Assessment Run 23 2008

Thyroid transcription factor-1 (TTF-1)

The slide to be stained for Thyroid transcription factor 1 (TTF-1) comprised:
1. Liver, 2. Colon adenocarcinoma, 3. Thyroid, 4. Lung, 5. Lung carcinoid,
6-7. Lung adenocarcinoma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a TTF-1 staining as optimal included:

- A strong, distinct nuclear staining of the pneumocytes type II, the Clara cells and the columnar epithelial cells of the terminal bronchi in the lung.
- A strong, distinct nuclear staining of all the follicular cells and C-cells in the thyroid gland.
- A strong nuclear staining of the majority of the neoplastic cells in the two lung adenocarcinomas and a weak to moderate, distinct nuclear staining of the majority of the neoplastic cells of the lung carcinoid.
- A negative reaction of the colon adenocarcinoma.

No staining of other cells should be seen except for a granular cytoplasmic reaction in liver cells and some other cells with mAb clone 8G7G3/1.

125 laboratories submitted stains. At the assessment 36 achieved optimal marks (29 %), 20 good (16 %), 44 borderline (35 %) and 25 poor marks (20 %).

The following Abs were used:
mAb clone **SPT24** (Novocastra n=62)
mAb clone **8G7G3/1** (Dako, n=37; NeoMarkers, n=11; Ventana, n=9; Zymed, n=2; Immunologic, n=2; BioCare, n=1; Sanova, n=1)

Optimal staining for TTF-1 in this assessment was only obtained with the mAb **SPT24** (Table 1).

Table 1. **Proportion of sufficient and optimal stains with the two TTF-1 clones.**

SPT24		**8G7G3/1**	
Sufficient	Optimal	Sufficient	Optimal
84% (52/62)	58% (36/62)	6% (4/63)	0% (0/63)

SPT24: The optimal protocols were all based on heat induced epitope retrieval (HIER). Typically an alkaline buffer was used, such as Tris-EDTA/EGTA pH 9.0 (22/36)*, Bond Epitope Retrieval Solution 2 (Bond, Leica Microsystems) (4/5), Target Retrieval Solution pH 9 (Dako), (4/5), or Cell Conditioning1 (BenchMark, Ventana) (3/9), but also Citrate pH 6.0 (2/5) and Cell Conditioning2 (BenchMark, Ventana) (1/1) could be used to obtain an optimal result.
The mAb was diluted in the range of 1:25 – 1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 52 out of 62 (84 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The most frequent causes of an insufficient staining were:
- Less successful primary Ab
- Too low concentration of the primary Ab

In accordance to the previous TTF-1 assessments the prevalent feature of the insufficient results was a false negative staining of the lung carcinoid when using the clone 8G7G3/1. Only 4 out of 63 stains based on the clone 8G7G3/1 were marked as good and 3 of these used both efficient HIER and a 3-step polymer/multimer detection system. For yet unexplained reasons the clone 8G7G3/1 gave different staining patterns concerning the well-known cytoplasmic cross-reaction of this clone in e.g. liver cells. In some protocols the cytoplasmic reaction was very intense and at the same time only a faint specific nuclear staining was seen while in other protocols the cytoplasmic reaction was almost absent and a moderate specific nuclear reaction was seen.
The insufficient results with clone SPT24 were characterized by a generally too weak reaction of the cells

expected to be demonstrated.
This was the third assessment of TTF-1. The proportion of sufficient results has varied (Table 2).

Table 2. **Proportion of sufficient results with TTF-1 in three runs.**

	Run 9 2003	Run 19 2007	Run 23 2008
Participants, n=	63	99	125
Sufficient results	60%	24%	45%

While the decline in percentage of sufficient results from run 9 to run 19 was mainly caused by more challenging material, the improvement from run 19 to run 23 reflects mainly the increase in the number of laboratories using the mAb clone SPT24 from 25/100 laboratories in run 19 to 62/125 laboratories in the current run. Regarding clone 8G7G3/1, NordiQC has not been able to identify any protocol to obtain an optimal staining of the lung carcinoid included in the multi-tissue blocks used in runs 19 and 23.
A comparative study of the two clones carried out in two Danish reference laboratories is described on **TTF-1**.

Normal lung is suitable for control: The nuclear staining should be as strong as possible without significant cytoplasmic reaction. The columnar epithelial cells of the terminal bronchioli appears to express less antigen than the pneumocytes type II and might be a more suited as a critical stain quality indicator for TTF-1.

Conclusion
The mAb clone SPT24 is the most robust and sensitive antibody for the demonstration of TTF-1. The mAb clone 8G7G3/1 has a lower sensitivity, especially as regards lung carcinoid tumours.

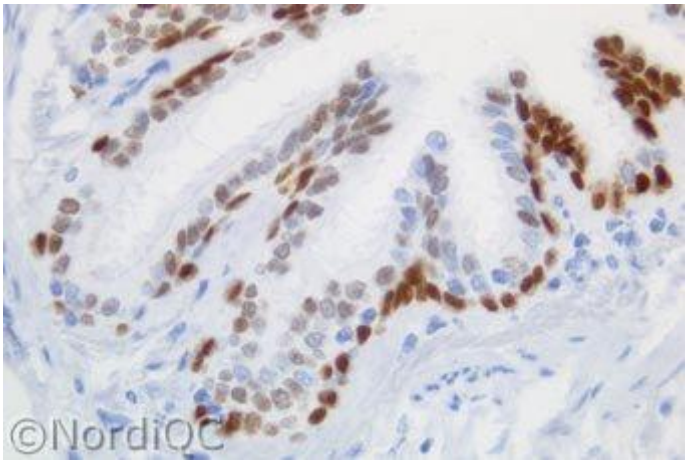

Fig. 1a
Optimal TTF-1 staining of the normal lung using the mAb clone SPT24. The basal epithelial cells lining the bronchial duct show a strong distinct nuclear and staining, while the luminal epithelial cells show a weak to moderate nuclear staining.

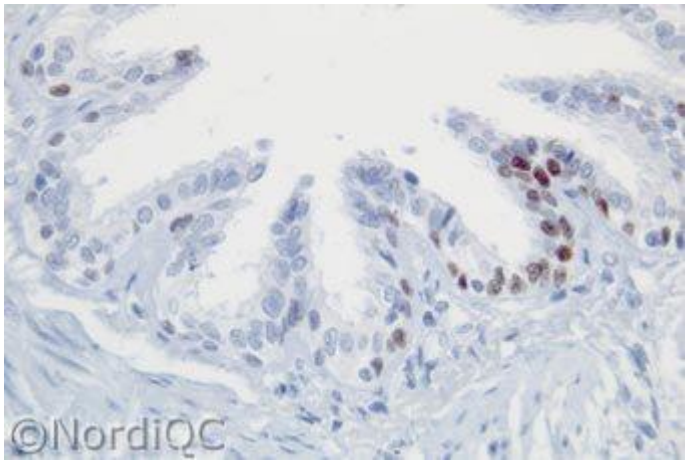

Fig. 1b
Insufficient TTF-1 staining of the normal lung using the mAb clone SPT24, but in a too low concentration – same field as in Fig. 1a. Only scattered epithelial cells show a weak to moderate nuclear staining. Also compare with Fig. 2b and 3b.

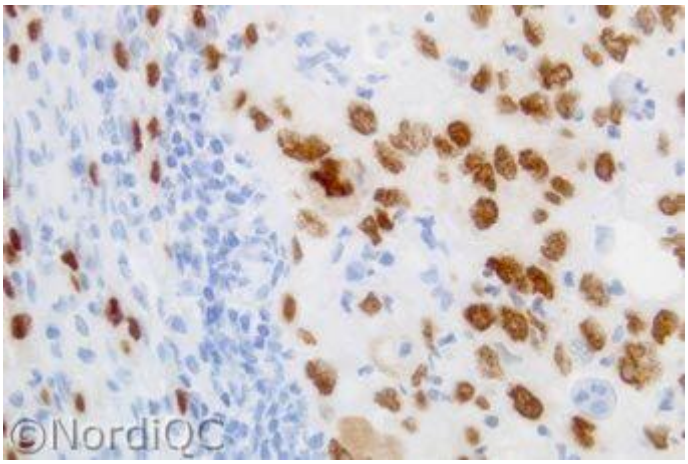

Fig. 2a
Optimal TTF-1 staining of the lung adenocarcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong distinct nuclear reaction with no background reaction.

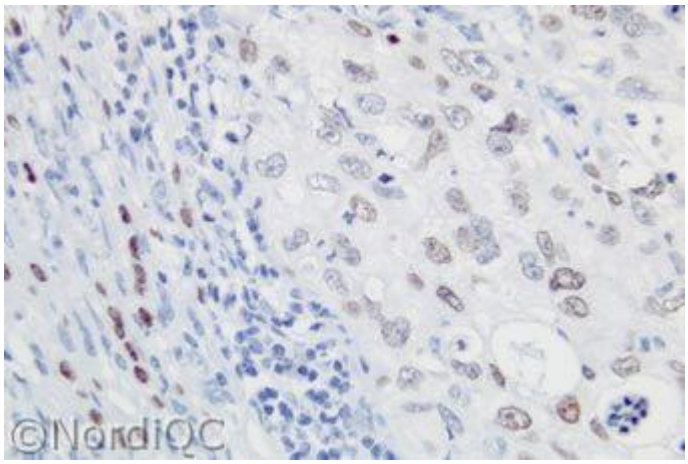

Fig. 2b
Insufficient TTF-1 staining of the lung adenocarcinoma using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 2a. Only the pneumocytes type II, left in the photo, show a distinct staining.

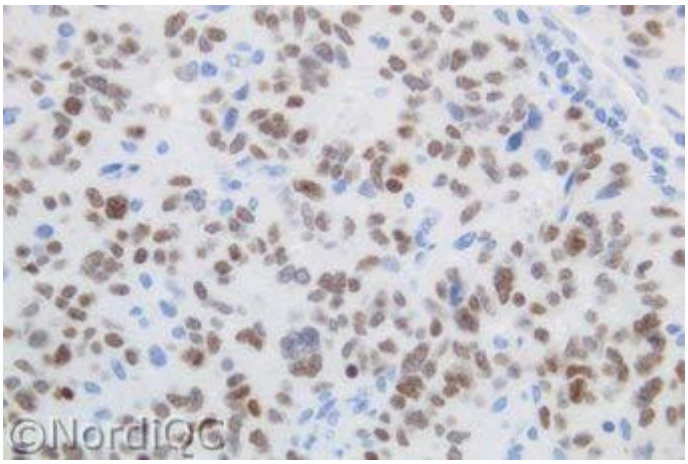

Fig. 3a
Optimal TTF-1 staining of the lung carcinoid. The majority of the neoplastic cells show a moderate, distinct nuclear staining (same protocol as in Figs. 1a and 2a).

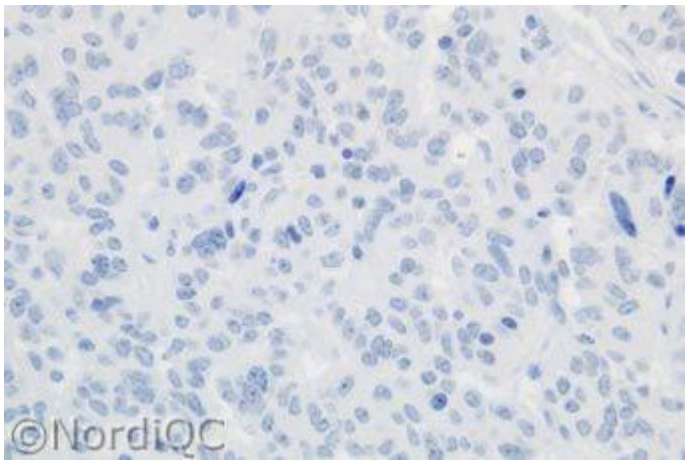

Fig. 3b
Insufficient staining for TTF-1 of the lung carcinoid – same field as in Fig. 2b. The neoplastic cells are all false negative (same protocol used in Figs. 1b and 2b).

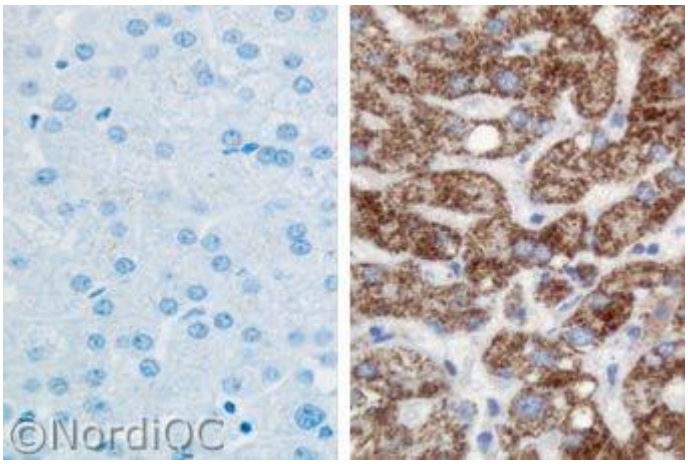

Fig. 4a. TTF-1 staining of the liver using the two different clones used in this assessment
Left: Staining of the liver using the mAb clone SPT24. The hepatocytes are negative.
Right: Staining of the liver using the mAb clone 8G7G3/1. The hepatocytes show a strong granular cytoplasmic staining.

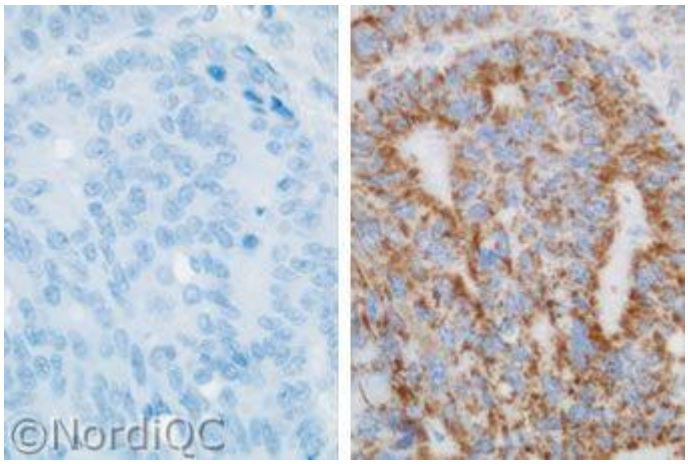

Fig. 4b. TTF-1 staining of the colon adenocarcinoma using the mAb clone SPT24 with two different detection systems with HIER.
Left: Using a polymer based detection system the neoplastic cells are all negative.
Right: Using a biotin based detection system, virtually all the neoplastic cells show a distinct granular cytoplasmic staining due to endogenous biotin.

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