Assessment Run 18 2006

Neurofilament protein (NFP)

The slide to be stained for Neurofilament protein (NFP) comprised:
1. Appendix, 2. Brain, 3. Carcinoid, 4. Neuroblastoma, 5. Adrenal gland.
All specimens were fixed in 10 % NBF.

Criteria for assessing a NFP staining as optimal was highly depending on the antibody used and included:

- A strong, distinct staining of normal axons (appendix, brain, adrenal gland, neuroblastoma) with mAb clone 2F11, and a heterogeneous staining with mAb cocktail of clones NFP7/DA2/RMd020.11.
- A strong, distinct staining of perikarya/ganglion cells (appendix, brain) with clones NFP7/DA2/RMd020.11, a moderate, distinct staining with clone 2F11.
- A distinct, heterogeneous staining of the carcinoid, neuroblastoma and adrenal gland (cortical zona glomerulosa and medullary phaeochromocytes) with clones NFP7/DA2/RMd020.11, while virtually no staining with clone 2F11.

57 laboratories participated in the assessment. One used an inappropriate antibody. Among the remaining 56 laboratories, 15 achieved optimal marks (27 %) , 31 good (55 %), 9 borderline (16 %) and 1 (2 %) poor marks.

The following Abs were used:
mAb clone **2F11** (Dako, n=43; Euro-Diagnostica, n=3; Ventana, n=2, Maxin, n=1, Monosan, n=1, Neomarkers, n=1; Sanbio, n=1)
mAb cocktail of clones **NFP7/DA2/RMd020.11** (Zymed, n=4)

With clone **2F11** an optimal staining result in this assessment was obtained in 13 out of 52 cases (25%). A further 31 laboratories obtained good marks. Thus a total of 44 staining results were sufficient (84%).
All protocols giving optimal results were based on Heat Induced Epitope Retrieval (HIER) in Tris-EDTA/EGTA pH 9 (9 of 30 were optimal), Cell conditioning 1 (Ventana CC1; 2 of 5 were optimal), EDTA pH9 (1 of 1 was optimal, Citrate pH6 (1 of 12 was optimal - after staining over-night) and Target retrieval solution (Dako TRS) pH 9.9 (1 of 1 was optimal).
The Ab dilution ranged from 1:25 - 1:2,000 depending on the total sensitivity of the system employed. No protocols using proteolytic pretreatment or omitting pretreatment resulted in an optimal stain.
With mAb cocktail of clones **NFP7/DA2/RMd020.11** an optimal staining result in this assessment was obtained in 2/4. Both used HIER. The Ab dilution ranged from 1:50 - 1:300.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER (citrate buffer),
- Inappropriate epitope retrieval (proteolysis)
- Omission of epitope retrieval
- Too high Ab concentration and/or endogenous biotin reaction causing false positive staining of epithelial cells and lymphocytes.

Almost all laboratories were able to demonstrate the cerebral nerves and large nerves of the appendix, while in the suboptimal protocols using clone 2F11, the small axons of the appendiceal muscularis propria, the adrenal gland and among tumour cells in the neuroblastoma were weakly demonstrated or false negative.
Appendix is a reliable control: the ganglion cells and axons should show the strongest staining possible without any staining of the smooth muscle cells or epithelium.

Conclusion
This was the first assessment of NFP. A specific epitope was not defined, as we wished to see which antibody was selected by the laboratories to demonstrate NFP in the multitissue block containing both normal tissues and tumours (generally known to express mainly unphosphorylated filaments).

Remarkably, the prevailing choice of Ab was mAb clone 2F11, which only decorates phosphorylated filaments generally known to leave the large majority of neuronal and neuroendocrine tumours unstained. Only four laboratories used the mAb cocktail of clones NFP7/DA2/RMd020.11, and none used clone N52 (Sigma), which may give a strong staining of some neuronal cells and tumours that are patchy stained or even negative with the cocktail mentioned above (see photos below).

For the demonstration of neuronal differentiation in tumours the laboratories should use mAb clone N52 and mAb cocktail of clones NFP7/DA2/RMd020.11 in tandem, while mAb clone 2F11 is only useful for the demonstration of normal axons.

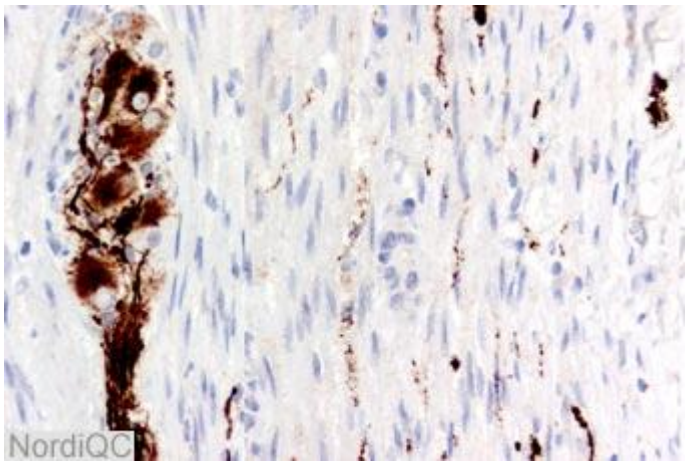

Fig. 1a
Optimal staining of appendix with clone 2F11. Distinct staining of both ganglion cells (left) and axons (right).

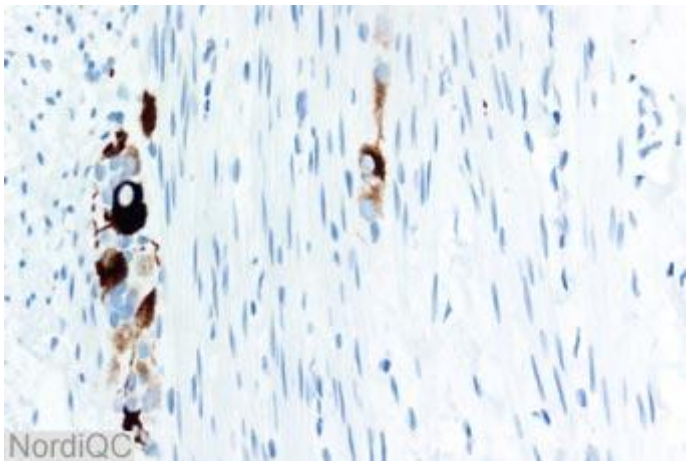

Fig. 1b
Optimal staining of appendix with clone NFP7/DA2/RMd020.11. Distinct staining of ganglion cells (left) but the axons in this field are virtually unstained.

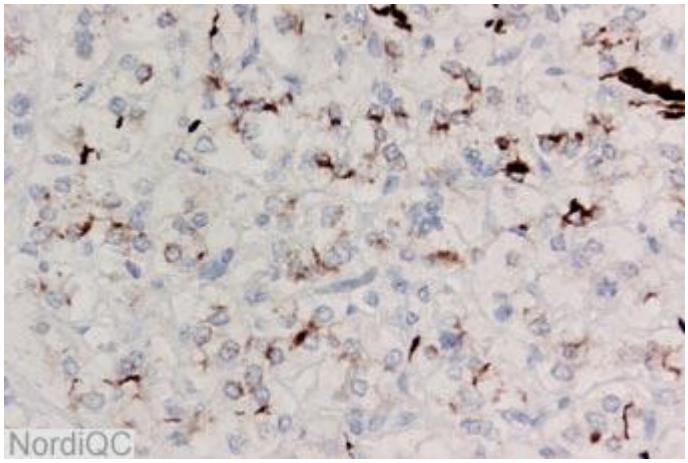

Fig. 2a
Optimal staining of adrenal medulla with mAB cocktails of clones NFP7/DA2/RMd020.11 (clone 2F11 shows the same pattern). Heterogeneous staining of phaeochromocytes is seen.

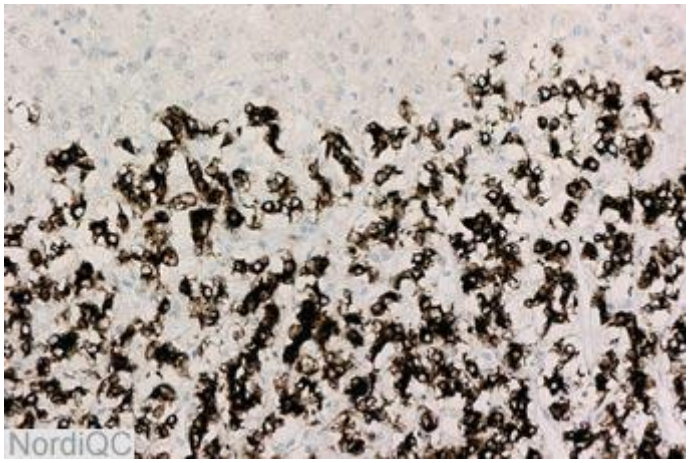

Fig. 2b
Optimal staining of adrenal medulla with clone N52 for comparison with Fig. 2a. Strong staining of phaeochromocytes. Note the unstained cells of zone reticularis above.

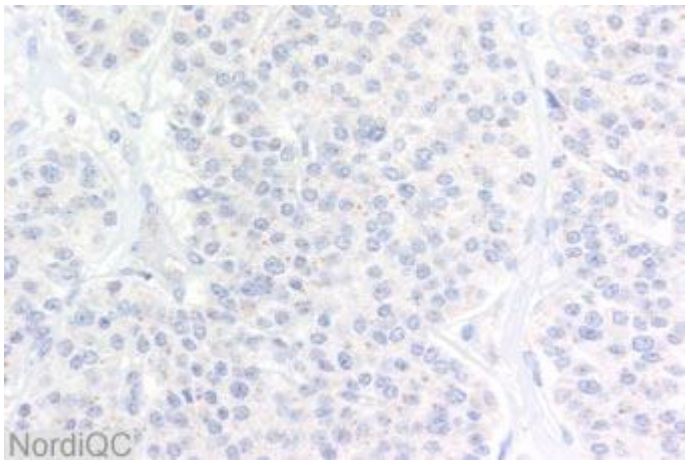

Fig. 3a
Optimal staining of the carcinoid tumour with clone 2F11. The cells are virtually unstained.

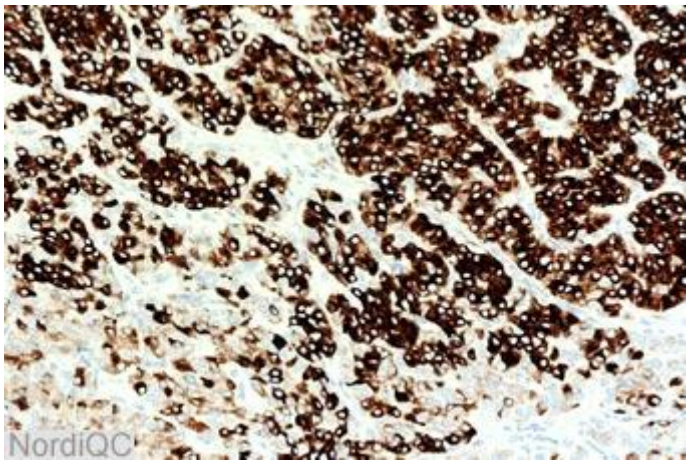

Fig. 3b
Optimal staining of the carcinoid tumour with clone NFP7/DA2/RMd020.11. Strong staining of most tumour cells is seen.

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