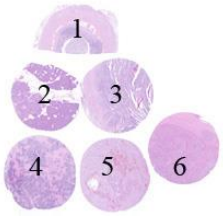
Assessment Run 53 2018

Chromogranin A (CGA)

Material



The slide to be stained for CGA comprised:

1: Appendix, 2: Pancreas, 3: Colon adenocarcinoma, 4: Small cell lung carcinoma (SCLC), 5: Thyroid medullary carcinoma, 6: Neuroendocrine tumour (Carcinoid)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CGA staining as optimal included:

- A strong and distinct cytoplasmic staining reaction of neuroendocrine cells in the appendiceal mucosa and islets of pancreas.
- An at least weak to moderate, distinct granular cytoplasmic staining reaction of normal ganglion cells and axons in the nerve plexus of appendix.
- An at least moderate, distinct cytoplasmic reaction of virtually all neoplastic cells in the neuroendocrine tumour and medullary thyroid carcinoma.
- An at least weak, distinct granular cytoplasmic staining reaction of the vast majority of neoplastic cells in the small cell lung carcinoma.
- No staining reaction of the appendiceal columnar epithelial cells, pancreatic exocrine cells and neoplastic cells in the colon adenocarcinoma.

Participation

Number of laboratories registered for CGA, run 53	306
Number of laboratories returning slides	296 (97%)

Results

296 laboratories participated in this assessment. 226 (76%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab
- Insufficient HIER
- Too low concentration of the primary Ab

Performance history

This was the seventh NordiQC assessment of CGA. The pass rate increased in this assessment compared to the previous runs and is, as shown in Table 2, the highest pass rate obtained until now.

Table 2**. Proportion of sufficient results for CGA in the seventh NordiQC run performed**

	Run 9 2003	Run 13 2005	Run 18 2006	Run 22 2008	Run 31 2011	Run 46 2016	Run 53 2018
Participants, n=	74	88	94	117	170	242	296
Sufficient results	39%	64%	70%	61%	75%	67%	76%

Increased use of successful Abs in this assessment was seen. In run 46, 2016, 61% of the laboratories used mAb clone LK2H10 (including a cocktail combined with mAb clone PHE5), compared to 76% in this run. As shown in Table 1, mAb clone LK2H10 is superior to e.g. mAb clones 5H7 and DAK-A3.

Conclusion

The mAb clone **LK2H10** was the most successful Ab for the demonstration of CGA. As concentrated (Conc) format within a laboratory developed assay, optimal results were obtained on all four main stainer platforms - Omnis (Dako), Autostainer (Dako), Bond (Leica) and BenchMark (Ventana) if HIER was performed in an alkaline buffer. mAb clone DAK-A3 gave inferior performance.
Normal appendix is recommendable as positive and negative tissue controls: Ganglion cells and axons in the peripheral nerves must show an at least weak to moderate distinct granular staining reaction, while smooth muscle cells and columnar epithelial cells should be negative. Neuroendocrine cells must show an intense staining reaction.

Table 1. **Antibodies and assessment marks for CGA, run 53**

Concentrated antibodies	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **5H7**	3	Leica	0	0	2	1	-	-
mAb clone **DAK-A3**	32	Dako/Agilent	1	3	19	9	13%	-
mAb clone **LK2H10**	1 2 1 40 3 1 7 1 2 1 1 19 2	Abcam Biogenex Bio SB Cell Marque Diagnostic Biosystem Hybritech Immunologic Invitrogen Millipore Monosan Progen Thermo Fisher Scientific Zytomed Systems GmbH	54	20	4	3	91%	97%
mAb clone **PHE5**	1	Unknown	0	0	0	1	-	-
mAb clones **LK2H10+PHE5**	8 1 11 1	Biocare Medical Invitrogen NeoMarkers Thermo Fisher Scientific	12	8	1	0	95%	94%
rmAb clone **SP12**	1	Thermo Fisher Scientific	0	0	0	1	-	-
pAb **A0430***	22	Dako/Agilent	5	11	3	3	73%	-
pAb **18-0094**	1	Invitrogen	0	0	1	0	-	-
pAb **RB-9003**	1	NeoMarkers	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **5H7, PA0430/PA0515**	6	Leica	0	1	1	4	-	-
mAb clone **LK2H10, 760-2519**	112	Ventana/Roche	72	25	7	8	87%	97%
mAb clone **LK2H10, 760-2519**[3]	2	Ventana/Roche	1	1	0	0	-	-
mAb clone **LK2H10, E001**	2	Linaris	0	2	0	0	-	-
mAb **LK2H10, AM126-5M**	1	Biogenex	0	1	0	0	-	-
mAb **LK2H10, 238M-90**	1	Cell Marque	1	0	0	0	-	-
mAb clone **LK2H10, MAD-000616QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clones **LK2H10, 8286-C010**	1	Sakura Finetek USA	1	0	0	0	-	-
mAb clones **LK2H10, BSB5345**	1	Bio SB	1	0	0	0	-	-
Ab clone EP3373, MAD-000564QD-7/N	1	Master Diagnostica	1	0	0	0	-	-
mAb clones **LK2H10+PHE5, PM010 AA**	1	Biocare Medical	1	0	0	0	-	-
mAb clones **LK2H10+PHE5, PM010 AA**[3]	1	Biocare Medical	1	0	0	0	-	-
clone **MX018, MAB0707**	1	Maixin	0	1	0	0	-	-
pAb **IR502***	1	Dako/Agilent	0	1	0	0	-	-
pAb **IR502**[3]	1	Dako/Agilent	0	0	1	0	-	-
Total	296		152	74	40	30	-	
Proportion			51%	25%	14%	10%	76%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system used on a different platform than it was developed for.

*discontinued products

Detailed analysis of CGA, Run 53
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **DAK-A3**: One protocol with an optimal result was based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako), 10 min. EnVision Flex was used as detection system. The dilution is unknown.

mAb clone **LK2H10**: Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (Dako) (27/32)*, Cell Conditioning 1 (CC1, Ventana) (19/24), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (5/6) or Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/6) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:1,000. Using these protocol settings, 67 of 69 (97%) laboratories produced a sufficient staining result (optimal or good).
*(number of optimal results/number of laboratories using this HIER buffer)

mAb clones **LK2H10+PHE5**: Protocols with optimal results were based on HIER using CC1 (Ventana) (7/9), TRS pH 9 (Dako) (2/4), BERS2 (Leica) (1/3) or BERS1 (Leica) 1/2 as retrieval buffer. The mAb was diluted in the range of 1:50-1:1,000. Using these protocol settings, 17 of 18 (94%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for CGA for the most commonly used antibody concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **LK2H10**	16/18** (89%)	0/4	10/13 (77%)	0/1	19/24 (79%)	0/1	5/6 (83%)	1/6
mAb clones **LK2H10+PHE5**	0/1	-	2/3	-	7/9 (78%)	-	1/3	1/2

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** Number of optimal results/number of laboratories using this buffer.

Ready-To-Use antibodies and corresponding systems

mAb clone **LK2H10**, product no. **760-2519**, Ventana, BenchMark GX/XT/Ultra:
Pprotocols using UltraView (760-500) as detection system were typically based on HIER using CC1 (efficient heating time 20-64 min.) and 8-32 min. incubation of the primary Ab.
Optimal protocols using OptiView (760-700) as detection system were typically based on HIER using CC1 (efficient heating time 16-64 min.) and 4-56 min. incubation of the primary Ab.
Using these protocol settings, 88 of 90 (98%) laboratories produced a sufficient staining result.

mAb clone **LK2H10**, product no. **8286-C010**, Sakura Finetek USA, Tissue-Tek Genie Advanced Stainer:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45 min.), 45 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit, DAB as detection system.

mAb clones **LK2H10+PHE5**, product no. **PM 010 AA**, Biocare Medical, intelliPATH:
One protocol with an optimal result was based on HIER using Reveal Decloaker (Pressure Cooker), 25 min. incubation of the primary Ab and MACH 4 HRP-Polymer as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for CGA for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS GX/XT/Ultra mAb LK2H10 **760-2519**	6/6 (100%)	4/6 (67%)	91/106 (86%)	68/106 (64%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments
In this assessment and in concordance with the previous NordiQC assessments of CGA, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 92% of the insufficient results (64 of 70). Virtually all laboratories were able to demonstrate CGA in high-level antigen expressing structures such as neoplastic cells of the medullary thyroid carcinoma and normal neuroendocrine cells in the appendix and pancreatic Langerhans islets. Demonstration of CGA in low-level expressing structures as neoplastic cells of the SCLC and peripheral nerves in the appendix was more challenging and required a carefully calibrated protocol.

The mAb clone LK2H10 was the most widely used antibody for demonstration of CGA and provided optimal results on all four main IHC platforms from Dako, Leica and Ventana, respectively (see Table 3). Used as a Conc within a laboratory developed (LD) assay, mAb clone LK2H10 gave an overall pass rate of 91% (74 of 81) of which 67% were optimal (see Table 1). The two main prerequisites for sufficient staining were use of HIER in an alkaline buffer and careful calibration of the titre of the primary Ab, whereas use of 3-step versus 2-step detection systems were of less importance. However, it was observed that use of 3-step polymer/multimer based detection systems seemed to provide higher proportions of optimal results, 37 of 47 (79%) compared to 2-step polymer/multimer based systems, 17 of 33 (52%).
The mAb clone cocktail LK2H10+PHE5 provided a pass rate of 95% (20 of 21) within a LD assay of which 57% were optimal (see Table 1).

mAb clone DAK-A3 was used by 32 participants and provided a significantly inferior performance compared to mAb clone LK2H10. Despite similar protocol settings, a disappointing pass rate of 13% (4 of 32) was seen. Insufficient results were typically characterized by a reduced staining intensity and proportion of cells demonstrated. Overall, too low sensitivity/affinity of the clone seemed to cause the inferior performance. Compared to the last run for CGA, a small decrease in use of mAb clone DAK-A3 was observed, where the clone was used by 15% (36 of 242) of the laboratories compared to 10% (32 of 296) in this run.

The RTU system from Ventana based on the mAb clone LK2H10 (760-2519) gave a high proportion of sufficient and optimal results as shown in Table 1. Optimal and sufficient results could be obtained both by using laboratory modified protocol settings and by the recommended protocol settings from Ventana.
It was observed that a significant higher proportion of optimal results were obtained by use of OptiView as detection system compared to the use of UltraView. With UltraView 49% (29 of 59) of the results were optimal, of which seven used UltraView amplification, compared to 84% (43 of 51) if OptiView was used.

In this assessment the mAb clone 5H7 (Leica/Novocastra) showed an inferior performance both as Conc and RTU format, as 90% (9 of 10) of protocols based on this clone gave insufficient results characterized by a too weak or false negative staining reaction. The protocol settings applied for the mAb clone 5H7 were typically based on HIER using a non-alkaline buffer. In this assessment, only 12% (36 of 296) used HIER in a non-alkaline buffer of which 50% (18 laboratories) obtained sufficient results.

Controls
In concordance with previous assessments for CGA, appendix is recommendable as positive and negative tissue control: An at least weak to moderate distinct granular staining must be seen in the axons and ganglion cells of the peripheral nerves. Neuroendocrine cells in the appendiceal mucosa should display a strong staining and diffusion of the staining in the vicinity of these cells has to be accepted. Enterocytes and smooth muscle cells should be negative.
In this context it must be stressed that pancreas cannot be used as positive tissue control even though recommended by some vendors. Endocrine cells in the pancreatic islets have a high level of CGA expression, which cannot reliable be used as control of sufficient sensitivity of the protocol. The low-level and limited expression of CGA in many neuroendocrine tumours and carcinomas can consequently lead to a false negative staining result in these tumours despite positive staining reaction in pancreas.

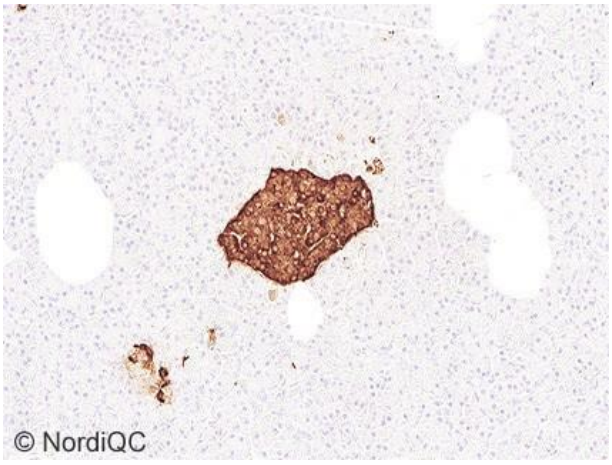


Fig. 1a
Optimal CGA staining of the pancreas using the mAb clone LK2H10 optimally calibrated, HIER at high pH and a 3-step polymer based detection system performed on Autostainer Link 48, Dako/Agilent.
The vast majority of endocrine islet cells show a strong and distinct cytoplasmic staining reaction.
Also compare with Figs. 2a - 5a – same protocol.

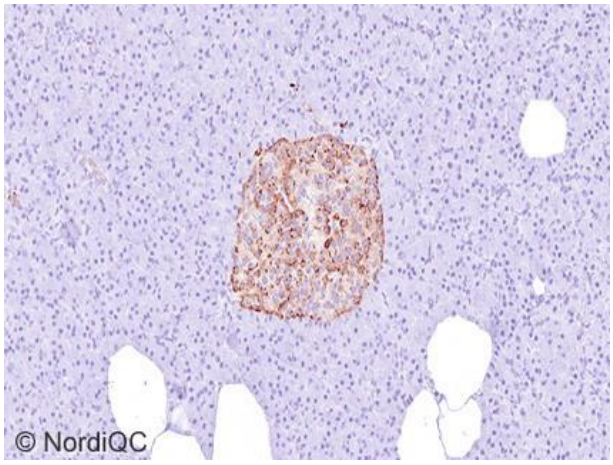


Fig. 1b
CGA staining of the pancreas using an insufficient protocol with too low sensitivity.
The protocol was based on the mAb clone DAK-A3, using similar protocol settings as Fig. 1a with HIER at high pH and performed on Autostainer Link 48, Dako/Agilent.
Also compare with Figs. 2b - 4b – same protocol.

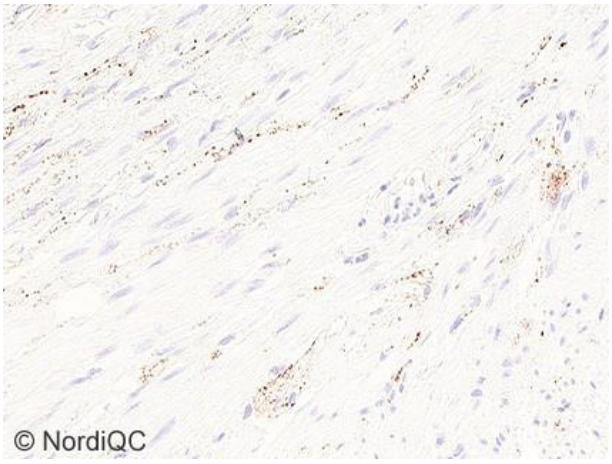


Fig. 2a
Optimal CGA staining of the appendix using same protocol as in Fig. 1a. A moderate and distinct granular cytoplasmic staining reaction of normal ganglion cells and axons in the nerve plexus is seen. No background staining is seen. Also compare with Figs. 3a - 5a – same protocol.

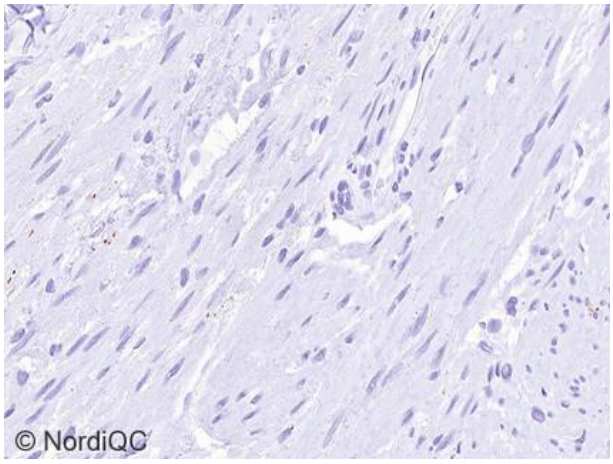
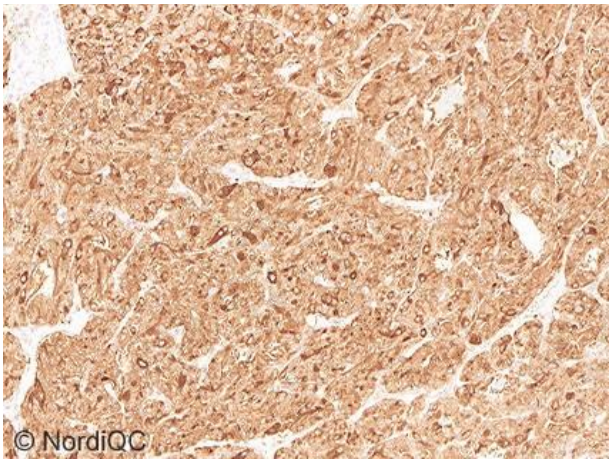


Fig. 2b
Insufficient CGA staining of the appendix using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only scattered ganglion cells and axons can be identified.
Also compare with Figs. 3b and 4b – same protocol.



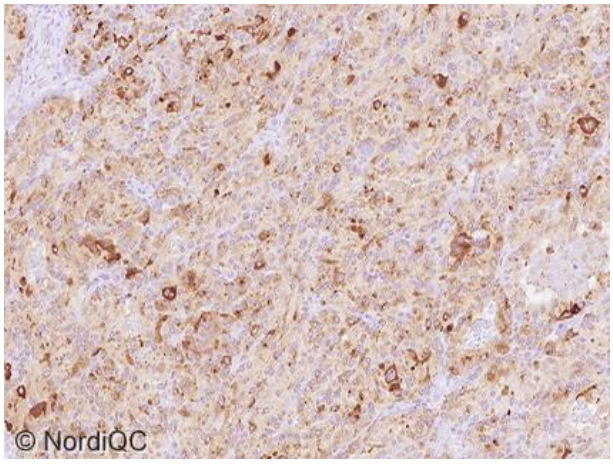


Fig. 3a
Optimal CGA staining of the thyroid medullary carcinoma using same protocol as in Figs. 1a and 2a.
Virtually all the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

Fig. 3b
Insufficient CGA staining of the thyroid medullary carcinoma using the same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.
A significant reduced staining intensity due to a clone with too low sensitivity.
Also compare with Fig. 4b – same protocol.

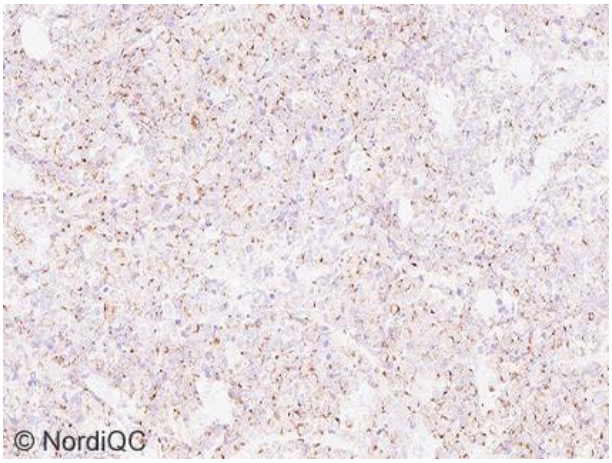


Fig. 4a
Optimal CGA staining of the SCLC using same protocol as in Figs. 1a – 3a.
Virtually all the neoplastic cells show a weak to moderate and cytoplasmic staining reaction with a dot-like accentuation. No background staining is seen.

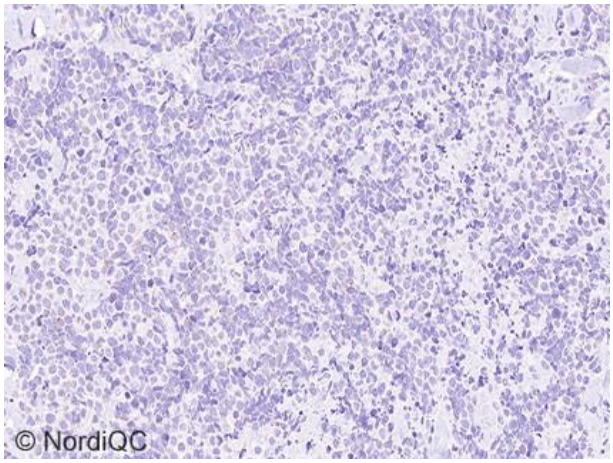


Fig. 4b
Insufficient CGA staining of the SCLC using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining reaction.

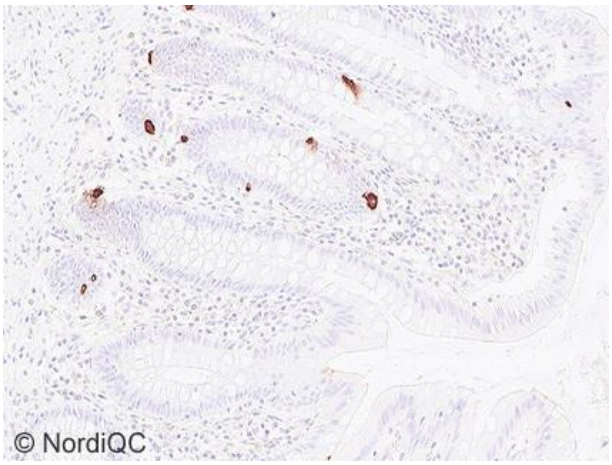


Fig. 5a
Optimal CGA staining of the appendix mucosa using same protocol as in Figs. 1a – 4a.
The neuroendocrine cells show an intense staining reaction. A weak diffusion of the signal is seen in the close vicinity of the positive cells, whereas all other epithelial cells are negative.

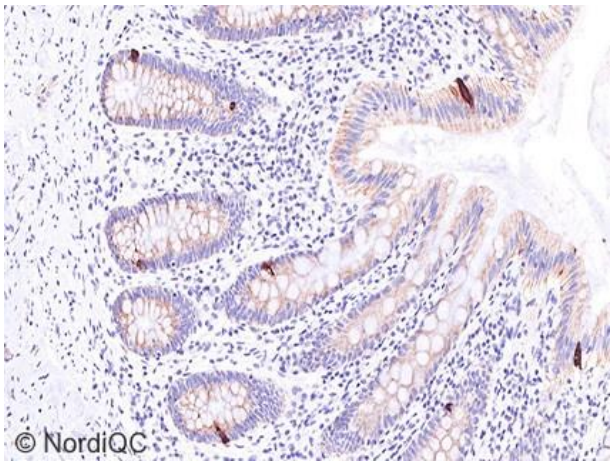


Fig. 5b
Insufficient CGA staining of the appendix mucosa using a protocol not calibrated appropriately.
The protocol was based on the mAb clone DAK-A3, using HIER at high pH, a 3-step polymer-based detection system and performed on Autostainer Link 48, Dako/Agilent.
An aberrant cytoplasmic staining of epithelial cells is observed compromising the interpretation.

HLK/LE/MV/RR 28.06.2018