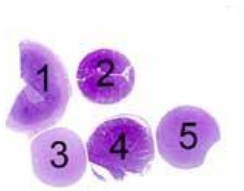
Assessment Run 9 2003

Chromogranin A (CGA)

The slide to be stained for Chromogranin A (CGA) comprised:
1: Appendix, 2: Pancreas, 3: Brain, 4: Lung small cell carcinoma, 5: Lung neuroendocrine carcinoma.

Criteria for assessing a CGA staining as optimal included: A moderate to strong distinct cytoplasmatic staining of appendix neuroendocrine cells, ganglion cells and neurons, the Langerhans' islets of pancreas, ganglion cells of cerebral cortex, and the majority of tumour cells in the small cell carcinoma and the neuroendocrine carcinoma. A slight background reaction in the pancreas or appendix epithelium was accepted (as this was interpreted as diffusion of either the antigen or the chromogenic solution).



74 laboratories submitted stainings. At the assessment 18 achieved optimal staining (24 %), 11 good (15 %), 18 borderline (24 %) and 27 (37 %) poor staining.

The following Abs were used:
mAb **DAK-A3** (DakoCytomation, n=18)
mAb **LK2H10** (NeoMarkers, n=4, Ventana, n=4, Novocastra, n=3, Boehringer Mannheim, n=3, BioGenex, n=1, Chemicon, n=1, Immunotech, n=1, or Zhongshan, n=1)
mAb **LK2H10+PHE5** (NeoMarkers, n=2)
pAb **A0430** (DakoCytomation, n=34)
pAb **18-0094** (Zymed, n=2)

Optimal stainings in this assessment were obtained with mAb LK2H10 (Fig. 1a and 2a), mAb LK2H10+PHE5, and pAb A0430 (giving the same staining pattern as illustrated in Fig 1a and 2a).

All laboratories achieving an optimal staining used HIER, most with Tris-EDTA/EGTA pH 9 (optimal in 15/18) or Citrate pH 6 (optimal in 3/15) as the heating buffer. Ten laboratories used no pre-treatment. Stainings from these were all assessed as insufficient. No laboratory used proteolytic pre-treatment.

The large majority of laboratories were able to demonstrate CGA in the neuroendocrine cells in the appendix and the pancreas, whereas the demonstration of CGA in the ganglion cells, neurons, and the neuroendocrine carcinoma and (in particular) small cell carcinoma was achieved only with a sensitive protocol using HIER as pre-treatment.

The most frequent causes of insufficient stainings (often in combination) were:
- Insufficient HIER (too short efficient heating time or no HIER)
- Too low concentration of the primary antibody
- Inappropriate choice of primary Ab.

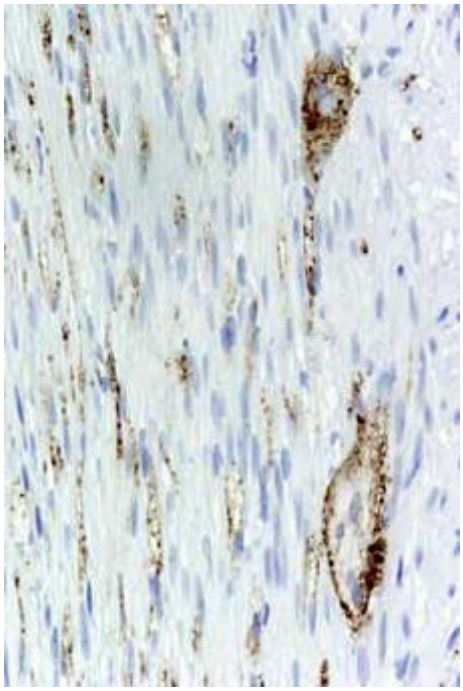


Fig. 1a
Optimal staining of the neural network of muscularis externa in a normal appendix for CGA using mAb LK2H10. Strong granular staining of ganglion cells and nerve fibres between the unstained smooth muscle cells.

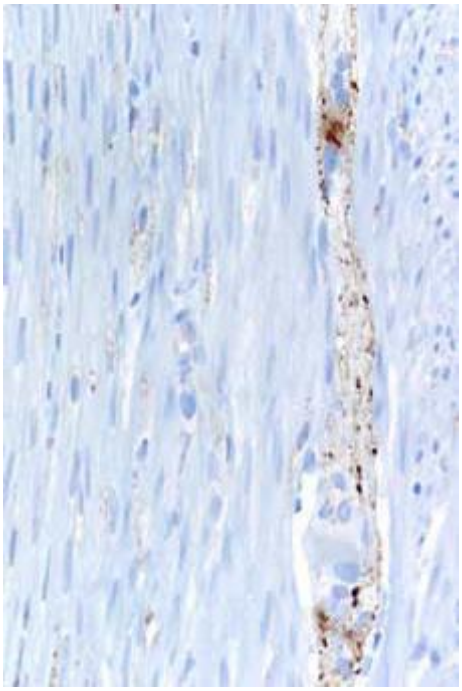


Fig. 1b
Good staining for CGA (same field and mAb as in Fig. 1a). Moderate, distinct granular staining of ganglion cells and weak but acceptable staining of nerve fibres between the unstained smooth muscle cells.

Fig. 1c
Insufficient staining for CGA (same field and mAb as in Fig. 1a) Weak staining of ganglion cells and no staining of nerve fibres.

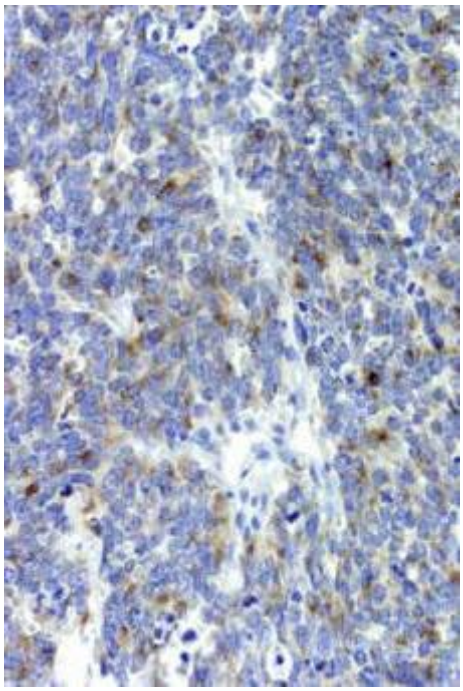


Fig. 2a
Optimal staining of a small cell lung carcinoma for CGA using mAb LK2H10. Moderate to strong dot-like staining of most neoplastic cells.

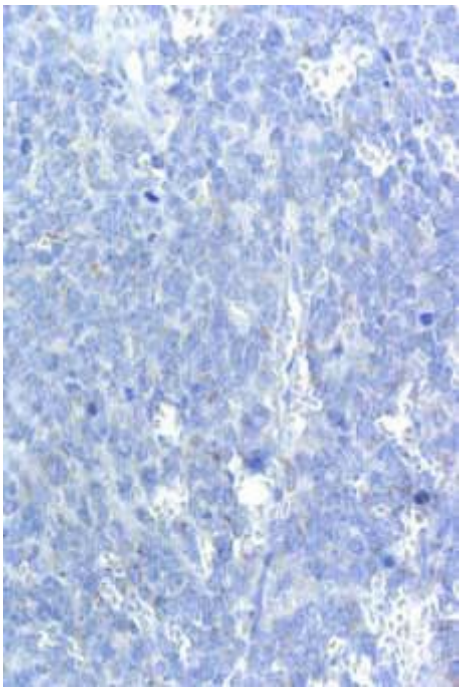


Fig. 2b
Good staining of a small cell lung carcinoma for CGA using mAb LK2H10. Weak but acceptable dot-like staining of many neoplastic cells.

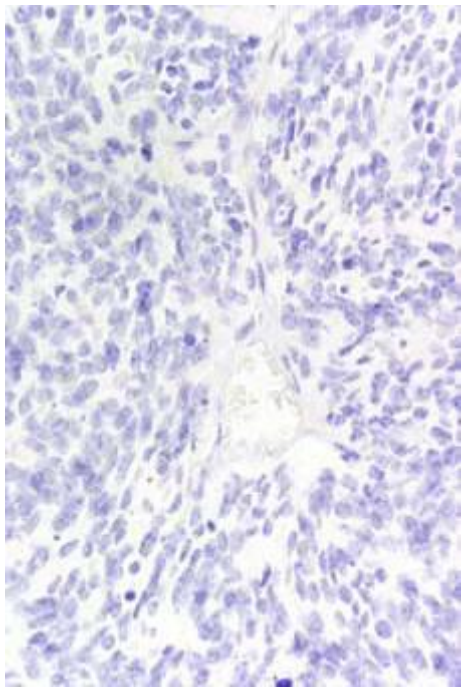


Fig. 2c
Insufficient staining of a small cell lung carcinoma for CGA using mAb LK2H10. No staining of the neoplastic cells.

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