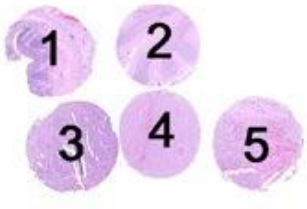
Assessment Run 31 2011

Chromogranin A (CGA)

The slide to be stained for CGA comprised:



1. Appendix, 2. Brain, 3. Colon adenocarcinoma, 4. Pancreas neuroendocrine carcinoma, 5. Thyroid medullary carcinoma.

All tissues were fixed in 10% 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 appendiceal mucosa.
- At least a weak to moderate, distinct granular cytoplasmic reaction of the normal ganglion cells and axons in the appendiceal Aurbach's plexus and the cortical neurons of the brain.
- At least a moderate, distinct cytoplasmic reaction in the majority of the neoplastic cells of the pancreatic neuroendocrine carcinoma and the medullary thyroid carcinoma.
- A negative reaction of the absorptive epithelial cells of the appendix and the the colon adenocarcinoma.

170 laboratories participated in this assessment. 75 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CGA, run 31**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **LK2H10**	13 5 2 2 1 1	NeoMarkers BioGenex Chemicon/Millipore Leica/Novocastra EuroProxima Zytomed	5	13	6	0	75 %	91 %
mAb clones **LK2H10 + PHE5**	8 3	NeoMarkers Biocare	3	5	3	0	73 %	80 %
mAb clone **DAK-A3**	16	Dako	0	2	12	2	13 %	-
mAb clone **5H7**	4	Leica/Novocastra	0	2	0	2	-	-
rmAb clone **SP12**	3 1 1 1	Spring Bioscience DSC Master Diagnostica NeoMarkers	0	0	5	1	0%	-
pAb **A0430**	53	Dako	36	15	2	0	96 %	100 %
pAb **18-0054**	2	Zymed	0	1	1	0	-	-
pAb **RB-9003-P**	1	NeoMarkers	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **LK2H10, 760-2519**	25	Ventana	9	13	1	2	88 %	100 %
mAb clone **LK2H10, E001**	3	Linaris	1	2	0	0	-	-
mAb clone **LK2H10, PM010**	1	Biocare	0	1	0	0	-	-
mAb clone **LK2H10, AM126-5M**	1	BioGenex	0	1	0	0	-	-
mAb clone **LK2H10, 238M-97**	1	Cell Marque	0	1	0	0	-	-
mAb clone **LK2H10, MS-324-R7**	1	NeoMarkers	0	1	0	0	-	-
mAb clone **5H7, PA0430**	2	Leica/Novocastra	0	0	1	1	-	-

pAb **IS502/IR502**	17	Dako	6	10	1	0	94 %	94 %
pAb clone **N1535**	1	Dako	0	1	0	0	-	-
Unknown	1	Zhongshan	0	0	1	0	-	-
Total	170		60	68	34	8	-	
Proportion			35 %	40 %	20 %	5 %	75 %	

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 **LK2H10**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (1/3)*, Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/2), Cell Conditioning 1 (CC1; BenchMark, Ventana) (1/5) or Citrate pH 6 (2/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:200– 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 out of 11 (91 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

mAb clones **LK2H10+PHE5**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/2), Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (1/1) or CC1 (BenchMark, Ventana) (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:300– 1:1.200 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 6 (80 %) laboratories produced a sufficient staining.

pAb **A0430**: The protocols giving an optimal result were based on HIER using either Tris-EDTA/EGTA pH 9 (5/8), Target Retrieval Solution pH 9 (TRS; 3-in-1) (Dako) (12/14), TRS pH 9 (Dako) (2/5), TRS pH 6.1 (Dako) (1/1), BERS1 (Bond, Leica) (1/1), BERS2 (Bond, Leica) (3/6), CC1 (BenchMark, Ventana)(11/14) or CC2 (BenchMark, Ventana)(1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:600– 1:4.000 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 47 (100 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **LK2H10** (prod. no. 760-2519, Ventana): The protocols giving an optimal result were based on HIER using mild or standard CC1, an incubation time of 20-44 min in the primary Ab and UltraView (760-500/760-501) as the detection system. 1 lab used amplification kit. Using these protocol settings all of 18 (100 %) laboratories produced a sufficient staining.

mAb clone **LK2H10** (prod. no. E001, Linaris): The protocol giving an optimal result was based on HIER using BERS2 (Bond, Leica), an incubation time of 24 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system.

pAb prod. no. **IS502/IR502** (Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1, Dako), an incubation time of 20-30 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 16 out of 17 (94 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Omission of HIER
- Biotin based detection system.

In this assessment and in concordance with the previous NordiQC assessments for CGA the prevalent feature of an insufficient staining was a too weak or false negative reaction of the cells expected to be demonstrated. A too weak or false negative staining was seen in 67 % of the insufficient results (28 out of 42). The majority of the laboratories were able to demonstrate CGA in the normal neuroendocrine cells in the appendiceal mucosa, whereas the demonstration of CGA in the neuroendocrine carcinomas, the cortical neurons in the brain and in particular the axons and ganglion cells of the peripheral nerves was more difficult and only seen for protocols with a high sensitivity and appropriate protocol settings, e.g. as obtained with a correct titre of the mAb clone LK2H10 or the pAb A0430 with HIER. All of 5 protocols based on the mAb clone LK2H10 omitting HIER were insufficient. The most widely used Ready-To-Use (RTU) formats and systems of the mAb clone LK2H10, 760-2519 Ventana and the pAb, IS502/IR502 Dako gave a very high proportion of sufficient results.

14 out of 16 protocols based on the mAb clone DAK-A3 gave an insufficient staining (false negative or too weak) and none were assessed as optimal despite the protocol settings were identical to the settings giving an optimal result for the other above mentioned Abs.
All of 6 stains based on the rmAb clone SP12 were assessed as insufficient.

In Table 2, the overall pass rates for the most widely used Abs in the latest four assessments are compared. The pAb A0430 (Dako) has in these four runs shown to be the most successful marker for CGA:

Table 2. **Cumulated pass rates for selected Abs for CGA**

	Run 13, 18, 22 & 31		
	Protocols Submitted	Protocols Sufficient	Pass rate
mAb clone LK2H10	128	100	78 %
mAb clone LK2H10+PHE5	25	18	72 %
mAb clone DAK-A3	68	4	6 %
pAb A0430	224	185	83 %

A combined false positive and false negative staining was seen when a too low titre of the primary Ab was used in conjunction with efficient HIER and a biotin based detection system. The rmAb clone SP12 gave a weak staining in structures with a low antigen expression as the peripheral nerves and the cortical neurons of the brain and at the same time an aberrant staining in inflammatory cells in the colon adenocarcinoma was seen (Fig. 4a & b).

In concordance to the previous assessments for CGA, appendix is a reliable control for CGA: An at least weak to moderate distinct granular staining must seen in the axons and ganglion cells of the peripheral nerves. A strong staining must be seen in the neuroendocrine cells in the appendiceal mucosa. A diffusion of the staining has to be accepted in the vicinity of these cells. The epithelial cells in the mucosa and the smooth muscle cells should be negative.

This was the 5th assessment of CGA in NordiQC. An increase in the overall pass rate compared to run 22, 2008 has been achieved (table 3), despite the large number of new participants.

Table 3. **Proportion of sufficient results for CGA in the 5 NordiQC runs performed**

	Run 9 2003	Run 13 2005	Run 18 2006	Run 22 2008	Run 31 2011
Participants, n=	74	88	94	117	170
Sufficient results	39 %	64 %	70 %	61 %	75 %

However, a significant difference regarding the pass rates was observed between the laboratories participating in the CGA assessment for the first time and laboratories also participating in the previous run 22, 2008: For the laboratories participating for the first time the pass rate was 58 % (40 out of 68), whereas the pass rate was 86 % (88 out of 102 laboratories) for the laboratories participating in both runs.

Conclusion
The mAb clones LK2H10 and the pAb A0430 were the most robust Abs for the demonstration of CGA. The mAb clone DAK-A3 should not be used due to a consistent poor performance in 5 consecutive runs for CGA. HIER, preferable in an alkaline buffer, is mandatory to obtain an optimal result. A sufficient result for CGA could be obtained by 91 – 100% of the participants when using one of these two markers either as a concentrate properly calibrated or as a RTU format/system. Normal appendix is a recommendable control tissue: The normal ganglion cells and axons in the peripheral nerves must show an at least weak to moderate distinct granular reaction, while the surrounding smooth muscle cells should be negative.

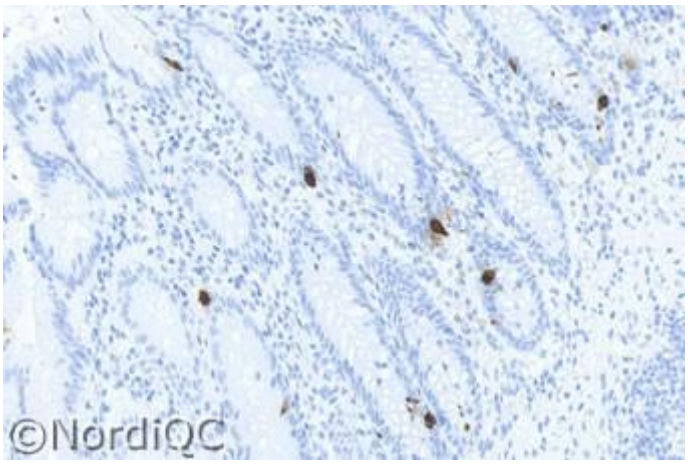


Fig. 1a
Optimal CGA staining of the appendix mucosa using the mAb clone LK2H10 optimally calibrated and with HIER in an alkaline buffer (x200). The neuroendocrine cells show a strong cytoplasmic staining reaction. A weak diffusion of the staining is seen in the vicinity of the positive cells.

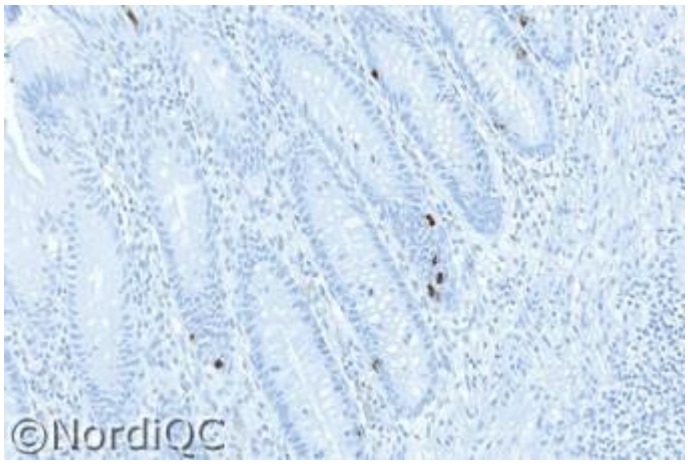


Fig. 1b
CGA staining of the appendix mucosa using an insufficient protocol based on the mAb clone LK2H10 without HIER (x200) – same field as in Fig. 1a. The neuroendocrine cells show a strong cytoplasmic staining reaction. However, compare with Figs. 2b & 3b – same protocol.

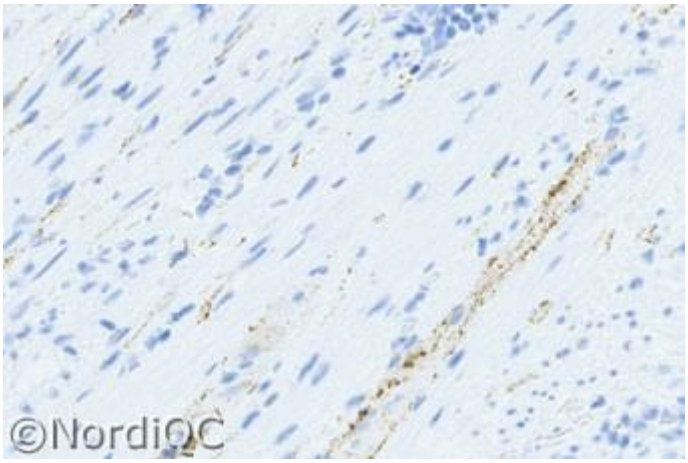


Fig. 2a
Optimal CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1a. (x400). Both the axons of the peripheral nerves and the ganglion cells show a weak to moderate distinct granular staining reaction, while the smooth muscle cells are negative.

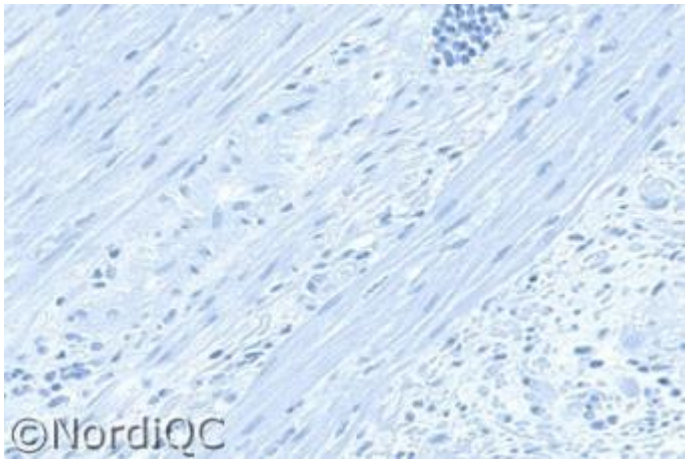


Fig. 2b
Insufficient CGA staining of the appendix, lamina muscularis propria using same protocol as in Fig. 1b. (x400). No staining reaction is seen in the axons of the peripheral nerves and the ganglion cells - same field as in Fig. 2a.

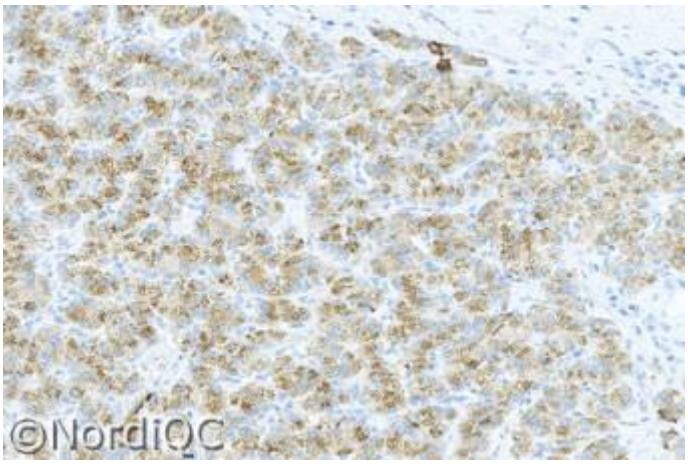


Fig. 3a
Optimal CGA staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a moderate cytoplasmic staining reaction.

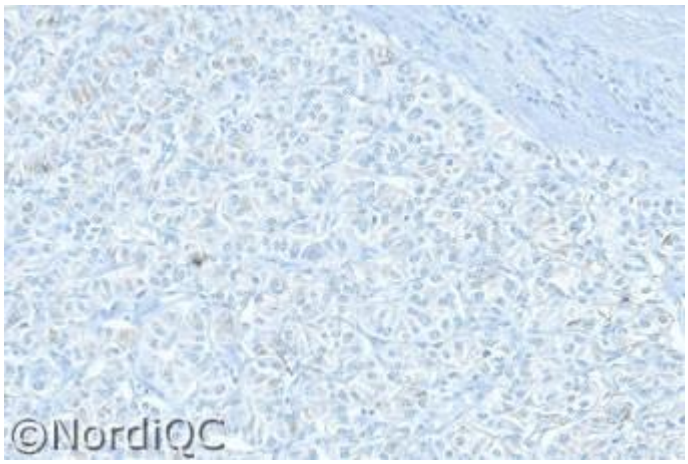


Fig. 3b
Insufficient CGA staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak and equivocal staining reaction – same field as in Fig. 3a.

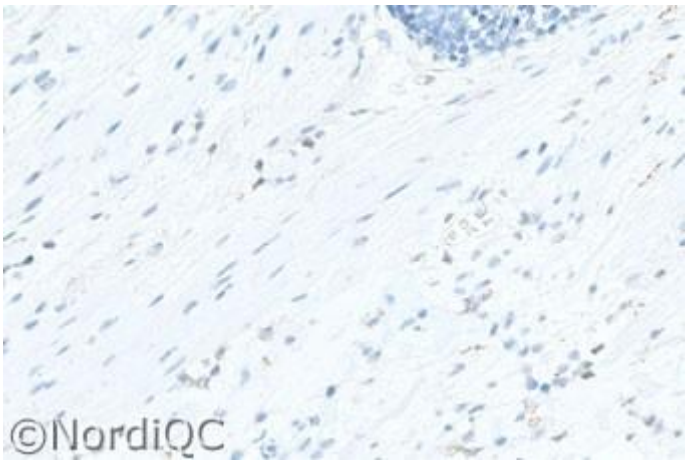


Fig. 4a
Insufficient CGA staining of the appendix, lamina muscularis propria using the rmAb clone SP12 with HIER in an alkaline buffer. Only a weak staining reaction is seen in the axons of the peripheral nerves and the ganglion cells – also compare with Fig. 4b, left – same protocol.

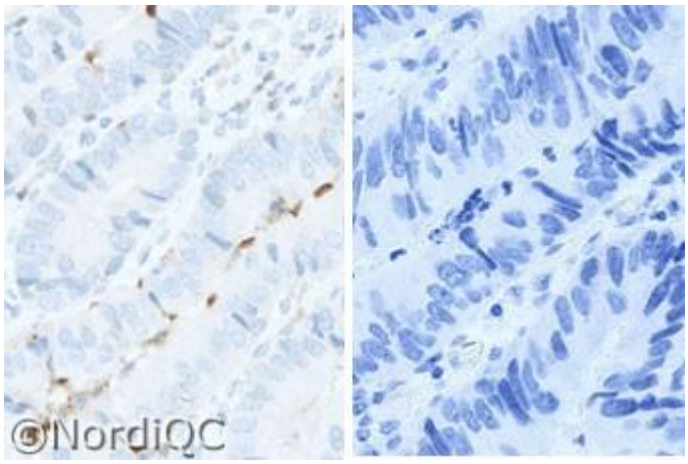


Fig. 4b
CGA staining of the colon adenocarcinoma.
Left: Insufficient staining using same protocol as in Fig. 4a based on the rmAb clone SP12 with HIER in an alkaline buffer. The inflammatory cells and scattered neoplastic cells show an aberrant cytoplasmic staining reaction.
Right: Optimal staining using same protocol as in Figs. 1a – 3a. No staining is seen.

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