## Assessment Run 33 2011

# Thyroid transcription factor (TTF-1)

### Material

The slide to be stained for TTF-1 comprised:


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

All tissues were fixed in 10% neutral buffered formalin.

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

- A strong, distinct nuclear staining reaction 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 reaction of all the follicular epithelial cells in the thyroid gland.
- A strong nuclear staining reaction of the majority of the neoplastic cells in the two lung adenocarcinomas and at least weak to moderate, distinct nuclear staining reaction of the majority of the neoplastic cells of the lung carcinoid.
- A negative staining reaction of the colon adenocarcinoma.
- A cytoplasmic staining in the hepatocytes was accepted when using the mAb clone 8G7G3/1

183 laboratories participated in this assessment. 60 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for TTF1, run 33**

| Concentrated Abs | N | Vendor | Optimal | Good | Borderl. | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **8G7G3/1** | 22<br>6<br>1 | Dako<br>Thermo/NeoMarkers<br>Zytomed | 0 | 1 | 27 | 1 | 3 % | - |
| mAb clone **SPT24** | 105<br>8<br>1<br>1<br>1 | Leica/Novocastra<br>Monosan<br>DCS<br>Immunologic<br>Master Diagnostica | 79 | 23 | 11 | 3 | 88 % | 89 % |
| **Ready-To-Use Abs** | | | | | | | | |
| mAb clone **8G7G3/1 790-4398** | 17 | Ventana | 0 | 0 | 17 | 0 | 0 % | - |
| mAb clone **8G7G3/1 IS/IR056** | 13 | Dako | 0 | 2 | 11 | 0 | 15 % | - |
| mAb clone **8G7G3/1 343M-96/97** | 3 | Cell Marque | 0 | 0 | 3 | 0 | - | - |
| mAb clone **8G7G3/1 PM087** | 1 | Biocare | 0 | 0 | 1 | 0 | - | - |
| mAb clone **SPT24 PA0364** | 3 | Leica/Novocastra | 3 | 0 | 0 | 0 | - | - |
| mAb clone **SPT24 MAB-0599** | 1 | Maxin | 0 | 1 | 0 | 0 | - | - |
| **Total** | 183 | | 82 | 27 | 70 | 4 | - | |
| **Proportion** | | | 45 % | 15 % | 38 % | 2 % | 60 % | |

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

Following central protocol parameters were used to obtain an optimal staining:

**Concentrated Abs**
mAb clone **SPT24**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (CC1; BenchMark, Ventana) (19/39)*, Target Retrieval Solution pH 9 (3-in-1) (TRS;Dako) (17/21), TRS pH 9 (Dako) (9/13), Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (13/16), Bond Epitope Retrieval Solution 1 (BERS1; Bond, Leica) (2/2), Diva Decloaker pH 6.2 (Biocare) (2/2), Tris-EDTA/EGTA pH 9 (14/17), EDTA/EGTA pH 8 (1/1) or Citrate pH6 (2/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:30-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 101 out of 113 (89 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

**Ready-To-Use Abs**
mAb clone **SPT24** (product.no. PA0364, Leica/Novocastra): The protocols giving an optimal result were based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 15 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings 3 out of 3 laboratories produced an optimal staining.

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

In concordance to the previous assessments for TTF-1 in NordiQC the prevalent feature of the insufficient results was a false negative staining of the cells/structures expected to be demonstrated. Virtually all laboratories were able to demonstrate TTF-1 in structures with a high antigen expression as the thyroid epithelial cells and the pneumocytes of the lung, whereas the demonstration of TTF-1 in cells with a reduced antigen expression as the epithelial cells of the terminal bronchi of the lung and in particular the neoplastic cells of the lung carcinoid was more challenging and could only by obtained by a correctly calibrated protocol. Most important to obtain an optimal and consistent staining for TTF-1 was the choice of the primary Ab as the mAb clone SPT24 was found to have a significant higher pass rate compared to the mAb clone 8G7G3/1. In this run a pass rate of 89 % was seen when the mAb clone SPT24 was used compared to a pass rate of 5 %, when the mAb clone 8G7G3/1 was used. This was the same pattern observed in the last NordiQC assessment for TTF-1, as shown in table 2, where the cumulated data and pass rates for the two Abs are compared.

Table 2. **the overall pass rate in the last 2 runs for the mAb clones SPT24 and 8G7G3/1**

| | **SPT24 All protocol settings** | | **8G7G3/1 All protocol settings** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Participants, n= | 87 % (158/182) | 65 % (118/182) | 6% (7/123) | 0% (0/123) |

In none out of 123 protocols an optimal staining for TTF-1 could be obtained by the use of the mAb clone 8G7G3/1 despite similar protocol settings as e.g. HIER, detection systems etc. were applied as for the mAb clone SPT24. The mAb clone 8G7G3/1 thus has shown to have a significant lower affinity for TTF-1 compared to the mAb clone SPT24.
The insufficient results with clone SPT24 were typically characterized by a too weak general staining and caused by e.g. too low titre of the primary Ab, insufficient HIER and/or use of a detection system with a too low sensitivity.
This was the 4th assessment of TTF-1 in NordiQC (Table 3) and a significant increase of the pass rate has been achieved during the last 3 runs, despite many new participants have enrolled.

Table 3. **Proportion of sufficient results for TTF-1 in the four NordiQC runs performed**

| | Run 9 2003 | Run 19 2007 | Run 23 2008 | Run 33 2011 |
|---|---|---|---|---|
| Participants, n= | 63 | 99 | 125 | 183 |
| Sufficient results | 60 % | 24 % | 45 % | 60 % |

The increase seems almost proportional to the increase in the number of laboratories using the mAb clone SPT24, which in this run was used by 120 laboratories (66%) compared to 62 laboratories (50 %) and 25 laboratories (25%) in the runs 23 and 19 respectively.

Normal lung was found to be the most robust positive control for TTF-1: Virtually all the pneumocytes type II and the columnar epithelial cells of the terminal bronchioli must show an as strong as positive nuclear staining reaction and only a weak cytoplasmic staining. Thyroid was found to be less reliable as the epithelial cells seem to express to much higher antigen expression and can not be used to evaluate the sensitivity of the protocol

used. However it was observed that the mAb clone 8G7G3/1 could give the expected staining for TTF-1 in the normal lung but still gave a false negative staining in the lung carcinoid, which indicates that the laboratories also must use lung carcinoids as positive control when the validation of the protocol is being established.

**Conclusion**

In this and in concordance with the previous assessments for TTF-1 the mAb clone SPT24 was found to be the most robust and sensitive marker for the demonstration of TTF-1. Lung tissue is recommendable as positive control provided that both the pneumocytes and the columnar epithelial cells of the terminal bronchi show a strong and distinct nuclear staining. The mAb clone 8G7G3/1 was found to have a significant lower sensitivity especially for lung carcinoid tumours.

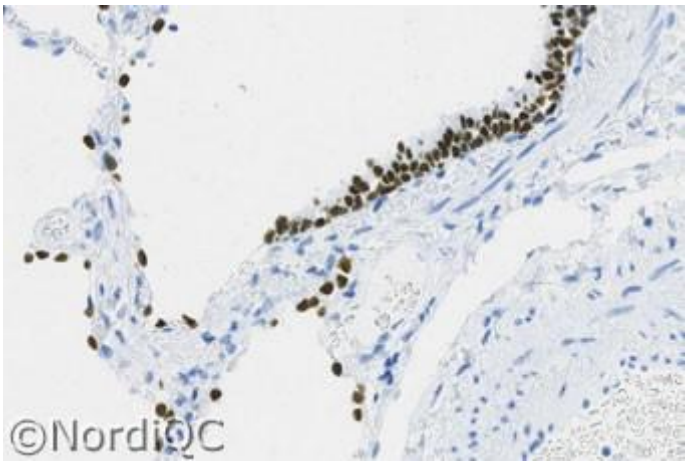


Fig. 1a
Optimal TTF-1 staining of the normal lung using the mAb clone SPT24 optimally calibrated. The epithelial cells lining the bronchial duct and the pneumocytes show a strong distinct nuclear staining reaction, while no background staining is seen.

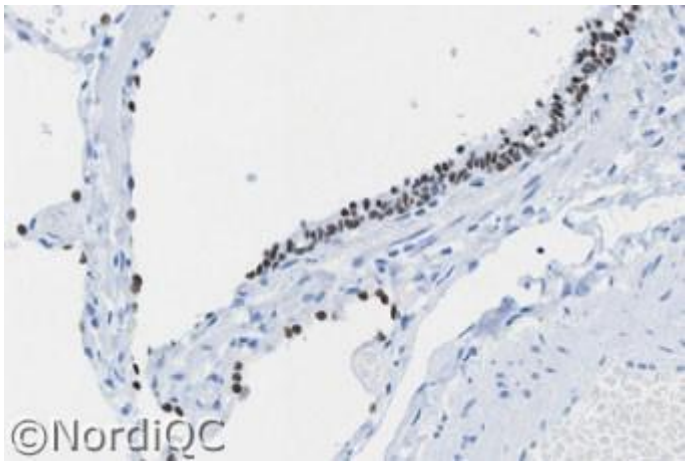


Fig. 1b
Insufficient TTF-1 staining of the normal lung using the mAb clone SPT24, but in a too low concentration – same field as in Fig. 1a. The epithelial cells of the bronchial duct and the pneumocytes cells show a reduced nuclear staining reaction. Also compare with Figs. 2b - 4b - same protocol.

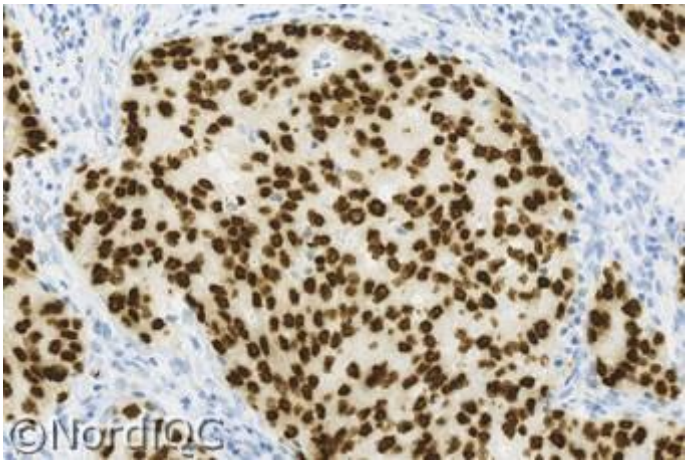


Fig. 2a
Optimal TTF-1 staining of the lung adenocarcinoma no.6 using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong distinct nuclear staining reaction with no background reaction.

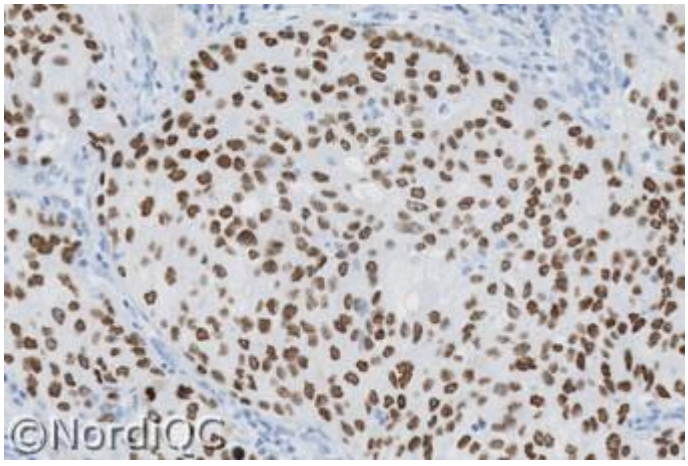


Fig. 2b
TTF-1 staining of the lung adenocarcinoma no. 6 using same insufficient protocol as in Fig. 1b – same field as in Fig. 2a. Virtually all the neoplastic cells are demonstrated, however also compare with Figs. 3b and 4b.

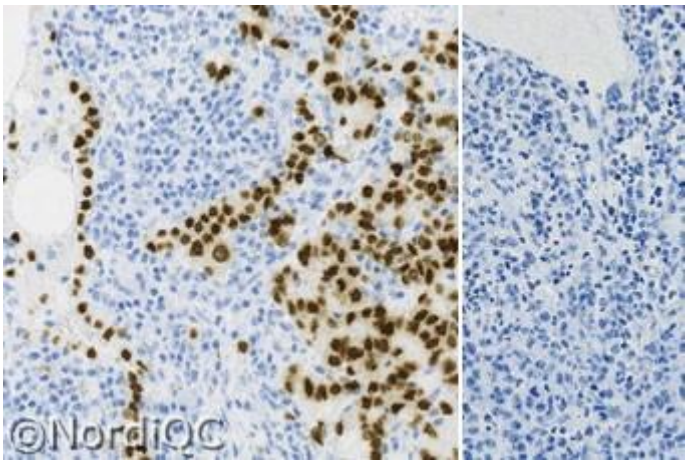


Fig. 3a
Left: Optimal TTF-1 staining of the lung adenocarcinoma no.7 using same protocol as in Figs. 1a. & 2a. Virtually all the neoplastic cells show a strong distinct nuclear staining reaction with no background reaction.
Right: Using same protocol, no staining is seen in the colon adenocarcinoma.

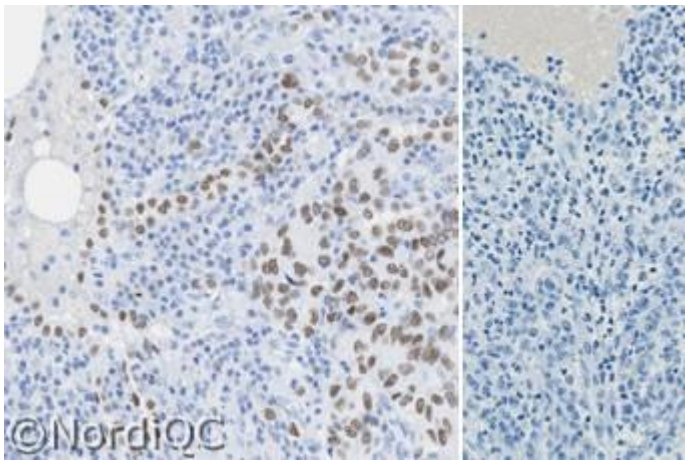


Fig. 3b
Left: Insufficient TTF-1 staining of the lung adenocarcinoma no.7 using same protocol as in Figs. 1b. & 2b. The neoplastic cells show a significant reduced staining reaction.
Right: Using same protocol, no staining is seen in the colon adenocarcinoma.

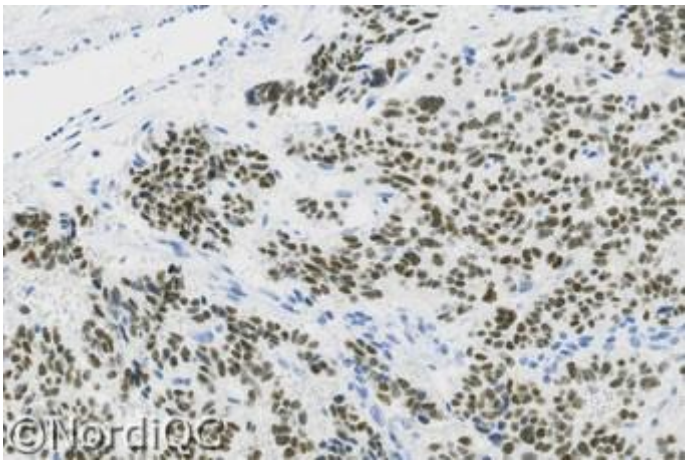


Fig. 4a
Optimal TTF-1 staining of the lung carcinoid. The majority of the neoplastic cells show a moderate to strong, distinct nuclear staining reaction (same protocol as in Figs. 1a - 3a).

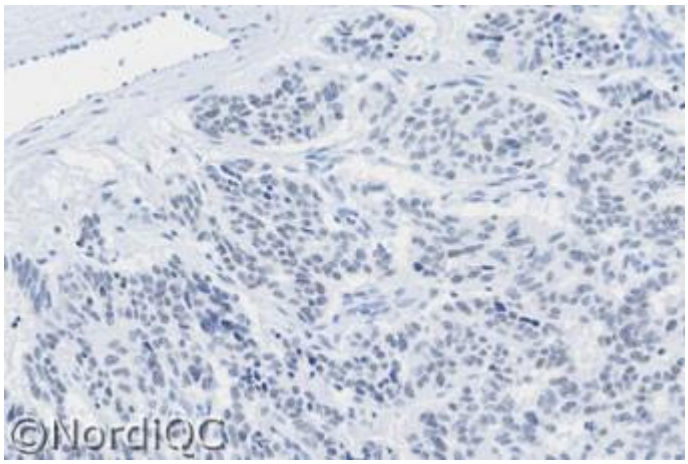


Fig. 4b
Insufficient staining for TTF-1 of the lung carcinoid – same field as in Fig. 4a. The neoplastic cells are all false negative - same protocol used as in Figs. 1b - 3b.

SN/MV/LE 27-11-2011