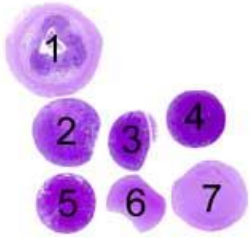
Assessment Run 13 2005

Chromogranin A (CGA)



The slide to be stained for Chromogranin A (CGA) comprised:
1: Appendix, 2: Large cell neuroendocrine lung carcinoma, 3 - 4: Small cell lung carcinoma, 5: Pancreas, 6: Brain (autopsy material fixed in about 6 weeks), 7: Brain (resection specimen). All tissues were fixed in neutral buffered formalin.

Criteria for assessing a CGA staining as optimal included: A strong and distinct cytoplasmic staining of the normal neuroendocrine cells in the appendical mucosa and islets of Langerhans, a moderate to strong distinct staining of normal ganglion cells and axons in the appendiceal Aurbach's plexus as well as cortical neurons and most tumour cells in the three carcinomas. All other cells should be negative. However, a faint staining of the appendiceal enterocytes was accepted.

88 laboratories participated in the assessment. 21 achieved optimal (24 %), 35 good (40 %), 17 borderline (19 %) and 15 (17 %) poor marks.

The following CGA antibodies were used:
mAb clone **LK2H10** (NovoCastra, n=6; Ventana, n=4; NeoMarkers, n=3; Boehringer Mannheim, n=2; BioGenex, n=2; Chemicon, n=2; Cell Marque, n=1; Immunon, n=1; Linaris, n=1)
mAb clone **DAK-A3** (DakoCytomation, n=15)
mAb clone **LK2H10+PHE5** (NeoMarkers, n=3)
mAb clone **SP12** (NeoMarkers, n=1)
mAb clone **PHE5** (BioGenex, n=1)
pAbs **A0430** (DakoCytomation, n=44) and **18-0094** (Zymed, n=2).

Optimal stains in this assessment were obtained with the clones LK2H10 (1/22), LK2H10+PHE5 (2/3) and the polyclonal A0430 (18/44).

All laboratories achieving an optimal staining used HIER with buffers as follows: Tris-EDTA/EGTA pH 9 (18 out 48 were optimal), Citrate pH 6 (2 out of 21) and Target Retrieval Solution low pH DakoCytomation (1 out of 3).

Using the polyclonal Ab A0430 in combination with HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining for 17 out of 31 laboratories (55 %), while using LK2H10+PHE5 and HIER with Tris-EDTA/EGTA pH 9 an optimal staining was obtained in 1 out of 3 protocols (33 %) and using LK2H10 with HIER in Tris-EDTA/EGTA pH 9 an optimal staining was achieved in 1 out of 8 protocols (13 %). Using DAK-A3 an optimal staining could not be achieved irrespective of the IHC method applied.

In the optimal staining the pAb A0430 was used in the range of 1:750 – 10.000, LK2H10+PHE5 in the range of 1:200 – 3.000 and LK2H10 was used in dilution of 1:1.000 (all ranges depending on the total sensitivity of the IHC protocol).

The most frequent causes of insufficient staining were:
- Omission of HIER (10 out of the 30 insufficient)
- Inappropriate choice of primary antibody (14 out of the 30 insufficient)
- Too diluted primary antibody

11 laboratories used a protocol without pre-treatment. 10 of these gave an insufficient staining.

In general almost all laboratories were able to demonstrate CGA in the neuroendocrine cells in the appendix and the pancreas, whereas the demonstration of CGA in the neurons and carcinomas was much more difficult. Consequently, the demonstration of CGA in the endocrine cells in the appendix and pancreas can not be used as a reliable positive control as these structures harbour a very high concentration of CGA. A calibration of the protocol on the basis of these cells gives a high risk of false negative reactions in the neoplasm's.

CGA was also assessed in run 9. In that run 74 laboratories participated, out of which 45 (61 %) obtained an

insufficient staining. Each laboratory was given a specific recommendation to improve their protocol. 43 laboratories, which obtained an insufficient result in run 9 submitted a new CGA-stain in run 13. 28 of the 43 laboratories followed the recommendations given, and 25 of these improved the score from insufficient to either good or optimal, while 3 were still insufficient. 15 laboratories did not follow the recommendations. 3 of these obtained a good result in run 13, whereas 12 still had an insufficient staining. The overall proportion of insufficient staining was reduced from to 61 % in run 9 to 36 % in run 13.

In this assesment it appeared that it is easier to obtain an optimal staining with the polyclonal A0430 than the monoclonal LK2H10. Clone DAK-A3 can not be recommeded. As regards the other Abs, there are too few users for a reliable evaluation.

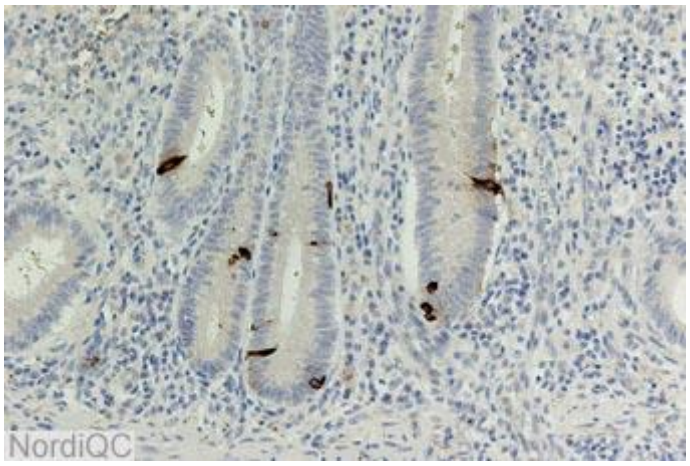


Fig. 1a
Optimal staining for CGA in the appendix. The normal neuroendocrine cells in the mucosa are strongly labelled with minimal reaction of the enterocytes.

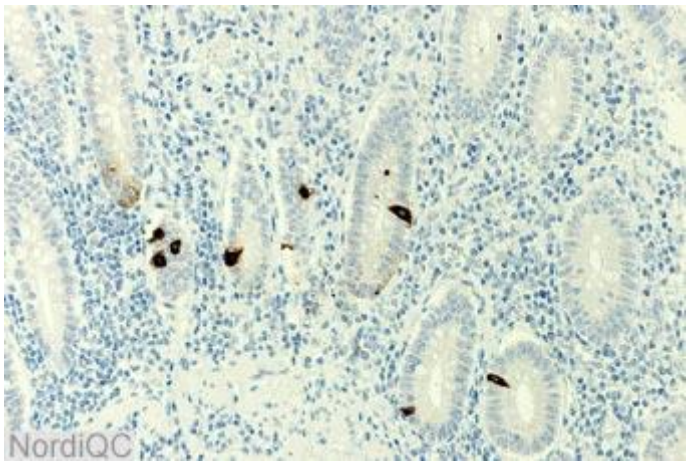


Fig. 1b
Staining for CGA in the appendix using an insufficient protocol. The normal neuroendocrine cells in the mucosa are strongly labelled with minimal reaction of the enterocytes. However, compare with figs. 2b, 3b and 4b.

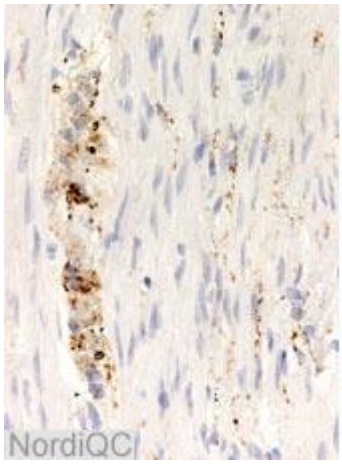


Fig. 2a
Optimal staining for CGA of the neural network in the muscularis externa in the appendix. The ganglion cells and axons are strongly stained without any reaction in the muscle cells.

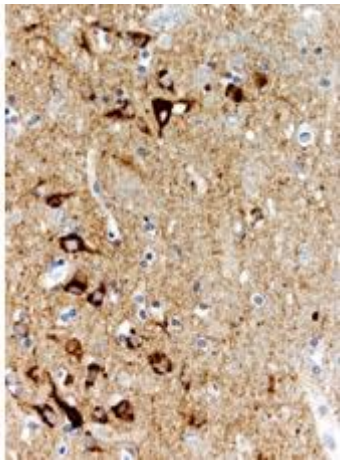


Fig. 3a
Optimal staining for CGA in the brain. The cortical neurons and axons are distinctively stained.

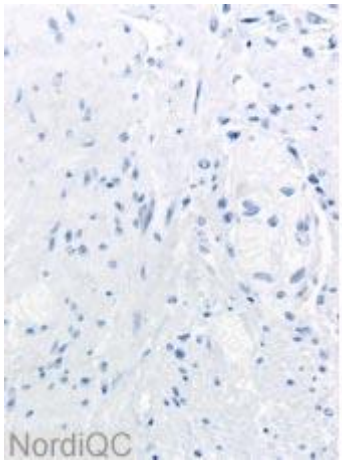


Fig. 2b
Insufficient staining for CGA in the appendix. The ganglion cells and axons are virtually negative (same protocol as fig. 1b.).

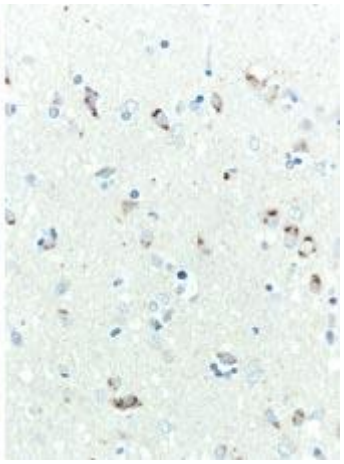


Fig. 3b
Insufficient staining for CGA in the brain. The cortical neurons show only a weak staining and the axons are negative (same protocol as fig. 1b.).

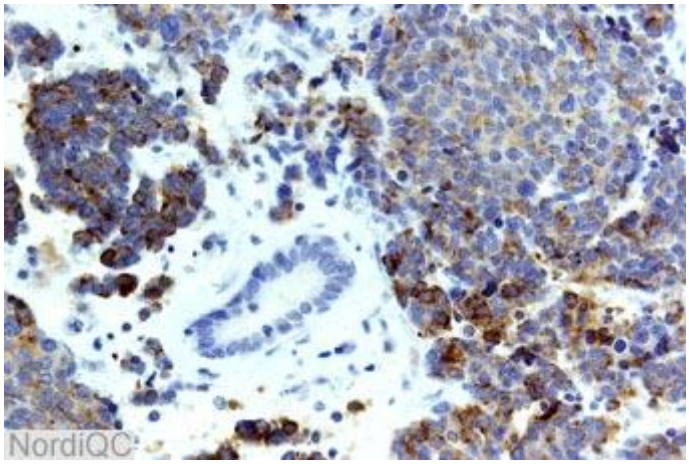


Fig. 4a
Optimal staining of a small cell lung carcinoma for CGA. The majority of the neoplastic cells show a moderate to strong granular cytoplasmic staining.

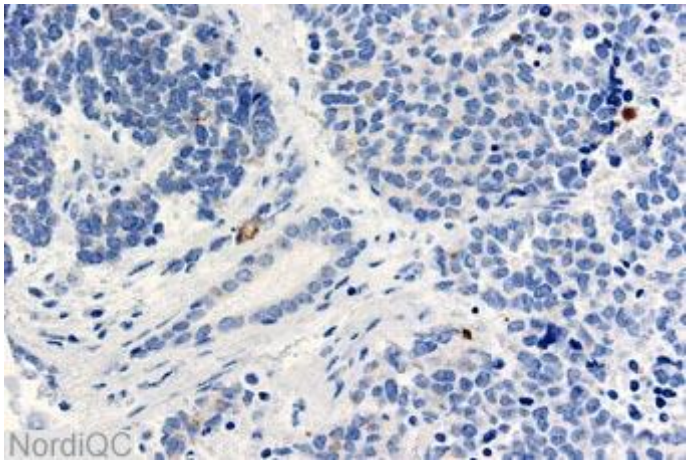


Fig. 4b
Insufficient staining of a small cell lung carcinoma for CGA. Only a few neoplastic cells are demonstrated (same protocol as in fig. 1b).

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