Assessment Run 6 2002

Calretinin (CR)

The slides to be stained for Calretinin (CR) contained an ovary (specimen 2; CR in surface epithelial cells and some stromal cells), appendix (specimen 3; CR in ganglia and axons), kidney (specimen 4; CR detected in proximal tubules only when using some pAbs) and adrenal cortex (specimen 6; CR in cortical cells), a testis/epididymis with malignant mesothelioma (specimen 1; CR in tumour cells), and lung adenocarcinoma (specimen 5; CR negative).

47 laboratories submitted stainings. The most used antibodies was mAb clone DAK Calret 1 (DakoCytomation; n=12), mAb clone 5A5 (Novocastra; n=8), and pAbs (SWant; n=11, and Zymed; n=11). Five laboratories used other mAbs and pAbs.

At the assessment, 9 were deemed optimal, 24 acceptable, 9 borderline and 5 poor.
The basis for an optimal result was an intense and distinct staining of both nuclei and cytoplasm of cells expected to stain.

An optimal staining could be obtained with all the four Abs mentioned. A marked difference in this study was staining of renal tubules with both pAbs, a staining which was not seen with med mAbs. The significance of this difference is uncertain.
The most suitable pre-treatment for all four markers was HIER in an alkaline buffer (Tris-EDTA or -EGTA).

The most frequent parameters giving suboptimal reactions were:
- insufficient HIER (citrate buffer pH 6),
- proteolytic pre-treatment, and
- inappropriate dilution of Ab according to the sensitivity of protocol causing either too weak staining or overstaining.

Too weak staining was most evident in peripheral nerves of the appendix, which is more suitable for control tissue than normal mesothelium. In the appendix, the enterocytes should not stain and may serve for negative control.

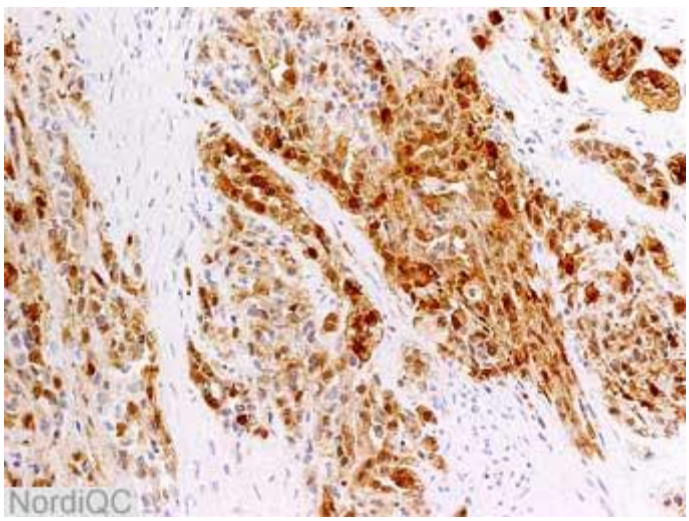

Fig.1a
Optimal staining using mAb DAK Calret 1. Malignant mesothelioma.Most tumor cells are strongly stained in cytoplasm and nuceli.

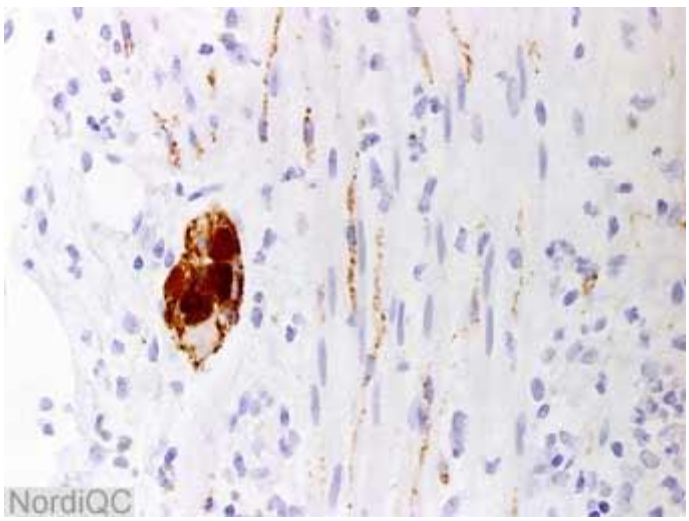

Fig.1b
Optimal staining using the same Ab and protocol as in Fig. 1a. Appendix with normal ganglia and axons showing a distinct staining.

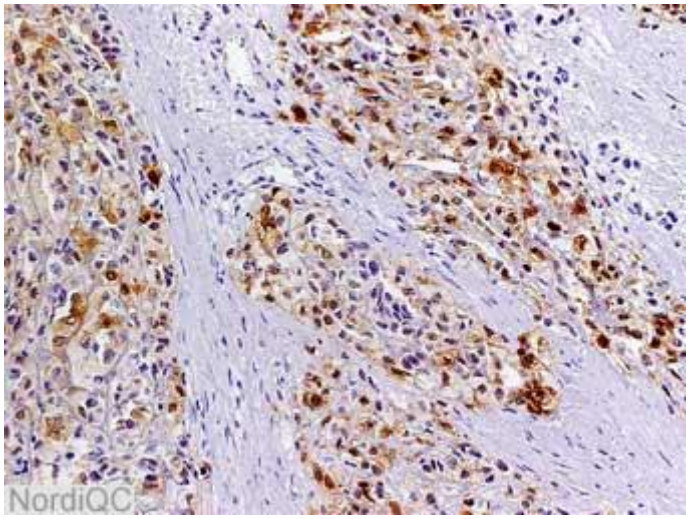

Fig. 2a
Acceptable staining. Same mAb and same field as in Fig. 1a. Some cells are weakly stained (due to a lower concentration of Ab).

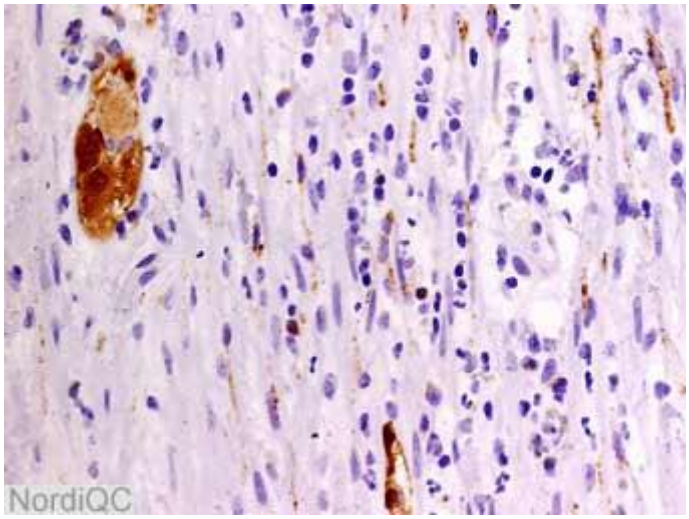

Fig. 2b
Acceptable staining using the same mAb and protocol as in Fig. 2a. Appendix with normal ganglia and axons showing staining a little less distinct than in Fig. 1b.

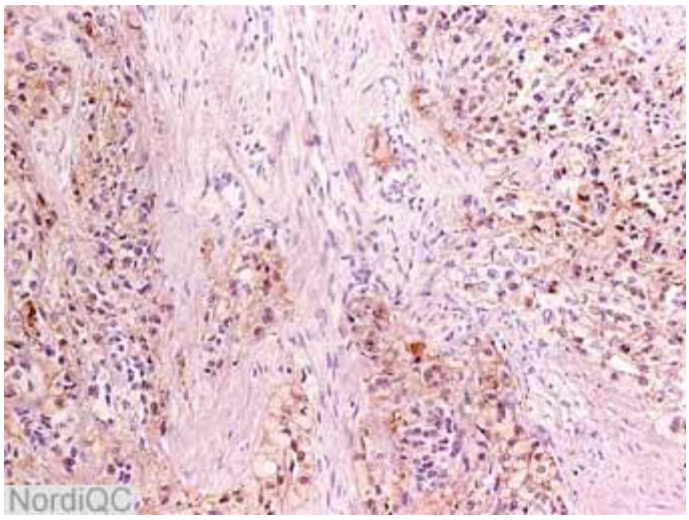

Fig. 3a
Insuffcient staining using a pAb to CR. Same field as in Fig. 1a and 2a. Most cells are weakly stained.

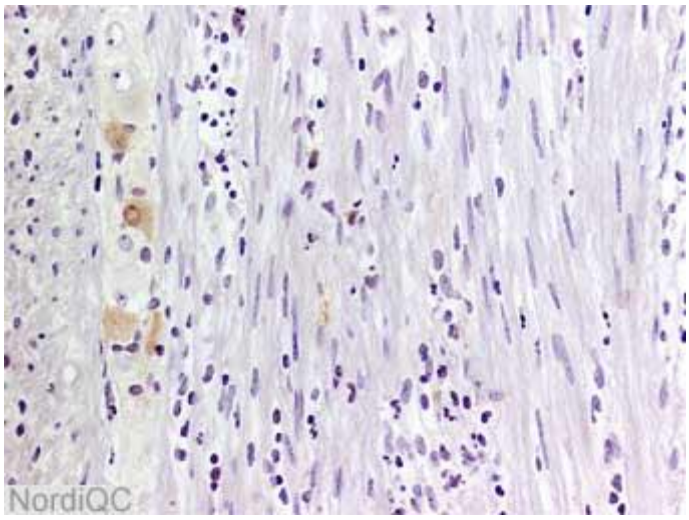

Fig. 3b
Insufficient staining using the same Ab and protocol as in Fig 3a. Same field as in Fig. 1b and 2b. The ganglia are weakly stained and the nerves almost unstained.

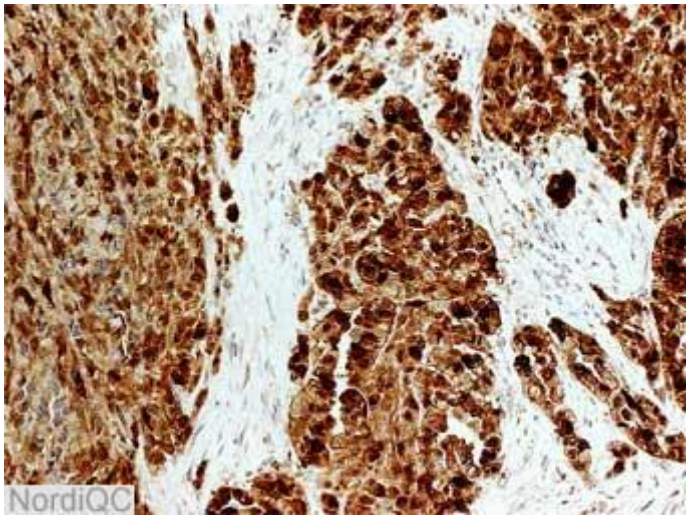

Fig. 4a
Insuffcient staing using a pAb to CR. Same field as in Fig. 1a, 2a and 3a. The tumour cells are strongly stained but some stromal cells are also stained (compare Fig. 4b).

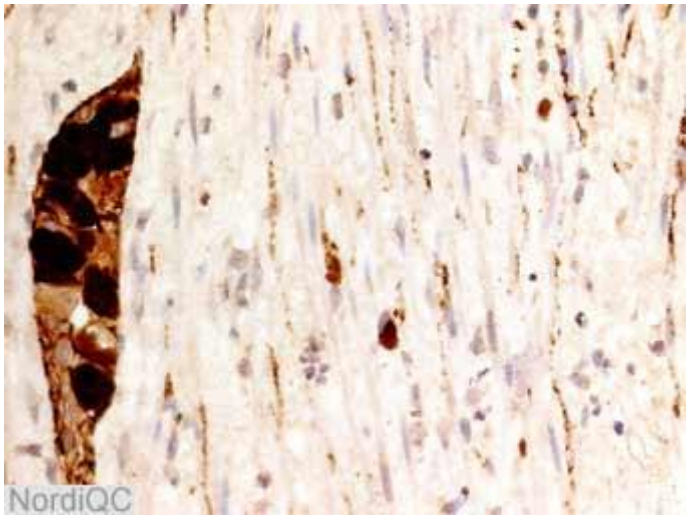

Fig. 4b
Insufficient staining using the same Ab and protocol as in Fig 4a. Same field as in Fig. 1b, 2b and 3b. The ganglia and axons are strongly stained but also smooth muscle cells stain.

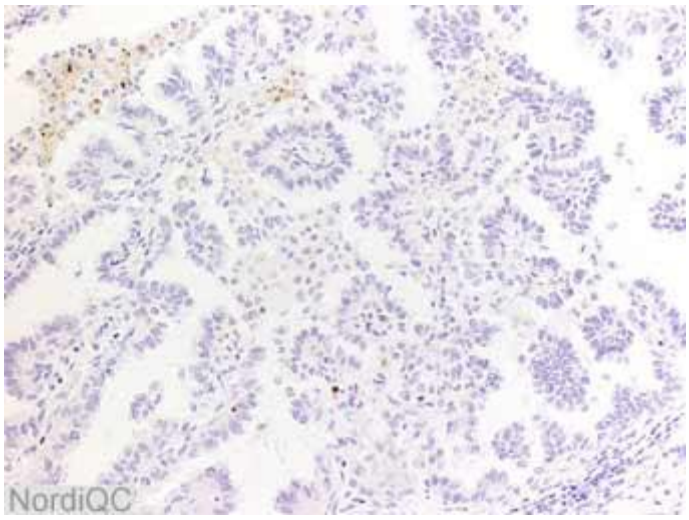

Fig. 5a
Staining from an optimal protocol using mAb 5A5. Lung adenocarcinoma.The tumour cells are unstained. Some histiocytes (upper left corner) are weakly stained.

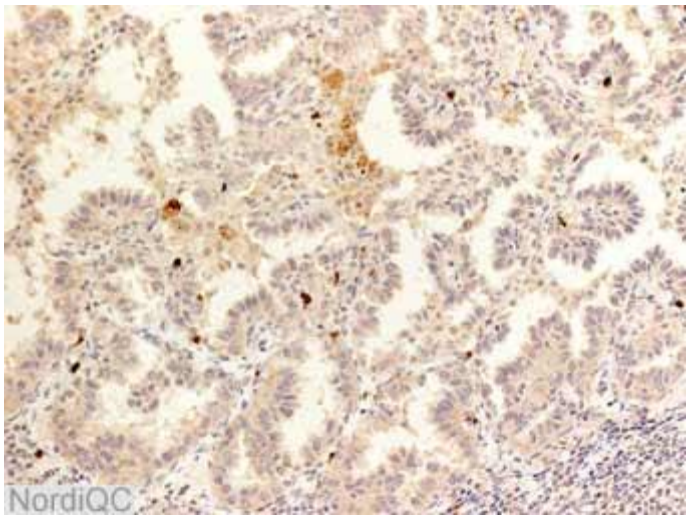

Fig. 5b
Insufficient staining using the same Ab and protocol as in Figs. 4a and 4b. Same field as in Fig. 5a. The tumor cells are false positive. Note that the staining is mostly cytoplasmatic with little nuclear staining.

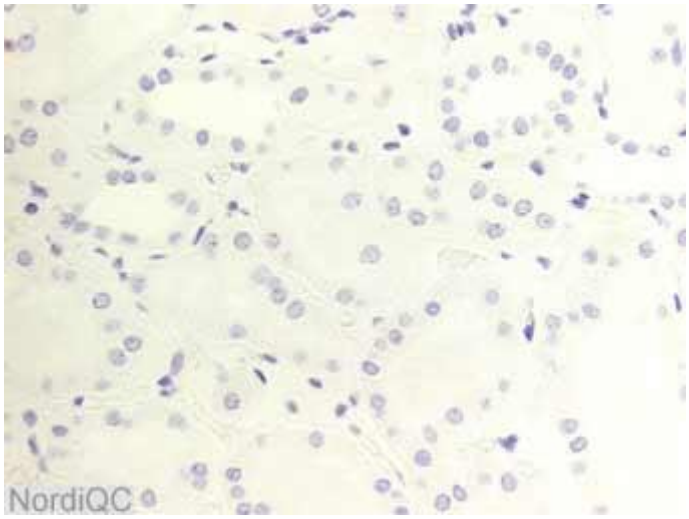

Fig. 6a
Staining from the same optimal protocol as in Fig. 5a. Normal kidney. No staining is seen.

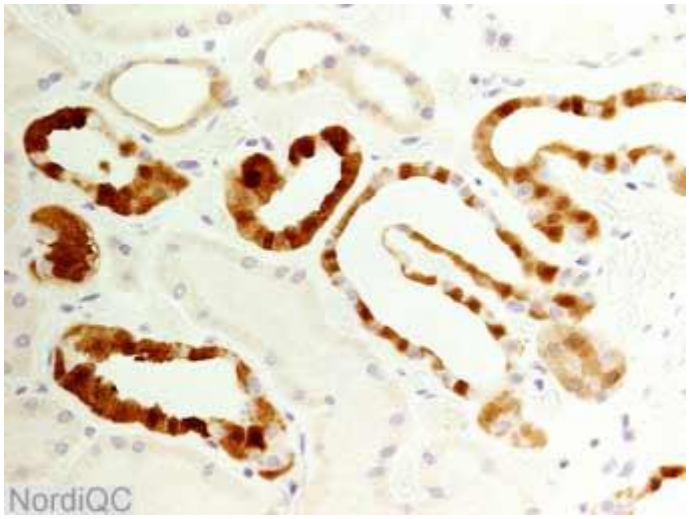

Fig. 6b
Staining from an optimal protocol using a pAb. Normal kidney. A strong staining of particularly the proximal tubules is seen.

SN/MV/LE 19-6-2004