Assessment Run 22 2008

Chromogranin A (CGA)

The slide to be stained for Chromogranin A (CGA) comprised:
1. Brain, 2. Appendix, 3. Colon adenocarcinoma, 4. Pancreas (from patient with blood group A), 5. Pancreas (from patiet with blood group 0), 6-7. Endocrine lung carcinomas, 8. Medullary thyroid carcinoma.
All tissues were fixed in 10% neutral buffered formalin.

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 in the islet cell in the pancreas*
- A moderate to strong distinct cytoplasmic reaction in 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 of the medullary thyroid carcinoma
- A moderate to strong distinct cytoplasmic reaction of the two endocrine lungcarcinomas
- A negative reaction of the neoplastic cells in the colon adenocarcinoma.

* A weak background reaction (due to diffusion of the antigen/chromogen and focal autolysis in the two pancreas specimens) was inevitable.

117 laboratories submitted stains. At the assessment 34 achieved optimal marks (29 %), 37 good (32 %), 25 borderline (21 %) and 21 poor marks (18 %).

The following Abs were used:
mAb clone **LK2H10** (Ventana, n=11; NeoMarkers, n=8; BioGenex, n=5; Linaris, n=4; Chemicon, n=1; Euro-Diagnostica, n=1; Novocastra, n=1)
mAb clone **LK2H10+PHE5** (NeoMarkers, n=5; BioCare Medical, n=1)
mAb clone **DAK-A3** (Dako, n=16)
mAb clone **5H7** (Novocastra, n=2)
rmAb clone **SP12** (NeoMarkers, n=4)
pAb **A0430** (Dako, n=57)
pAb **N1535** (Dako, n=1)
pAb **18-0094** (Zymed, n=1)

Optimal staining for CGA in this assessment was obtained with the mAb clone **LK2H10** (8 out of 30), the mAb clone **LK2H10+PHE5** (2 out of 6) and the pAb **A0430** (24 out of 56).

LK2H10: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (3/10)*, Cell Conditioning1 (BenchMark, Ventana; 4/10) or Citrate pH 6 (1/4). The mAb was typically diluted in the range of 1:500 – 1:1.000 depending on the total sensitivity of the protocol employed or as a Ready-To-Use antibody. Using these protocol settings 17 out of 20 (85 %) laboratories produced a sufficient staining (optimal or good).
*number of optimal results/number of laboratories using this buffer

LK2H10+PHE5: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (2/4). The mAb was diluted in the range of 1:200 – 1:5.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining.

A0430: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (20/37), Cell Conditioning1 (BenchMark, Ventana) (2/7), Bond Epitope Retrieval Solution 1 (Bond, Vision Biosystems) (1/4) or Target Retrieval Solution pH 9 (FLEX TRS high pH, Dako) (1/3). The pAb was typically diluted in the range of 1:250 – 1:4.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 33 out of 39 (85 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Omission of HIER

In this assessment and in concordance with the observations in previous CGA assessments almost all laboratories were able to demonstrate CGA in the normal neuroendocrine cells in e.g. pancreas, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining in the neurons and axons as well as the three neuroendocrine carcinomas.
A too weak or false negative staining was seen in 98 % of the insufficient results (45 out of 46).
In all the assessments for CGA, appendix have shown to be a reliable control, as a moderate to strong staining of the the Aurbach's plexus (neurons and axons) being the critical stain quality indicator for a proper immunohistochemical demonstration of CGA in the tumours.

The choice of Ab and epitope retrieval are central parameters for a successful demonstration of CGA.
In Table 1, the overall pass rates are compared for the most widely used Abs in the latest three assessments:

Table 1	Run 13 2005		Run 18 2006		Run 22 2008		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone LK2H10	22	14	19	14	31	25	72	53 (74%)
mAb clone LK2H10+PHE5	3	3	5	3	6	4	14	10 (71%)
mAb clone DAK-A3	15	2	11	0	16	0	52	2 (4%)
pAb A0430	44	37	53	42	57	39	154	118 (77%)

Thus, in 3 successive assessments only 4% of the slides stained with mAb clone DAK-A3 showed a sufficient result (compared to 71-77% for the 3 other Abs). In accordance with this, tests in the NordiQC laboratory have shown that it is not possible to obtain satisfactory results with DAK-A3. In spite of the clear demonstration of its insensitivity in the previous runs, 16 labs used DAK-A3 in the current run. NordiQC will again urge the producer to withdraw the clone from the market an inform the costumers about the problem.

Omission of HIER is another important protocol parameter. 7 out of 8 protocols using mAb clone LK2H10 or pAb A0430 without HIER in the current run gave insufficient results.

This was the 4th NordiQC assessment of CGA. The proportion of sufficient results in the four runs are shown in Table 2.

Table 2	Run 9 2003	Run 13 2005	Run 18 2006	Run 22 2008
Participants, n	77	88	94	117
Sufficient results	49%	64%	70%	61%

Major reasons for the unsatisfactory low proportion of sufficient staining results are the use of the low affinity mAb DAK-A3 and omission of HIER.

68 laboratories participating in all 4 runs for CGA showed an increase in the proportion of sufficient results from 41 % (run 9) to 74 % (run 22). 89 laboratories participated in both run 18 and run 22. For these, the proportion of sufficient results were 71% and 73%, respectively. The decline in the over all proportion of sufficient stains from run 18 to run 22 is consequently due to a large number of new participants submitting CGA stains for the first time.

Conclusion
The mAb clones LK2H10 and LK2H10 + PHE5 and the pAb A0430 are useful Abs for the demonstration of CGA. HIER, preferentially in an alkaline buffer, and a proper calibration of Ab titer are mandatory to obtain an optimal result. Normal appendix is a recommendable control tissue: the ganglion cells and axons in the Aurbach's plexus must show an at least moderate, distinct granular reaction, while the surrounding muscle cells should be unstained.

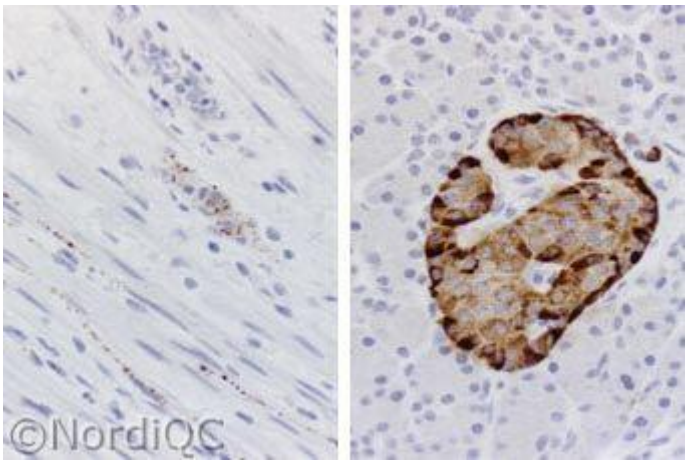

Fig. 1a
Optimal staining for CGA using the pAb A0430 with HIER.
Left, appendix: Both the axons of the peripheral nerves and the ganglion cells in muscularis propria show a distinct granular reaction, while the smooth muscle cells are negative.
Right, pancreas: Virtually all the endocrine cells of the Langerhans islet show a distinct staining.

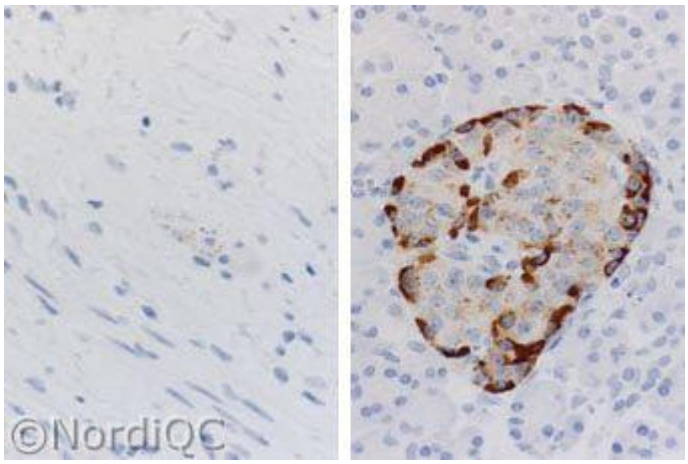

Fig. 1b
CGA staining using an insufficient protocol (mAb DAK-A3) – same field as in Fig 1a.
Left, appendix: The peripheral nerves are demonstrated, but the number of positive cells is reduced and show a weaker reaction compared to the reaction in Fig 1a.
Right, pancreas: Only the glucagon producing cells show a strong and distinct staining.
Also compare with Fig. 2b & 3b – same protocol.

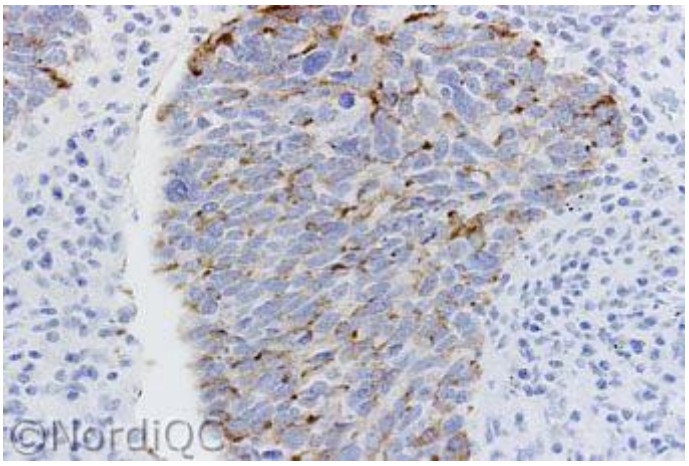

Fig. 2a
Optimal CGA staining of the endocrine lung carcinoma no. 6 using same protocol as in Fig. 1a. The majority of the neoplastic cells show a distinct staining and a scattered dot-like reaction.

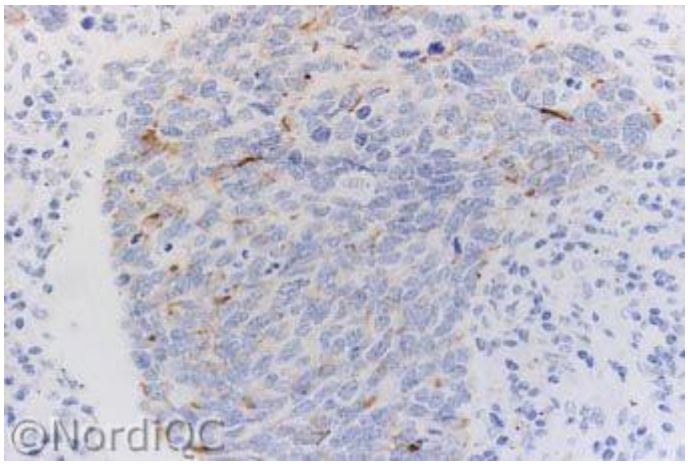

Fig. 2b
Insufficient CGA staining of the endocrine lung carcinoma no. 6 using same protocol as in Fig. 1b. The number of positive cells is reduced and show a weaker reaction compared to the reaction in Fig 2a. – same field.
Also compare with Fig 3b.

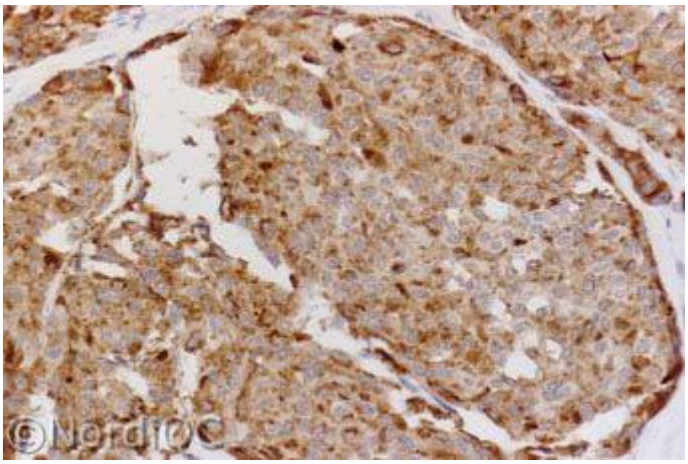

Fig. 3a
Optimal CGA staining of the medullary thyroid carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a distinct cytoplasmic staining with a scattered dot-like reaction.

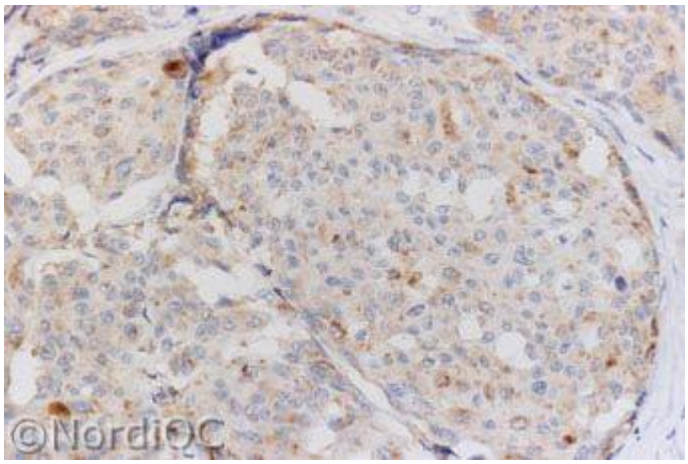

Fig. 3b
Insufficient CGA staining of the medullary thyroid carcinoma using same protocol as in Fig. 1b. Only scattered cells show a weak dot-like staining. Same field as in Fig 3a.

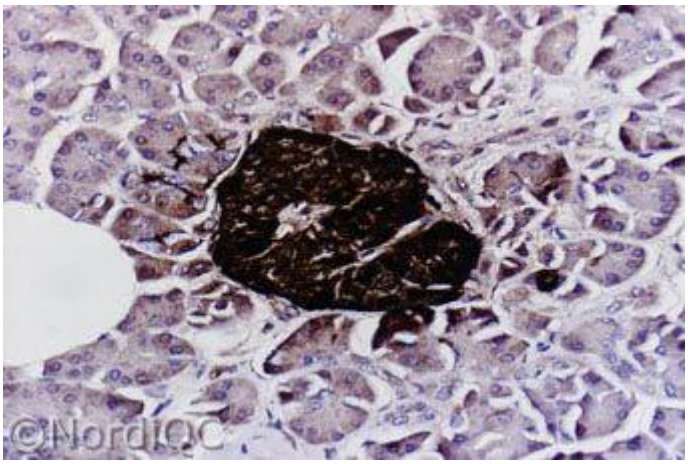

Fig. 4a
Insufficient staining for CGA of the pancreas. The endocrine cells show a strong reaction, but the morphology is severely impaired, due to excessive HIER. Also compare with Fig 4b.

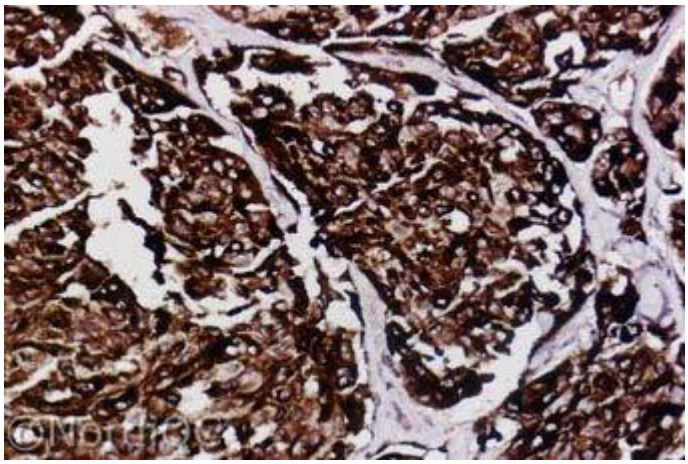

Fig. 4b
Insufficient CGA staining of the medullary thyroid carcinoma using same protocol as in Fig. 4b. The neoplastic cells are demonstrated, but as in Fig 4a the morphology is severly impaired.

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