NordiQC

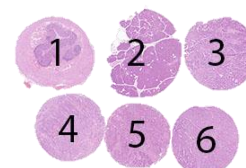
Assessment Run 70 2024

Chromogranin A (CGA)

Updated 03.05.2024

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CGA, typically used in the diagnostic work-up of neuroendocrine tumors. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for CGA (see below).

Material
The slide to be stained for CGA comprised:



1. Appendix, 2. Pancreas, 3. Colon adenocarcinoma, 4-5. Small cell lung carcinoma (SCLC) 6. Neuroendocrine tumour (Colon neuroendorine tumour (NET)).

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 to strong, distinct cytoplasmic reaction of virtually all neoplastic cells in the neuroendocrine tumour.
- An at least weak, distinct granular cytoplasmic staining reaction of the vast majority of neoplastic cells in both small cell lung carcinomas.
- No staining reaction of the appendiceal columnar epithelial cells, pancreatic exocrine cells and neoplastic cells in the colon adenocarcinoma.

KEY POINTS FOR CGA IMMONUASSAYS
- The mAb clone **LK2H10** is recommendable both as a concentrated Ab or an RTU.
- The mAb clones **LK2H10 + PH05** seems promising, but data is limited.
- The mAb clones **DAK-A3** and **5H7** cannot be recommended due to inferior performances.
- Efficient HIER should be performed in an alkaline buffer.
- Use a sensitive 3-step detection system.

Participation

Number of laboratories registered for CGA, run 70	415
Number of laboratories returning slides	370 (89%)

All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

Results
370 laboratories participated in this assessment. One laboratory used an inappropriate antibody. This was not included in the analysis. Of the remaining 369 laboratories 276 (75%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3 and 4).

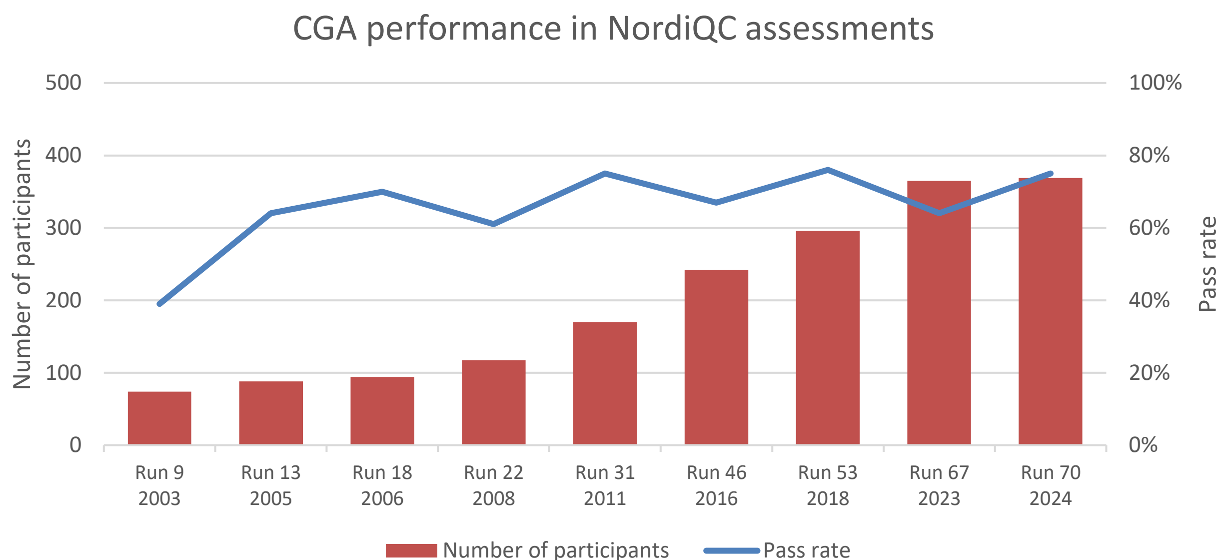
The most frequent causes of insufficient staining reactions were:
- Less successful primary Ab
- Use of a 2-step detection system
- Insufficient HIER

Accredited by DANAK under registration number 616 to proficiency testing

Performance history

This was the nineth NordiQC assessment of CGA. The pass rate increased to 75% in this assessment compared to the previous run 67 and similar to the level seen in run 53 as shown in Graph 1.

Graph 1. **Proportion of sufficient results for CGA in the nine NordiQC runs performed.**



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 reaction and diffusion of the staining in the vicinity of these cells has to be accepted. Columnar epithelial cells 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 analytical 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.

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 if HIER was performed in an alkaline buffer with a 3-step detection system. The corresponding and widely used Ready-To-Use system (45% of all participants) from Ventana/Roche based on mAb clone LK2H10 gave an overall pass rate of 90% and 100% when applied within vendor recommended protocol settings. The mAb clones DAK-A3 and 5H7 gave significantly inferior performances, despite similar protocol settings being applied as for mAb clone LK2H10. The two clones were in total used by 14% of the participants providing an unacceptable pass rate of 8%, no optimal results.

Table 1a. **Overall results for CGA, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	144	41	57	33	13	68%	28%
Ready-To-Use antibodies	225	108	71	34	12	80%	48%
Total	369	149	128	67	25		
Proportion		40%	35%	18%	7%	75%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CGA, run 70**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **5H7**	3	Leica Biosystems	0	0	0	3	-	-
mAb clone **DAK-A3**	34	Dako/Agilent	0	7	21	6	21%	0%
mAb clone **LK2H10**	1 2 1 59 4 2 2 2 1 14 1	Abcam Biogenex Bio SB Cell Marque Diagnostic Biosystems Immunologic Millipore Monosan Progen Biotechnik GmbH Thermo Fisher Scientific Zytomed Systems GmbH	39	39	9	2	88%	44%
mAb clones **LK2H10+PHE5**	6 2 1	Biocare Medical NeoMarkers Thermo Fisher Scientific	2	7	0	0	100%	22%
mAb clone **IHC544**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **EP38**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **SP12**	1	Thermo Fisher Scientific	0	1	0	0	-	-
rmAb clone **BP6129**	1	Biolynx Biotechnology	0	0	1	0	-	-
rmAb clone **QR096**	1	Quartett	0	1	0	0	-	-
Ab clone **CGA-ATHA**	1	Gennova	0	0	0	1	-	-
pAb **A0430***	2	Dako/Agilent	0	0	1	1	-	-
Unknown	1	Unknown	0	1	0	0	-	-
Total	144		41	57	33	13		
Proportion			28%	40%	23%	9%	68%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
*discontinued products

Table 1c. **Ready-To-Use antibodies and assessment marks for CGA, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **5H7 PA0430/PA0515 (VRPS)[3]**	9	Leica Biosystems	0	0	9	0	0%	0%
mAb clone **5H7 PA0430/PA0515 (LMPS)[4]**	6	Leica Biosystems	0	1	4	1	17%	0%
mAb clone **LK2H10 760-2519 (VRPS)[3]**	21	Ventana/Roche	10	11	0	0	100%	48%
mAb clone **LK2H10 760-2519 (LMPS)[4]**	146	Ventana/Roche	88	41	12	5	88%	60%
mAb clone **LK2H10 BMS018**	4	Zytomed Systems GmbH	2	2	0	0	-	-
mAb **LK2H10 AM126**	1	Biogenex	0	1	0	0	-	-
mAb **LK2H10 238M-90/98**	16	Cell Marque	6	6	3	1	75%	38%
mAb clone **LK2H10 MAD-000616QD**	2	Master Diagnostica/Vitro SA	0	0	0	2	-	-
mAb clone **LK2H10 8286-C010**	3	Sakura Finetek	0	2	1	0	-	-
mAb clone **LK2H10 E001**	1	Linaris	0	1	0	0	-	-
mAb clone **LK2H10 PDM067**	2	Diagnostic Biosystems	0	0	1	1	-	-
mAb clones **LK2H10+PHE5 PM010 AA**	6	Biocare Medical	2	4	0	0	100%	33%
mAb clone **317F1D8 PA069**	1	Abcarta	0	1	0	0	-	-
mAb clone **MX018, MAB-0707**	2	Fuzhou Maixin	0	0	2	0	-	-
mAb clone **IHC544 IHC544-7**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **EP3373 MAD-000565QD-7N**	1	Master Diagnostica/Vitro SA	0	0	0	1	-	-
rmAb clone **EP38 RMPD066**	1	Diagnostic Biosystems	0	0	0	1	-	-
pAb **IR502***	1	Dako/Agilent	0	0	1	0	-	-
pAb **412751**	1	Nichirei Bioscience	0	1	0	0	-	-
Total	225		108	71	34	12		
Proportion			48%	32%	15%	5%	80%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Product used on another platform than developed for
*discontinued products

Detailed analysis of CGA, Run 70

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **LK2H10**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS, Dako/Agilent) High pH (17/47)*, TRS Low pH (2/4) (Dako/Agilent), Cell Conditioning 1 (CC1, Ventana/Roche) (13/21), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (5/8) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (2/7) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:1,000. Using these protocol settings, 78 of 88 (89%) 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/Roche) (1/4) or BERS1 (Leica Biosystems) (1/2) as retrieval buffer. The mAb was diluted in the

range of 1:100-1:800. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CGA for the most commonly used antibody concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**[1]		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark**[2]		**Leica Biosystems Bond**[3]	
	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**	3/11 (27%)	1/2	14/36 (39%)	0/1	13/21 (62%)	-	5/8 (63%)	2/7 (29%)
mAb clones **LK2H10+PHE5**	-	-	-	-	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.
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra plus
3) Bond III

Ready-To-Use antibodies and corresponding systems
mAb clone **LK2H10**, product no. **760-2519**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Optimal protocols using UltraView (760-500) as detection system were typically based on HIER using CC1 (efficient heating time 36-64 min.) and 12-36 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 4-40 min. incubation of the primary Ab.
Using these protocol settings, 147 of 155 (95%) laboratories produced a sufficient staining result.

Table 3 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 3. **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 BenchMark mAb LK2H10 **760-2519** **UltraView**	16/16 (100%)	10/16 (63%)	51/59 (86%)	22/59 (37%)
VMS BenchMark mAb LK2H10 **760-2519** **OptiView**	5/5 (100%)	4/5 (80%)	76/84 (90%)	66/84 (79%)
Leica BOND mAb 5H7 **PA0515**	0/9 (0%)	0/9 (0%)	1/6 (17%)	0/6 (0%)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

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 87% of the insufficient results (81 of 93). Virtually all laboratories were able to demonstrate CGA in high-level antigen expressing structures such as neoplastic cells of the neuroendocrine tumour and normal neuroendocrine cells in the appendix and pancreatic Langerhans islets. Demonstration of CGA in low-level expressing structures as neoplastic cells of the SCLCs (especially tissue core no. 4) and peripheral nerves in the appendix was more challenging and required a carefully calibrated protocol. The remaining insufficient results were caused by either false positive staining reaction (2%) or poor signal-to-noise ratio (11%).

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/Agilent, Leica Biosystems and Ventana/Roche, respectively (see Table 2). Used as a conc. within a laboratory developed (LD) assay, mAb clone LK2H10

gave a significantly increased pass rate of 88%, 44% optimal compared to the latest assessment run 67, with a pass rate of 68%, 30% optimal and almost back to the level seen in run 53, with a pass rate of 91%, 67% optimal. The main prerequisites for sufficient staining were use of HIER in an alkaline buffer, careful calibration of the titre of the primary Ab and a 3-step detection system. 74% (66 of 89) of the laboratories used a 3-step detection system, giving a pass rate of 97% (64 of 66), 55% optimal (n=36). If using a 2-step detection system, a significantly lower pass rate of 61% (9 of 34) was obtained, 13% optimal (n=3).

mAb clone **DAK-A3** was used by 34 participants and provided a significantly inferior performance compared to mAb clone LK2H10. Despite similar protocol settings, a disappointing pass rate of 21% (7 of 34) was seen. The majority of insufficient results were characterized by a reduced staining intensity and proportion of cells demonstrated. Overall, too low analytical sensitivity/affinity of this clone seemed to cause the inferior performance. The observation and results were concordant to the data seen in runs 46, 53 and 67. Laboratories using this clone should consider change to another Ab as mAb clone LK2H10 and recalibrate and validate the IHC assay.

The mAb clone cocktail **LK2H10+PHE5** as a conc. and RTU provided a pass rate of 100% (15 of 15) within a LD assay of which 26% were optimal (see Tables 1b and 1c). The four optimal protocols were based on a 3-step detection system.

The RTU system from Ventana/Roche based on the mAb clone **LK2H10** (**760-2519**) gave a high proportion of sufficient and optimal results as shown in Table 1c. Optimal and sufficient results could be obtained both by using laboratory modified protocol settings and by the recommended protocol settings from Ventana (see Table 3). The vast majority of laboratories modified the protocol. The most common modifications were prolonged HIER and/or adjustment of the incubation time of primary Ab.
It was observed that a significant higher proportion of optimal results were obtained by use of a 3-step detection system. With UltraView 33% (21 of 67) of the results were optimal compared to 64% (7 of 11) if applying UltraView amplification or 79% (70 of 89) if OptiView was used.

In this assessment the mAb clone **5H7** (Leica Biosystems) showed an inferior performance both as conc. and RTU format, as only a 6% (1 of 18) pass rate was obtained. Insufficient results were 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. Three laboratories used HIER in an alkaline buffer, one with a sufficient staining result.
The observation and results for mAb clone 5H7 were concordant to the data seen in runs 46, 53 and 67. Laboratories using this clone should consider change to another Ab as mAb clone LK2H10 and recalibrate and validate the IHC assay.

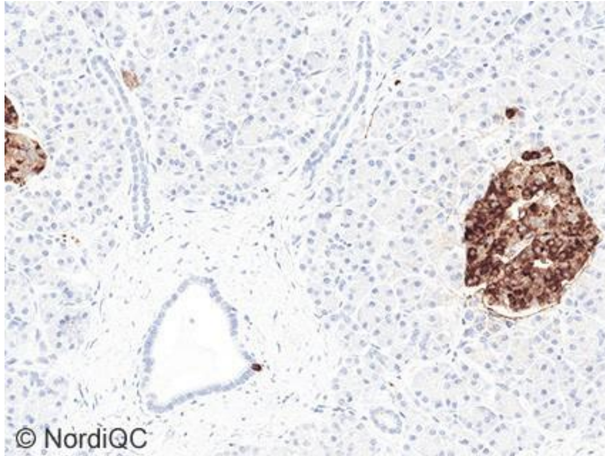


Fig. 1a
Optimal CGA staining of the pancreas using the mAb clone LK2H10 as RTU format (Ventana/Roche, 760-2519) using a modified protocol with HIER at high pH for 48 min., 24 min. incubation of the primary Ab and OptiView as detection system performed on BenchMark Ultra.
The vast majority of endocrine islet cells show a strong and distinct cytoplasmic staining reaction.
Also compare with Figs. 2a - 6a – same protocol.

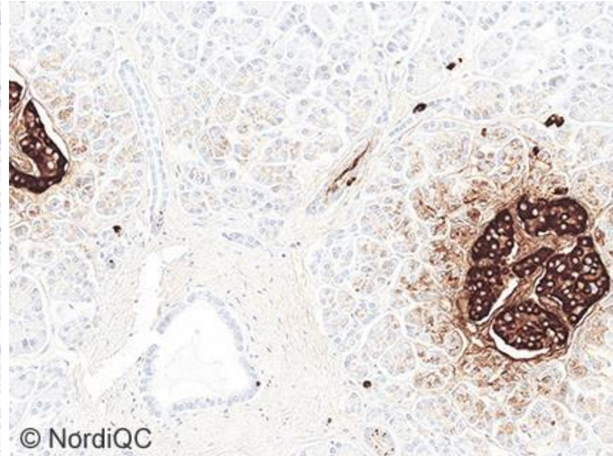


Fig. 1b
CGA staining of the pancreas using an insufficient protocol with overall too low analytical sensitivity.
The protocol was based on the mAb clone LK2H10 as RTU format (Ventana/Roche, 760-2519) using a modified protocol with insufficient HIER at high pH for only 8 min., 32 min. incubation of the primary Ab and OptiView as detection system performed on BenchMark Ultra.
Also compare with Figs. 2b - 4b – same protocol.

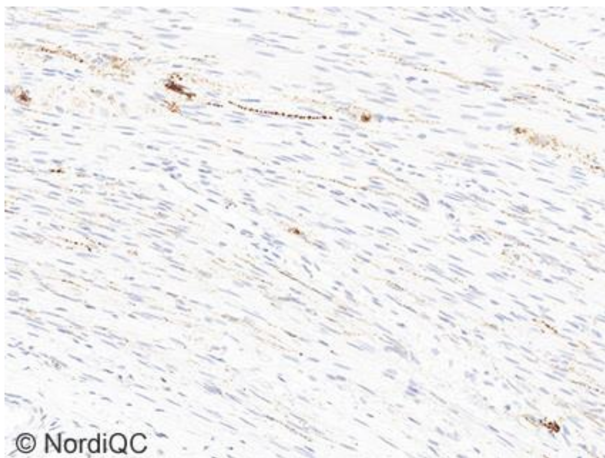


Fig. 2a
Optimal CGA staining of the appendix using same protocol as in Fig. 1a. A moderate and distinct granular cytoplasmic staining reaction of normal ganglion cells and axons in the nerve plexus is seen. No background staining is seen. Also compare with Figs. 3a - 5a – same protocol.

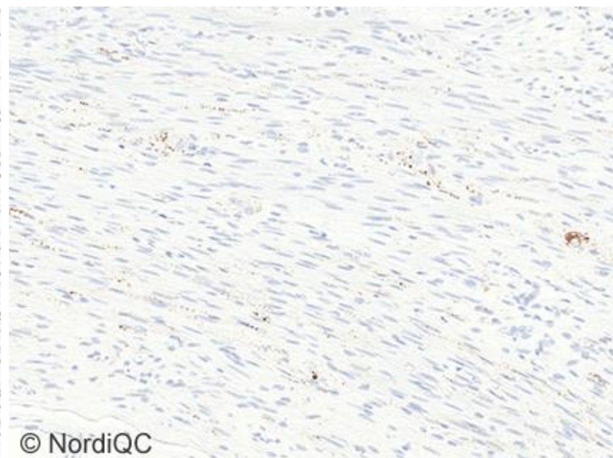


Fig. 2b
CGA staining of the appendix using same protocol as in Fig. 1b – same field as in Fig. 2a.
Ganglion cells and axons shows a weaker staining reaction than expected.
Also compare with Figs. 3b and 4b – same protocol.

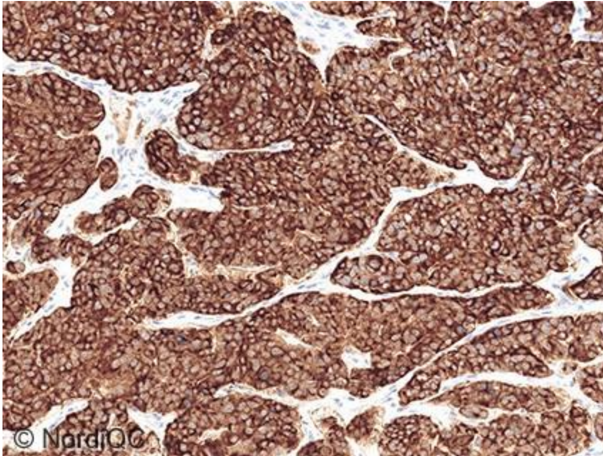


Fig. 3a
Optimal CGA staining of the neuroendocrine tumor using same protocol as in Figs. 1a and 2a.
Virtually all the neoplastic cells show a strong and distinct staining reaction.

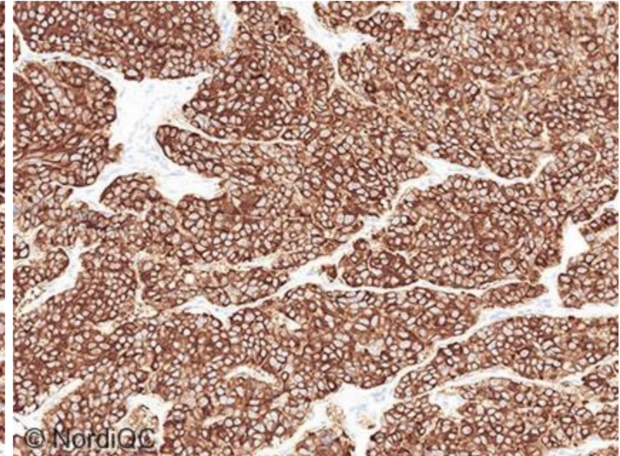


Fig. 3b
CGA staining of the neuroendocrine tumor using the same insufficient protocol as in Figs. 1b and 2b – same field as in Fig. 3a.
Also compare with Fig. 4b – same protocol.

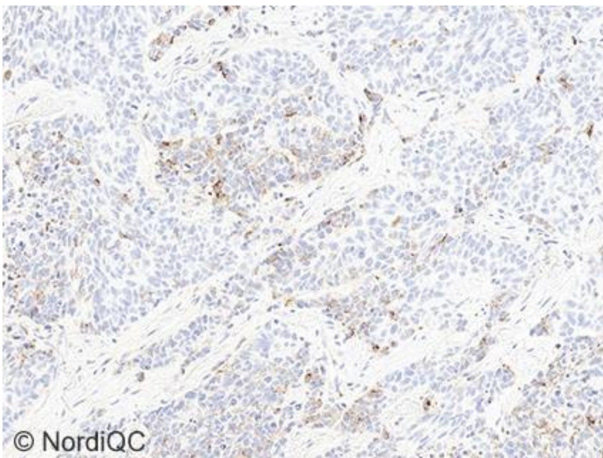


Fig. 4a
Optimal CGA staining of the SCLC, tissue core no. 4, using same protocol as in Figs. 1a – 3a.
The neoplastic cells show a weak to moderate dot-like accentuation. No background staining is seen.

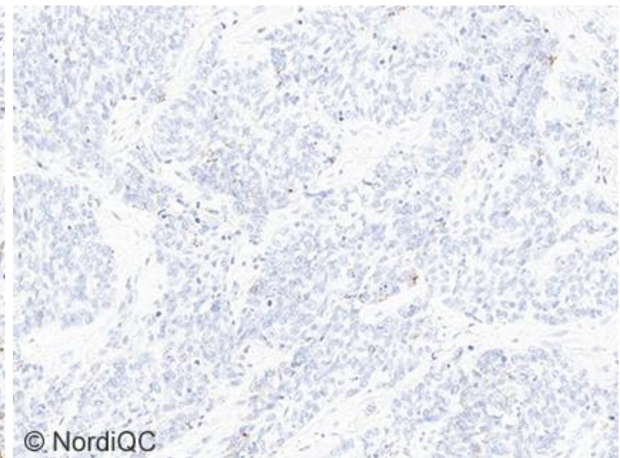


Fig. 4b
Insufficient CGA staining of the SCLC, tissue core no. 4, using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
Virtually all neoplastic cells are false negative.

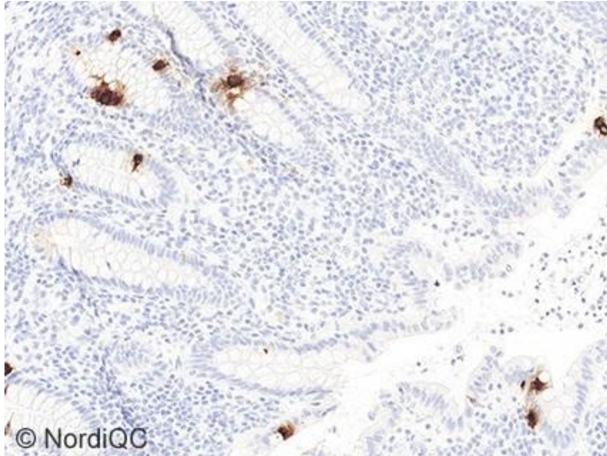


Fig. 5a
Optimal CGA staining of the appendix mucosa using same protocol as in Figs. 1a – 4a.
The neuroendocrine cells show an intense staining reaction. A weak diffusion of the signal is seen in the close vicinity of the positive cells, whereas all other epithelial cells are negative.

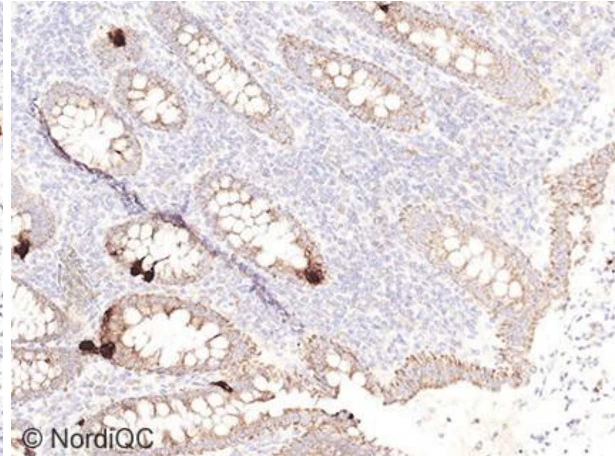


Fig. 5b
Insufficient CGA staining of the appendix mucosa using a protocol not calibrated appropriately.
The protocol was based on the mAb clone DAK-A3, using HIER at high pH, a 3-step polymer-based detection system and performed on Autostainer Link 48 (Dako/Agilent).
An aberrant cytoplasmic staining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

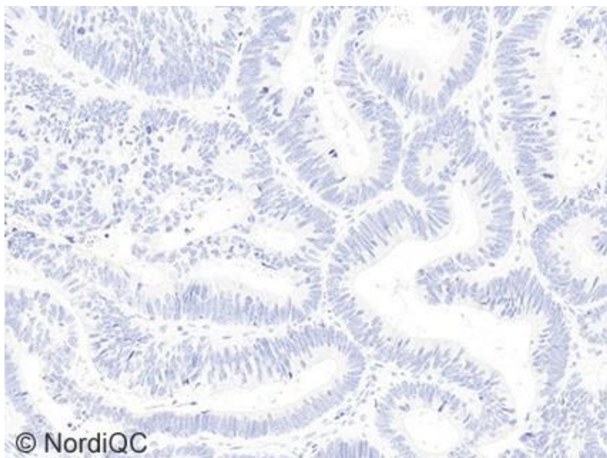


Fig. 6a
Optimal CGA staining of the colon adenocarcinoma using same protocol as in Figs. 1a – 5a.
No staining reaction is seen (however, scattered neuroendocrine cells can be observed).

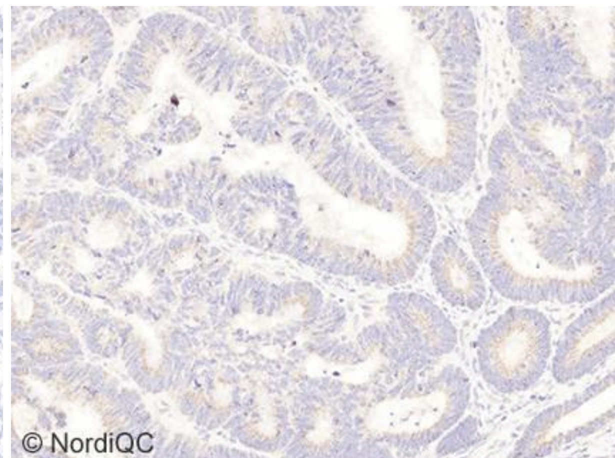


Fig. 6b
Insufficient CGA staining of the colon adenocarcinoma using same protocol as in Fig. 5b – same field as in Fig. 6a.
Virtually all neoplastic cells are false positive.

HLK/LE/SN 18.04.2024

Version	Description of change and reason	Date	Authorized by
2	Table 1c has been updated as an incorrect RTU product (8269-C010) from Sakura Finetek was submitted by one participant in version 1.	03.05.2024	HLK/SN