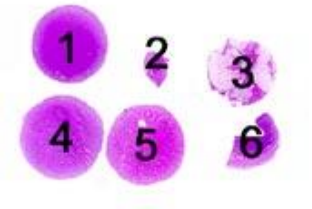
Assessment Run 17 2006

Calretinin (CR)

The slide to be stained for Calretinin (CR) comprised:
1. Appendix, 2. Kidney, 3. Lung adenocarcinoma, 4. Epithelioid malignant mesothelioma, 5. Biphasic malignant mesothelioma, 6. Granulosa cell tumour. All specimens were fixed in 10 % NBF.



Criteria for assessing a CR staining as optimal included:

- A strong, distinct cytoplasmic and nuclear staining of the peripheral nerves (both axon and ganglion) and macrophages in the appendix.
- A strong, distinct cytoplasmic and nuclear staining of both the normal mesothelial cells and the majority of the cells in the two mesotheliomas.
- A moderate, distinct cytoplasmic and nuclear staining of the granulosa cell tumour.
- A negative staining of the kidney and negative or only focal staining of the lung adenocarcinoma.

82 laboratories submitted stains. At the assessment 17 achieved optimal marks (21 %), 29 good (35 %), 26 borderline (32 %) and 10 poor marks (12 %).

The following Abs were used:
mAb clone **2E7** (Immunologic, n=2)
mAb clone **5A5** (Novocastra, n=20; Immunomarkers n=1)
mAb clone **DAKCalret1** (Dako, n=32)
pAb **18-0211** (Zymed, n=17)
pAb **7699/4** (Swant, n=4)
pAb **760-2700** (Ventana, n=4)
pAb **E070** (Linaris, n=1)
pAb **ILM 7696** (Immunologic, n=1)

Optimal staining for CR in this assessment was obtained with the mAbs clone **5A5** (4 out of 20) and clone **DAKCalret1** (6 out of 32), and the pAb **18-0211** (7 out of 17).

Using the mAb clone **5A5** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 or EDTA pH 8. The mAb was diluted in the range of 1:10 – 1:100 depending on the total sensitivity of the protocol employed. Using these settings 9 out of 13 (69 %) laboratories produced a sufficient staining (optimal or good), 4 were assessed as optimal (31%).

Using the mAb clone **DAKCalret1** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 as the heating buffer. The mAb was diluted in the range of 1:100 – 1:400 depending on the total sensitivity of the protocol employed. Using these settings 20 out of 27 (74 %) laboratories produced a sufficient staining, 6 were assessed as optimal (22%).

Using the pAb **18-0211** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 or citrate pH 6 as the heating buffer. The pAb was diluted in the range of 1:50 – 1:1,500 depending on the total sensitivity of the protocol employed. Using these settings 12 out of 15 (80 %) laboratories produced a sufficient staining, 7 were assessed as optimal (47%).

The most frequent causes of insufficient staining were:
- Less successful primary Abs
- Too low concentration of the primary Ab
- Too high concentration of the primary Ab
- False positive reaction due to endogenous biotin

In the assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the granulosa cell tumour. The theca cell component in the tumour was almost always positive, but only in the correctly calibrated protocols the neoplastic granulosa cells were demonstrated. The two mesotheliomas showed

different levels of CR expression, as the epithelioid tumour was strongly positive and CR was demonstrated by almost all laboratories, whereas only the laboratories with correctly calibrated protocols were capable of demonstrating CR in the biphasic mesothelioma expressing limited amounts of CR.

It should be noticed that a slight difference in the reactivity pattern of the 2 mAbs and pAb was observed: pAb **18-0211** focally decorated renal tubules in optimal stains. Also with this Ab, unidentified crystals were seen throughout the kidney specimen (this did not affect the interpretation or assessment).

CR was also assessed in Run 6 2002, in which 14/47 (30%) laboratories had an insufficient staining. The increase to 44 % insufficient staining in Run 17 is mainly due to the inclusion of tumours (granulosa cell tumour and biphasic mesothelioma) with low expression of CR. The epithelioid mesothelioma with high CR expression used as the positive quality indicator in Run 6 was also included in Run 17, where almost all laboratories obtained a strong reaction. Thus, the multitissue block used in Run 17 presented a greater challenge for the laboratories. However, using one of the 3 Abs mentioned above and appropriate protocol settings, 41/55 (75 %) obtained a sufficient staining.

Conclusions:
The mAb clones **5A5** and **DAKCalret1** and the pAb **18-0211** are applicable Abs for CR. No significant difference was seen concerning the reaction pattern of these three in the tumours selected for this assessment. HIER, especially in an alkaline buffer, is highly recommended for optimal performance for all 3 Abs. Appendix can be used as positive control. However, to serve as a reliable control and to reduce the proportion of false negative reactions, the nerves must be as strongly stained as possible, while no staining of the enterocytes should be seen.

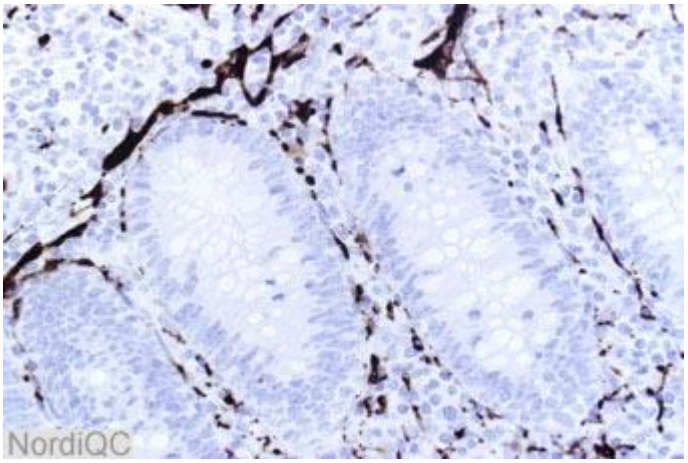


Fig. 1a
Optimal staining for Calretinin in the appendix. The peripheral nerves and macrophages show a distinct cytoplasmic and nuclear staining. No staining is seen in the epithelial cells.

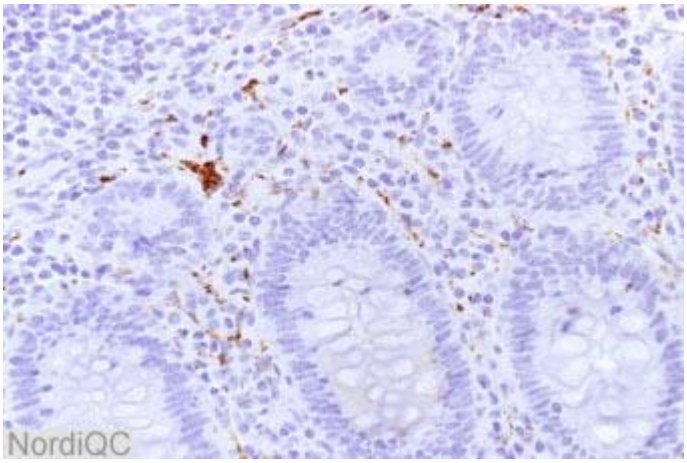


Fig. 1b
Staining for Calretinin in the appendix using an insufficient protocol. The cells expected to stain are weakly demonstrated. However, compare with Fig. 2b – same protocol.

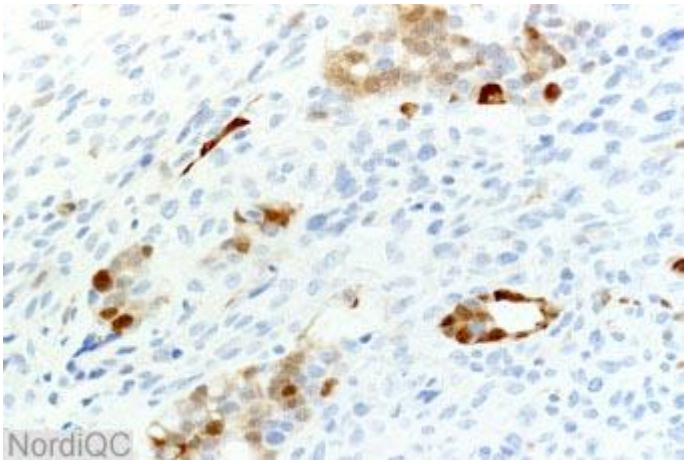


Fig. 2a
Optimal staining for Calretinin of the biphasic malignant mesothelioma. The majority of the neoplastic cells show a strong and distinct staining (same protocol used in Fig. 1a).

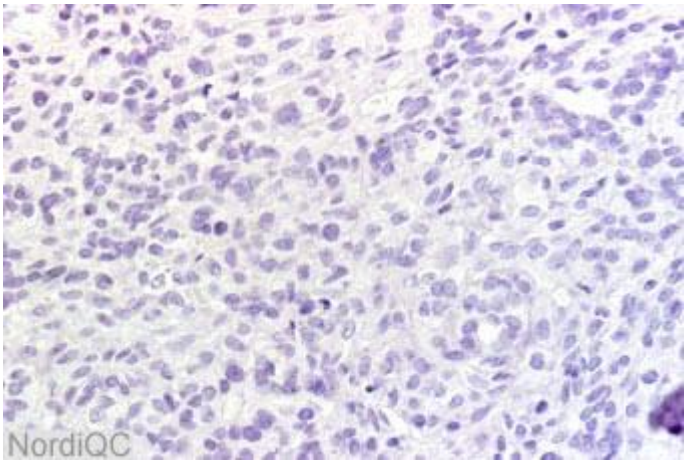


Fig. 2b
Insufficient staining for Calretinin of the biphasic malignant mesothelioma (same field as in Fig 2a). All the neoplastic cells are negative (same protocol used in Fig. 1b).

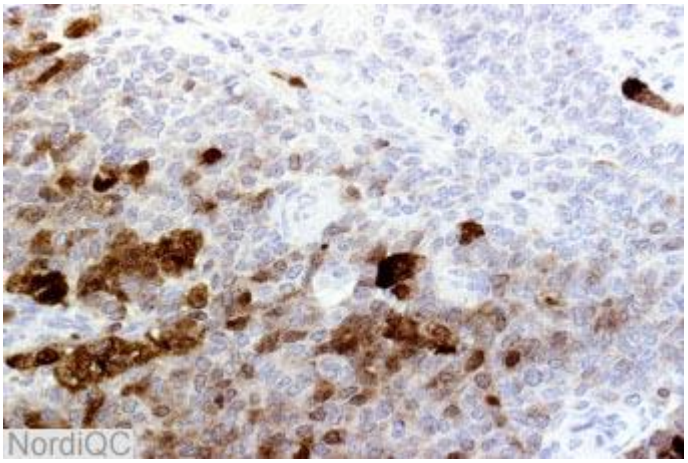


Fig. 3a
Optimal staining for Calretinin of the granulosa cell tumor. The majority of the neoplastic cells show a moderate but distinct cytoplasmic and nuclear staining.

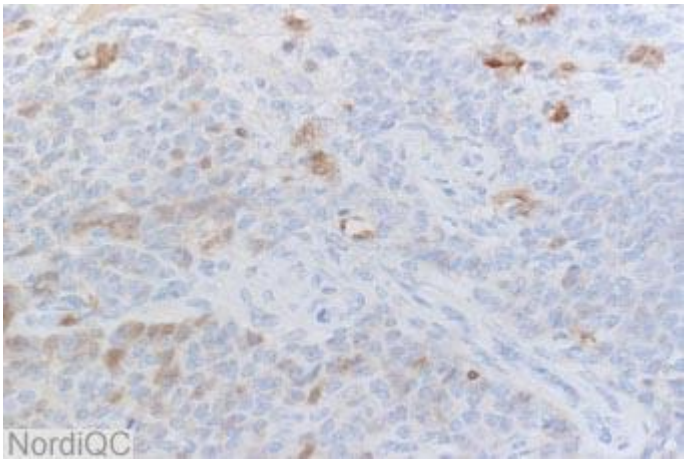


Fig. 3b
Insufficient staining for Calretinin of the granulosa cell tumor (same field as in Fig 3a). The neoplastic cells are virtually negative and only the theca cells are stained.

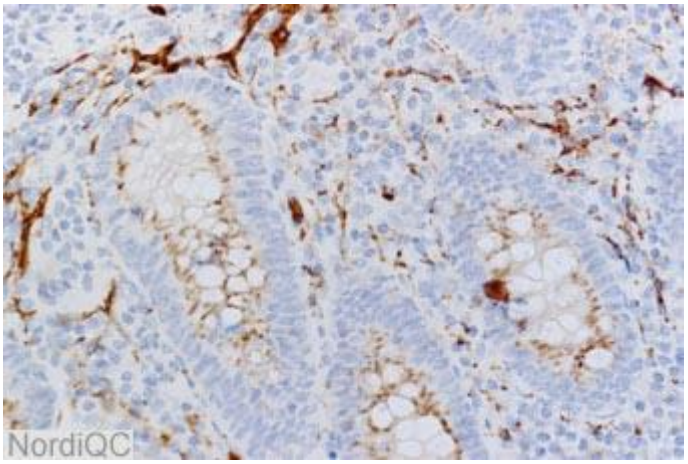


Fig. 4a
Staining for CR using the pAb 760-2700 in the appendix. The peripheral nerves and macrophages show a distinct cytoplasmic and nuclear staining but also a cross reactiony of the enterocytes is seen. No staining based on the Ab was marked as optimal.

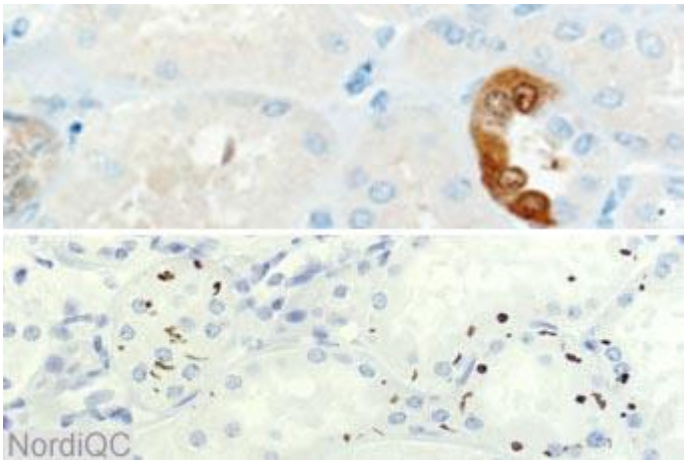


Fig. 4b
Top: Using a pAb as 7699/4 the renal tubules focally showed a positive staining.
Bottom: Using the pAb 18-0211 a crystal like reaction was seen in the renal tubules, however the reaction did not affect the interpretation and the Ab could be used to obtain an optimal staining.

SN/MV/LE 23-6-2006