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

The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab (the mAb clone 8G7G3/1)
- Too low concentration of the primary Ab

In concordance with the previous NordiQC assessments for TTF1, the prevalent feature of insufficient results was a false negative staining of cells/structures expected to be demonstrated. Virtually all laboratories were able to demonstrate TTF1 in structures with a high 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 no. 4 were more challenging and could only be obtained when using a correctly calibrated protocol. Most important for optimal and consistent staining for TTF1 was the choice of the primary Ab: The mAb clone SPT24 and the recently introduced rmAb SP141 had a significant higher pass rate compared to the mAb clone 8G7G3/1. In this run, pass rates of 94 % and 92 % were seen for mAb clone SPT24 and rmAb SP141, respectively. In comparison, a pass rate of 13 % was seen when the mAb clone 8G7G3/1 was used. This pattern was also observed in the previous NordiQC assessment for TTF1. Cumulated data and pass rates for the last 3 runs (Run 23, 33 and 39) are shown in table 3.

Table 3: **The overall pass rate in the last 3 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	90 % (299/332)	67 % (222/332)	92 % 11/12	92 % 11/12	8 % (15/187)	0 % (0/187)

* Because rmAb clone SP141 is only recently introduced, data represents the current run only (Run 39)

In the last 3 TTF1 assessments, none of 187 submitted protocols based on the mAb clone 8G7G3/1 produced an optimal staining despite protocol settings similar to those used for the mAb clone SPT24 as e.g., HIER and detection systems. The mAb clone 8G7G3/1 has thus shown to have a significantly lower affinity for TTF1 compared to the mAb clone SPT24 and the rmAb clone SP141. The few insufficient results with clone SPT24 were characterized by a too weak general staining typically caused by a too low titer of the primary Ab, insufficient HIER and/or use of a detection system with low sensitivity.

This was the 5th assessment of TTF1 in NordiQC. Data from the last four assessments are shown in table 4.
A significant increase of the pass rate has been achieved during these runs, despite many new participants enrolled.

Table 4. **Proportion of sufficient results for TTF1 in the last four NordiQC runs performed**

	Run 19 2007	Run 23 2008	Run 33 2011	Run 39 2013
Participants, n=	99	125	183	227
Sufficient results	24 %	45 %	60 %	71 %

The increase in pass rate is closely related to the increase in the proportion of laboratories using either the mAb clone SPT24 or the rmAb SP141. In this run, mAb clone SPT24 or the rmAb SP141 was used by 162 (71 %) compared to 120 laboratories (66 %) and 62 laboratories (50 %) in the runs 33 and 23 respectively.

Control
Normal lung was found to be the most robust positive tissue control for TTF1. The nuclear staining should be as strong as possible without significant cytoplasmic reaction. The columnar epithelial cells of the terminal bronchioles appear to express a reduced level of TFF1 antigen compared to the type II pneumocytes and the basal epithelial cells of the terminal bronchioles. This makes the columnar epithelial cells suited as critical stain quality indicator for TTF1. A strong nuclear staining reaction in type II pneumocytes and basal epithelial cells of the terminal bronchioles in combination with a moderate to strong nuclear staining reaction in the columnar epithelial cells of the terminal bronchioles is expected in normal lung (Fig. 2a and Fig. 2b). However, data from previous TTF1 assessments indicate that lung carcinoids – typically expressing low amounts of TTF1 – should also be included as positive control when the initial validation of a TTF1 protocol is being established.
Thyroid is a less reliable as control, since thyroid epithelial cells express high levels of TTF1, making it difficult to evaluate the sensitivity of the protocol used (Fig. 1a and Fig. 1b).

Conclusion
In this run, and in concordance with previous NordiQC assessments for TTF1, the mAb clone SPT24 was a very robust and sensitive marker for the demonstration of TTF1. The more recently introduced rmAb clone SP141 also seems to be a very sensitive marker, with 11 of 12 participating laboratories achieving optimal marks. Once again no laboratories using the mAb clone 8G7G3/1 achieved optimal mark as the antibody had a significant lower sensitivity compared to the other two clones, resulting in an alarming overall pass rate of only 13%. Lung tissue is recommendable as positive control: Columnar epithelial cells of the terminal bronchi must show moderate to strong, distinct nuclear staining and type II pneumocytes and basal epithelial cells a strong, distinct nuclear staining.
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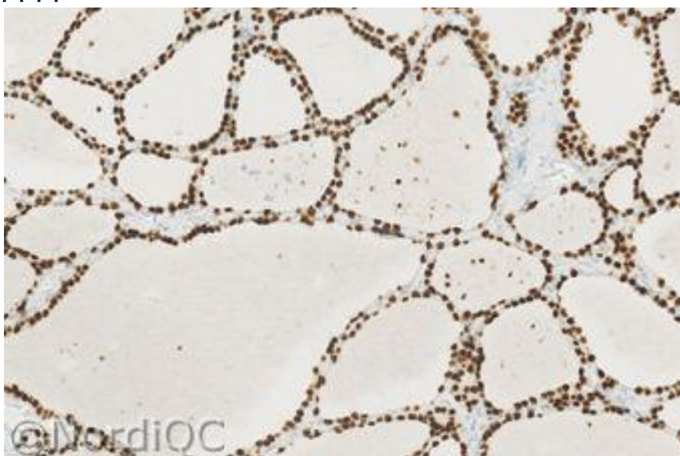

Fig. 1a

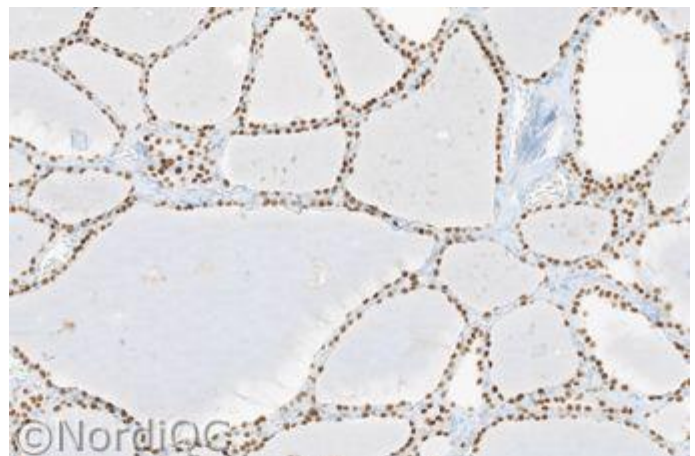

Fig. 1b

Optimal TTF1 staining of the thyroid gland using the rmAb clone SP141 (Ventana, RTU) optimally calibrated with HIER in an alkaline buffer (CC1) and performed on the BenchMark Ultra, Ventana. A strong nuclear staining reaction is seen in virtually all follicular epithelial cells. No background staining is seen.

Insufficient TTF1 staining of the thyroid gland using the mAb clone 8G7G3/1 (Ventana, RTU) with HIER in an alkaline buffer (CC1) and performed on the BenchMark Ultra, Ventana. 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, 3b and 4b – same protocol.

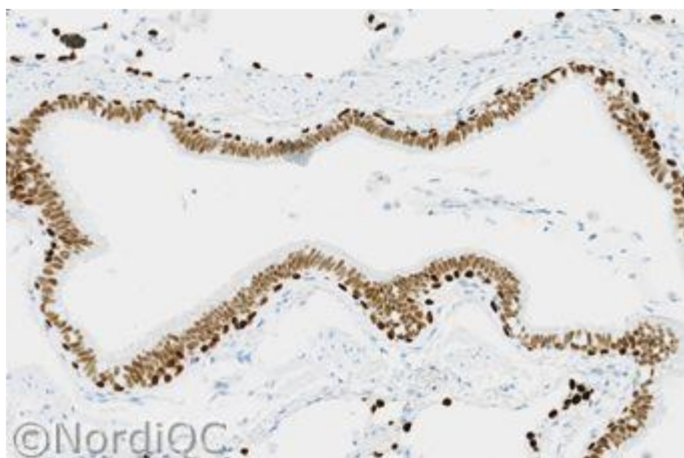

Fig. 2a
Optimal TTF1 staining of the lung using same protocol as in Fig. 1a. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show a strong distinct nuclear staining reaction, whereas the columnar epithelial cells show a moderate nuclear staining reaction. No background staining is seen.

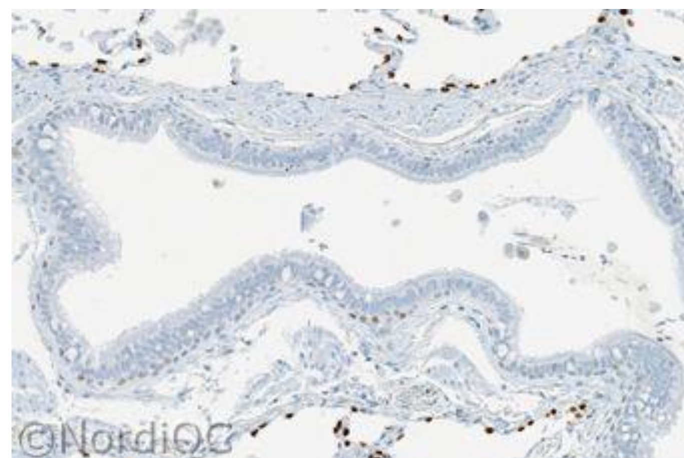

Fig. 2b.
Insufficient TTF1 staining of the lung using same protocol as in Fig. 1b. The type II pneumocytes and the basal epithelial cells lining the terminal bronchioles show only a weak to moderate positive nuclear staining reaction and no reaction is seen in the columnar epithelial cells - same field as in Fig. 2a.

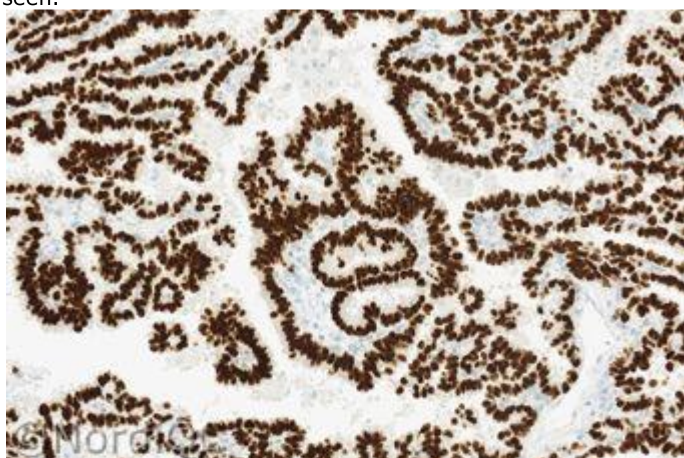

Fig. 3a
Optimal TTF1 staining of the lung adenocarcinoma no. 7 (high level expression of TTF1) using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct nuclear staining reaction. No background staining is seen.

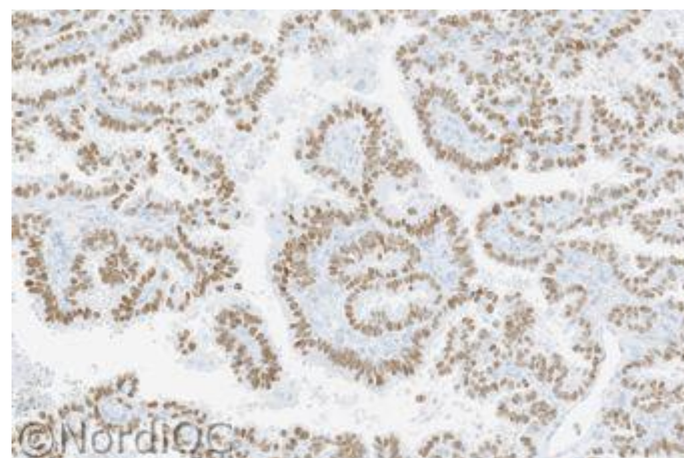

Fig. 3b
Insufficient TTF1 staining of the lung adenocarcinoma no. 7 using same protocol as in Figs. 1b & 2b. Despite a high level of TTF1 expression of the neoplastic cells only a moderate nuclear staining reaction is seen – same field as in Fig. 3a.

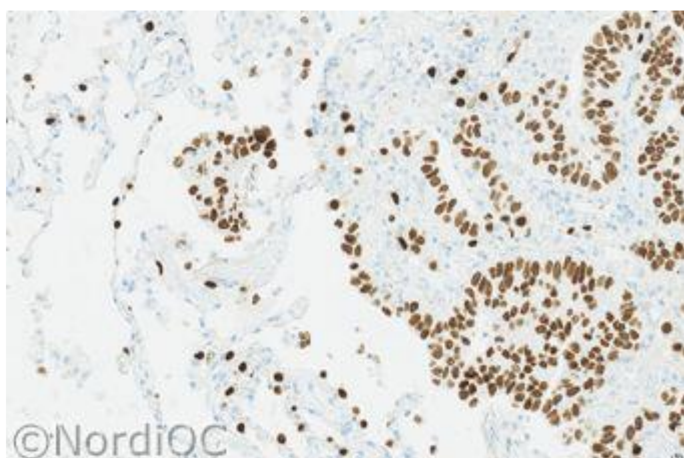

Fig. 4a
Optimal TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1a, 2a & 3a. Tumour (right side) with adjacent normal lung tissue. Virtually all the neoplastic cells

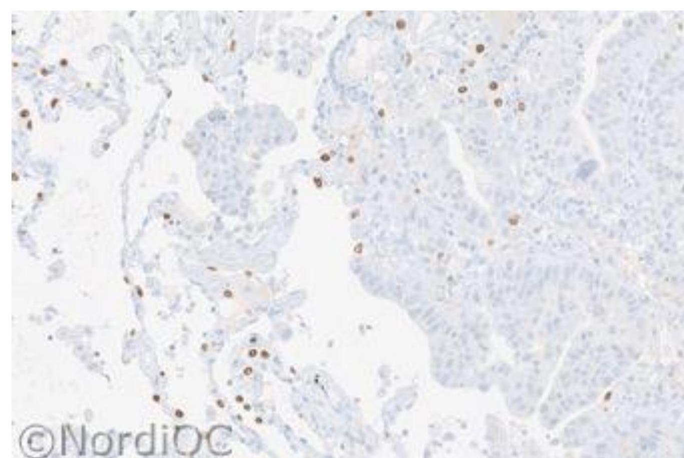

Fig. 4b
Insufficient TTF1 staining of the lung adenocarcinoma no. 4 using same protocol as in Figs. 1b, 2b & 3b. Despite a moderate positive staining reaction in the majority of type II

show a moderate to strong nuclear staining reaction. Strong reaction is also seen in all type II pneumocytes. No background staining is seen.

pneumocytes - both in the normal tissue and within the tumour tissue - virtually all neoplastic cells are negative – same field as in Fig. 4a.

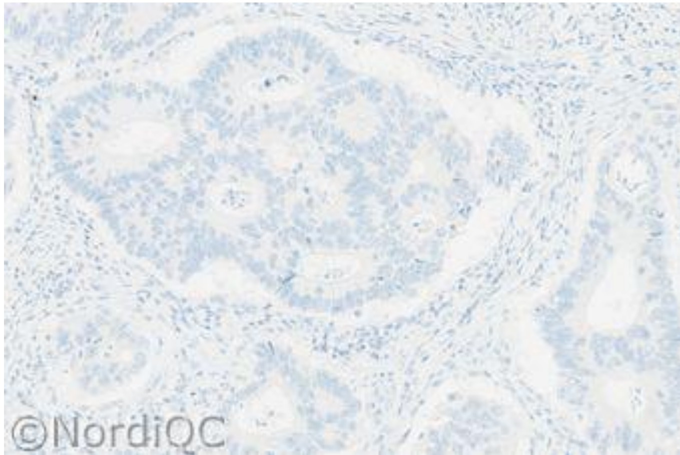

Fig. 5a
Optimal TTF1 staining of the colon adenocarcinoma using same protocol as in Figs. 1a - 4a. No staining is seen in the colon adenocarcinoma.

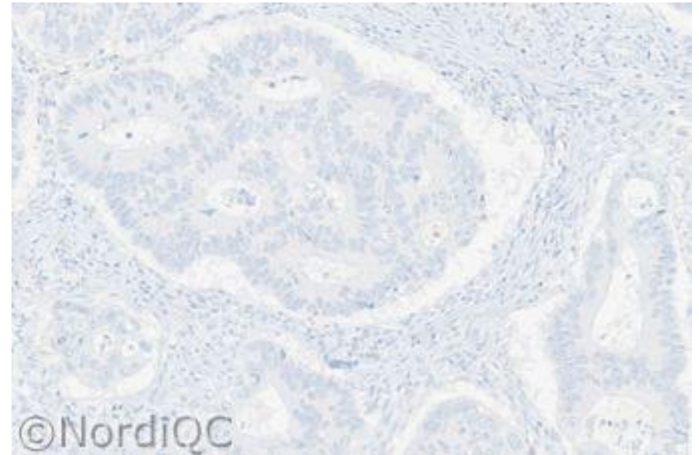

Fig. 5b
TTF1 staining of the colon adenocarcinoma using same protocol as in Figs. 1b - 4b. No staining is seen in the colon adenocarcinoma.

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