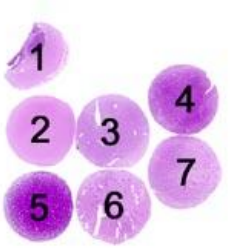
Assessment Run 18 2006

Chromogranin A (CGA)



The slide to be stained for Chromogranin A (CGA) comprised:
1. Appendix, 2. Brain, 3. Large cell neuroendocrine lung carcinoma, 4. Small cell lung carcinoma (SCLC), 5. Merkel cell carcinoma, 6. Colon adenocarcinoma, 7. Thyroid medullary carcinoma.
All specimens were fixed in 10 % NBF.

Criteria for assessing a CGA staining as optimal included:

- A strong and distinct cytoplasmic reaction of the normal neuroendocrine cells in the appendiceal mucosa and the colon adenocarcinoma.
- At least a weak to moderate but distinct granular cytoplasmic reaction of the normal ganglion cells and axons in the appendiceal Aurbach's plexus as well as the cortical neurons in the brain.
- A strong, distinct cytoplasmic reaction in the majority of cells of the SCLC, the Merkel cell carcinoma, the large cell neuroendocrine carcinoma and the thyroid medullary carcinoma.
- A negative reaction of the colon adenocarcinoma.

A weak focal background reaction due to diffusion of the antigens was accepted in the vicinity of a strong, specific CGA reaction.

94 laboratories submitted stains. At the assessment 30 achieved optimal marks (32 %), 36 good (38 %), 21 borderline (22 %) and 7 (8 %) poor marks.

The following Abs were used:
mAb clone **5H7** (Novocastra, n=1)
mAb clone **DAK-A3** (Dako, n=11)
mAb clone **LK2H10** (Ventana, n=9, NeoMarkers; n=4, BioGenex, n=2; Boehringer, n=1; Chemicom, n=1; Linaris, n=1; Novocastra, n=1))
mAb clone **LK2H10+PHE5** (NeoMarkers, n=2; BioGenex, n=2; Biocare, n=1)
rmAb clone **SP12** (NeoMarkers, n=1)
pAb **18-0094** (Zymed, n=2)
pAb **A0430** (Dako, n=53)
pAb **N1535** (Dako, n=1)
pAb **RB-9003** (Neomarkers, n=1)

Optimal staining for CGA in this assessment was obtained with the mAbs clones **LK2H10** and **LK2H10+PHE5**, and the pAb **A0430** as follows:

LK2H10: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9, Cell Conditioning 1 (CC1 Ventana) or Citrate pH 7.3. Clone LK2H10 could both be applied as a Ready-To-Use product and as a concentrate diluted in the range of 1:100 – 3,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 17 laboratories (82 %) produced a sufficient staining (optimal or good), 8 of them were optimal (47 %).

LK2H10+PHE5 (cocktail clones): the protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 as the HIER buffer. The mAb was diluted 1:3,000. Using similar protocol settings 3 out of 3 laboratories (100 %) produced a sufficient staining (optimal or good).

A0430: the protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 or Cell Conditioning 1 (CC1 Ventana). A0430 was typically applied in range of 1:1,000 - 1:5,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 37 out of 41 laboratories (90 %) produced a sufficient staining (optimal or good), 20 of them were optimal (49 %).

Grouped together, 54 out of 61 laboratories (89 %) using HIER and one of the three above mentioned markers for CGA obtained sufficient marks.

The most frequent causes of an insufficient staining were:
- Less successful primary antibody

- Inappropriate epitope retrieval (omission of HIER: 8 out of 8 protocols resulted in a too weak or false negative staining)
- Too low concentration of the primary antibody.

In the assessment and in concordance with observations in previous CGA assessments almost all laboratories were able to demonstrate CGA in the normal neuroendocrine cells, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the SCLC and the Merkel cell carcinoma.

A too weak or false negative staining was seen in 79 % of the insufficient results (22 out of 28, while in 11 % (3 out of 28) a too strong staining was observed giving a false positive staining, and in 11 % (3 out of 28) both a false negative and false positive reaction was noticed.

As observed in previous assessments peripheral nerves seem to be a reliable and valid quality indicator for the immunohistochemical demonstration of CGA, as the sufficient results all showed a distinct reaction of CGA in axons and ganglion cells in the Aurbach's plexus in the appendix. If these structures were negative, several of the tumours were partly or completely false negative.

CGA was also assessed in run 9 and run 13. In the latter, 88 laboratories participated out of which 36 % (32 laboratories) obtained an insufficient mark. Each laboratory was given a specific recommendation to improve their protocol. 25 laboratories, which obtained an insufficient result in run 13, submitted a new CGA stain in run 18. 18 of them followed the recommendation, of which 17 improved to good or optimal (94 %). 7 laboratories did not follow the recommendation and only 2 of these (29 %) obtained a sufficient staining in run 15.

The proportion of insufficient results have been reduced from 61 % in run 9 2003 to 30 % in the present run. In total, specific recommendations have been given to 68 laboratories. In 46, the advices have been followed, giving sufficient staining in 42 (91 %).

Conclusion

The mAb clones LK2H10 and LK2H10 + PHE5 and the pAb A0430 are all useful for the demonstration of CGA. HIER was mandatory to obtain an optimal result. The concentration of the primary Ab should be carefully calibrated. Normal appendix is an appropriate control tissue: the normal ganglion cells and axons in the Aurbach's plexus must show a distinct granular reaction while the surrounding muscle cells are unstained.

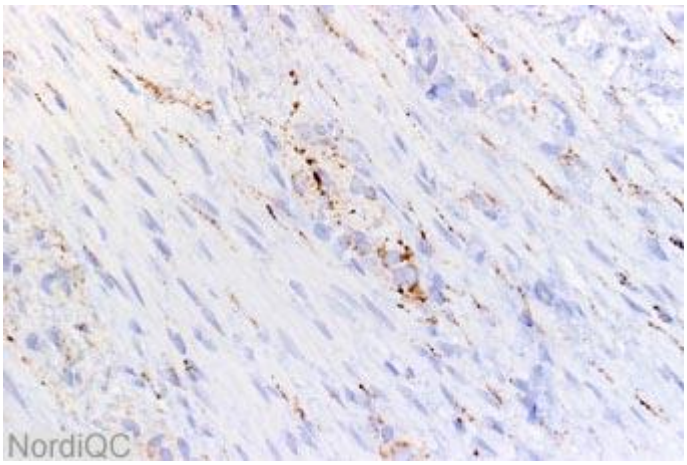


Fig. 1a
Optimal staining for CGA of the appendix using the pAb A0430 and HIER. The axons and ganglion cells of the peripheral nerves in tunica muscularis show a distinct and granular cytoplasmic reaction, while the smooth muscle cells are negative.

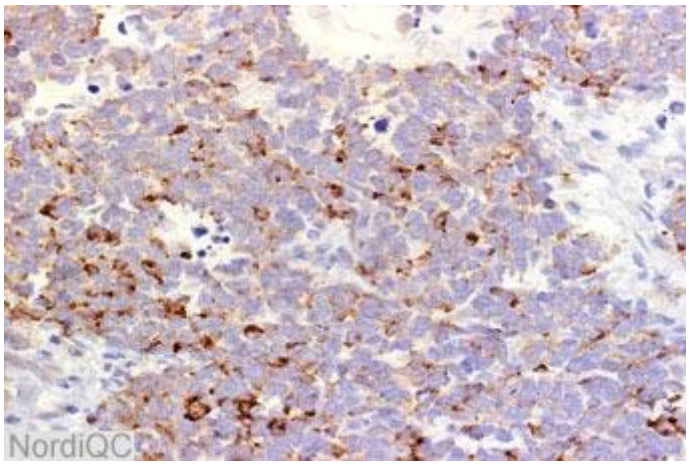


Fig. 1b
Optimal staining for CGA of the Merkel cell carcinoma. The majority of the neoplastic cells show a moderate and distinct dot like reaction. Same protocol as Fig 1a.

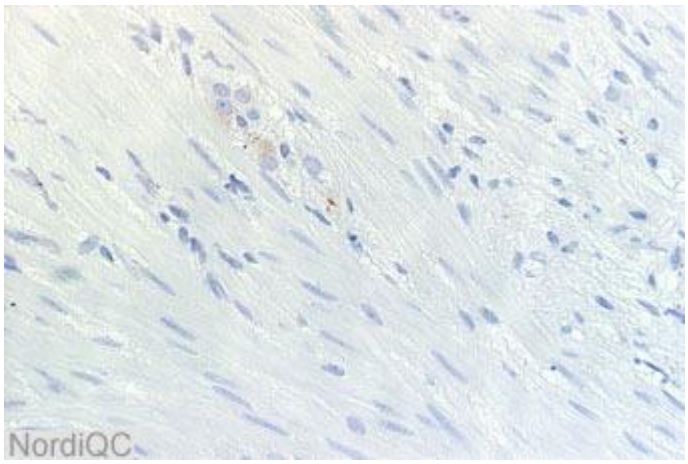


Fig. 2a
Insufficient staining for CGA of the appendix, same field as Fig 1a.. The peripheral nerves are only weakly demonstrated. The weak reaction was due to omission of HIER and a too low concentration of the pAb A0430.

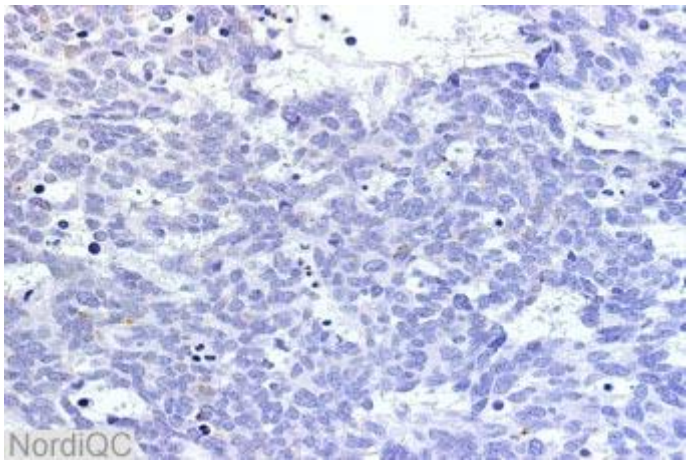


Fig. 2b
Insufficient staining for CGA of the Merkel cell carcinoma, same field as Fig 1b. All the neoplastic cells are virtually false negative. Same protocol as Fig 2a.

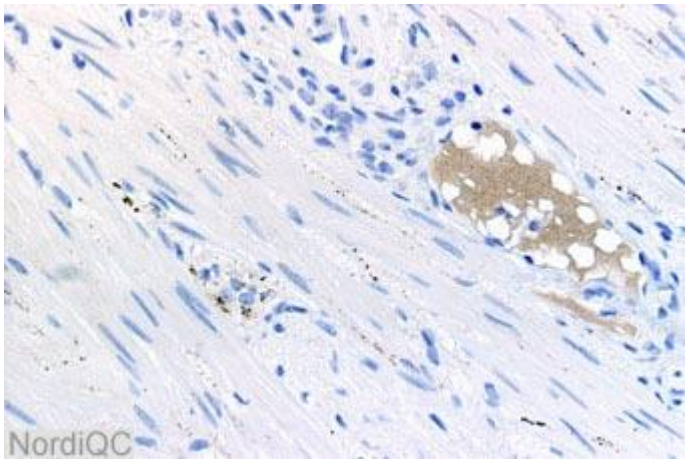


Fig. 3a
Staining for CGA of the appendix, same field as Fig 1a. using an insufficient protocol based on HIER and the mAb clone DAK-A3 in a high concentration. The axons are demonstrated, however compare with Fig 4a and 4b.

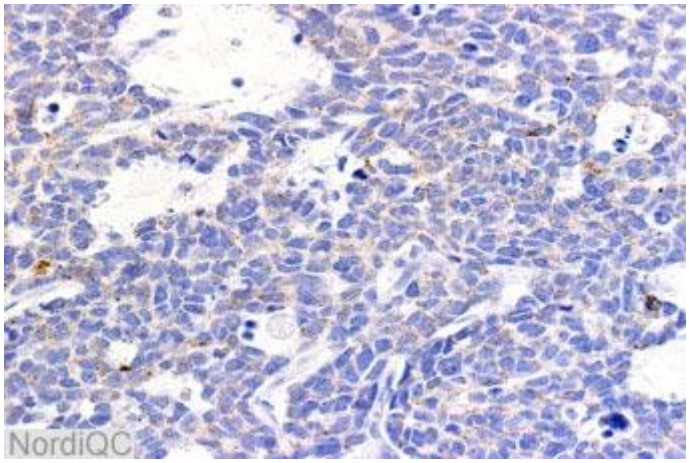


Fig. 3b
Staining for CGA of the Merkel cell carcinoma. Focally the neoplastic cells show a moderate and distinct dot like reaction using same protocol as Fig 3a. However compare with Fig 4a and 4b.

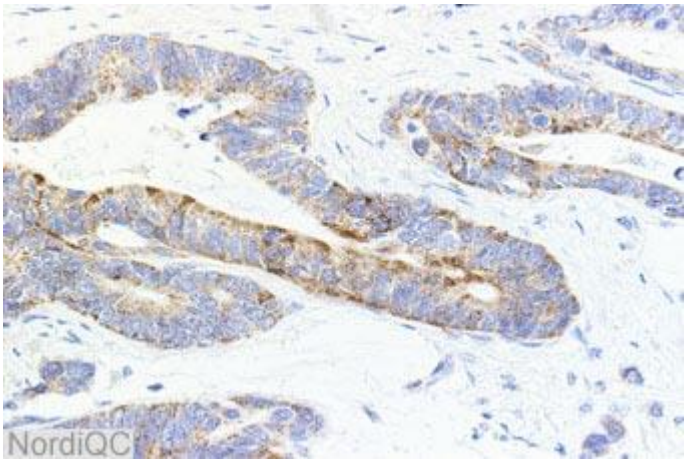


Fig. 4a
Insufficient staining for CGA of the colon carcinoma using same protocol as in Fig 3a and 3b. All the neoplastic cells show a false positive cytoplasmic reaction, compare with Fig 4b.

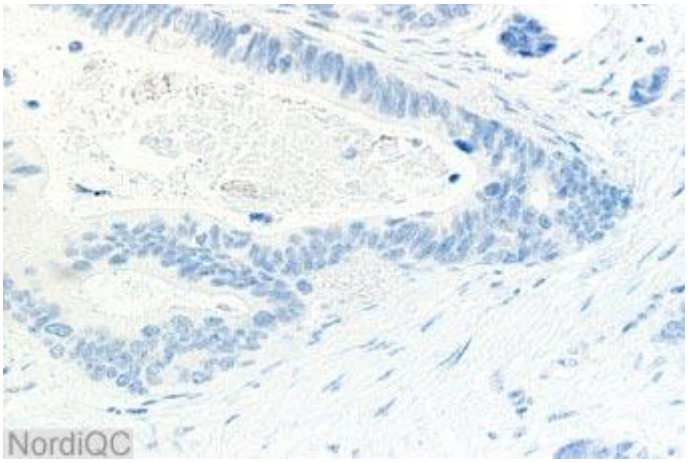


Fig. 4b
Optimal staining for CGA of the colon carcinoma, using a protocol based on HIER and the mAb clone LK2H10. The neoplastic cells are negative.

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