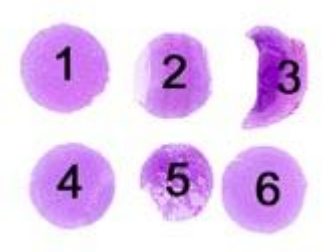
## Assessment Run 23 2008

# Calretinin (CR)

The slide to be stained for Calretinin (CR) comprised:
1. Kidney, 2. Adrenal gland, 3. Appendix, 4. Ductal breast carcinoma,
5. Epithelioid malignant mesothelioma, 6. Biphasic malignant mesothelioma.
All tissues were fixed in 10% neutral buffered formalin.




Criteria for assessing a CR staining as optimal included:

- A strong, distinct cytoplasmic and nuclear staining of the peripheral nerves (ganglion cells and axons) and macrophages in the appendix.
- A weak to moderate, distinct cytoplasmic and nuclear staining of the cortical epithelial cells of the adrenal gland.
- A moderate to strong, distinct cytoplasmic and nuclear staining of the majority of the neoplastic cells of the two mesotheliomas.
- A negative or only a focal staining of the epithelial cells of the kidney.
- A negative or only a focal staining of the neoplastic cells of the ductal breast carcinoma.

117 laboratories submitted stains. 6 used an inappropriate Ab (particularly Chromogranin A). In the assessment of the remaining 111 laboratories, 50 achieved optimal marks (45%), 39 good (35%), 18 borderline (16%) and 4 (4%) poor marks.

The following Abs were used:
mAb clone **DAKCalret1** (Dako, n=44)
mAb clone **5A5** (NeoMarkers, n=1; Novocastra, n=22)
mAb clone **2E7** (ImmunVision, n=2)
mAb clone **CRT01** (Zytomed, n=1)
rmAb clone **SP13** (NeoMarkers, n=2)
pAb **18-0211** (Zymed, n=21)
pAb **760-2700** (Ventana, n=11)
pAb **7699/4** (Swant, n=4)
pAb **AB5054** (Chemicon, n=2)
pAb **E070** (Linaris, n=1)

Optimal staining for **CR** in this assessment was obtained with the mAb clones **DAKCalret1** (26 out of 44), **5A5** (14 out of 23) and the pAb **18-0211** (10 out of 21).

All optimal protocols, independent of the Abs, were based on heat induced epitope retrieval (HIER) typically using an alkaline buffer such as Tris-EDTA/EGTA pH 9 or equivalent: CC1 (Ventana), BERS-2 (Leica Microsystems) and Target Retrieval Solution S2375 (Dako).
The mAb clone **DAKCalret1** was typically used in the range of 1:20 – 500 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) Ab.
The mAb clone **5A5** was typically used in the dilution 1:10 - 100 depending on the total sensitivity of the protocol employed.
The pAb **18-0211** was typically used in the range of 1:50- 1.000 depending on the total sensitivity of the protocol employed.
Table 1 shows the cumulated data from the latest three CR assessments.

Table 1. **Cumulated results from three CR assessments comparing Abs used by at least three participants.**

| | **Run 17, 19 & 23 All protocol settings** | | | **Run 17, 19 & 23 Optimal protocol settings*** | | |
|---|---|---|---|---|---|---|
| | **Protocols** | **Sufficient** | **Optimal** | **Protocols** | **Sufficient** | **Optimal** |
| mAb clone DakCalret1 | 114 | 86 (75%) | 40 (35%) | 103 | 82 (80%) | 40 (39%) |
| mAb clone 5A5 | 61 | 39 (64%) | 21 (34%) | 53 | 39 (74%) | 21 (40%) |
| pAb 18-0211 | 54 | 42 (78%) | 21 (39%) | 47 | 40 (82%) | 21 (45%) |

| | | | | | | |
|---|---|---|---|---|---|---|
| pAb 760-2700 | 22 | 7 (32%) | 0 (0%) | 21 | 7 (32%) | 0 (0%) |
| pAb-7699/4 | 11 | 5 (45%) | 1 (9%) | 11 | 5 (46%) | 1 (9%) |

*HIER in an alkaline buffer such as Tris-EDTA/EGTA pH 9 or equivalent (such as CC1, BERS-2 and TRS S2375), and an appropriate dilution of the antibody.

As seen in the table the mAb clones DakCalret1 and 5A5 and the pAb 18-0211 are the most robust Abs.

The most frequent causes of an insufficient staining were:
- Less successful primary Ab
- Insufficient HIER (e.g., Citrate pH 6)
- Too low concentration of the primary antibody.

In this assessment the prevalent feature of an insufficient staining was a general too weak or false negative staining of the structures expected to be demonstrated and in particular of the neoplastic cells of the mesotheliomas and the epithelial cells in the adrenal glad. For the first time an adrenal glad was incorporated in the multitissue block and tested for its potential as control tissue for CR. The adrenal gland appeared to be superior to the appendix as the adrenal cortical cells expressed less antigen than the peripheral nerves and thus provided better information about the sensitivity of the protocol.
A false positive (FP) staining was occasionally observed, mainly when using the Ready-To-Use pAb 760-2700 and typically with efficient HIER. The FP reaction was seen as a distinct granular cytoplasmic reaction of the appendiceal enterocytes and a diffuse cytoplasmic reaction of the epithelial cells in the kidney and the neoplastic cells of the ductal breast carcinoma. The nuclei of these cells were negative indicating the FP staining was either due to a cross reactivity of the primary Ab or a too high sensitivity of the protocols applied.
This was the 4th assessment of CR. As shown in Table 2, the proportion of sufficient stains has increased to an acceptable level in spite of many new laboratories.

Table 2. **Proportion of sufficient results in four CR assessments.**

| | Run 6 2002 | Run 17 2006 | Run 19 2007 | Run 23 2008 |
|---|---|---|---|---|
| Participants, n= | 47 | 82 | 87 | 111 |
| Sufficient results, % | 70 % | 56 % | 56 % | 80 % |

The specifically tailored recommendations given to 32 laboratories with an insufficient staining result in run 19 did have a positive impact: 20 followed the recommendations, of which 19 now obtained a sufficient result (95%). 11 laboratories did not change their protocol, and 4 improved to sufficient (36%).

**Conclusions:**
The mAb clones **DAKCalret1** and **5A5** and the pAb **18-0211** are recommendable Abs for CR. HIER, especially in an alkaline buffer, is highly recommended for optimal performance with all 3 Abs. Adrenal gland is useful for control: The large majority of cortical epithelial cells must show a weak to moderate nuclear and cytoplasmic staining reaction.

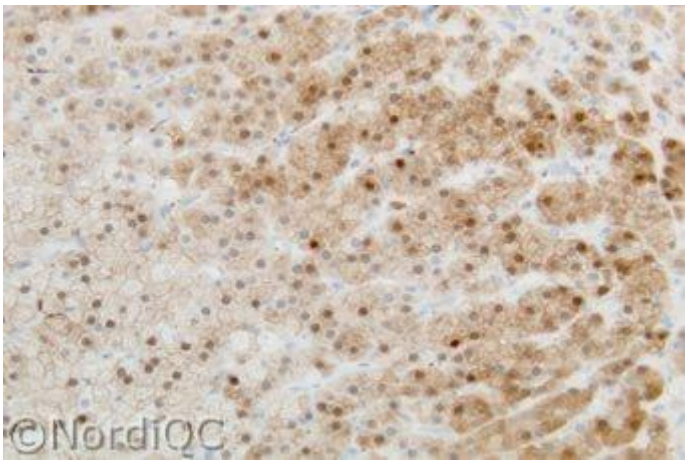



Fig. 1a
Optimal CR staining of the adrenal gland using the mAb clone DAK-Calret1 with HIER. The cortical epithelial cells show a distinct nuclear and cytoplasmic staining.

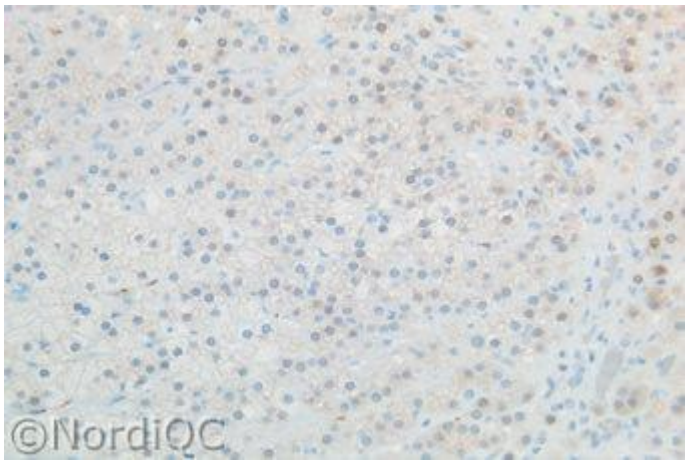



Fig. 1b
Insufficient CR staining of the appendix using the mAb clone DAK-Calret1 with HIER, but in a too low concentration – same field as in Fig. 1a. Only scattered cortical epithelial cells show a weak and diffuse staining. Also compare with Fig. 2b.

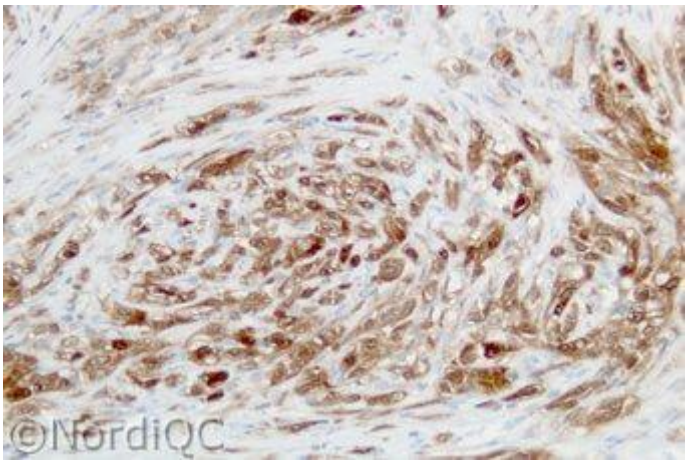



Fig. 2a
Optimal CR staining of the biphasic malignant mesothelioma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong distinct reaction with no background reaction.

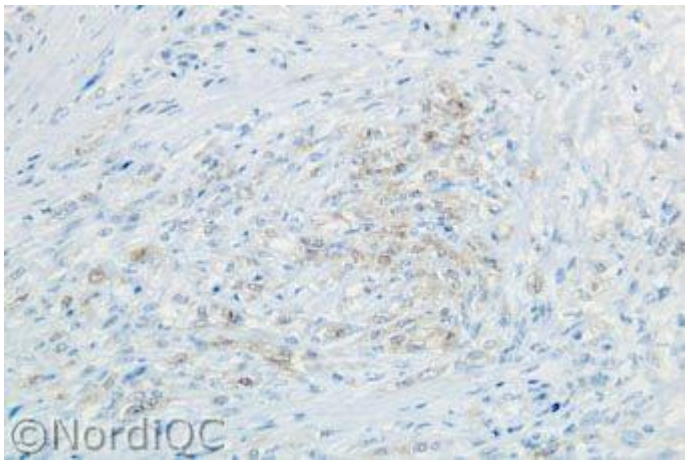



Fig. 2b
Insufficient CR staining of the biphasic malignant mesothelioma using same protocol as in Fig. 1b. The neoplastic cells are weakly stained or unstained – same field as in Fig. 2a.

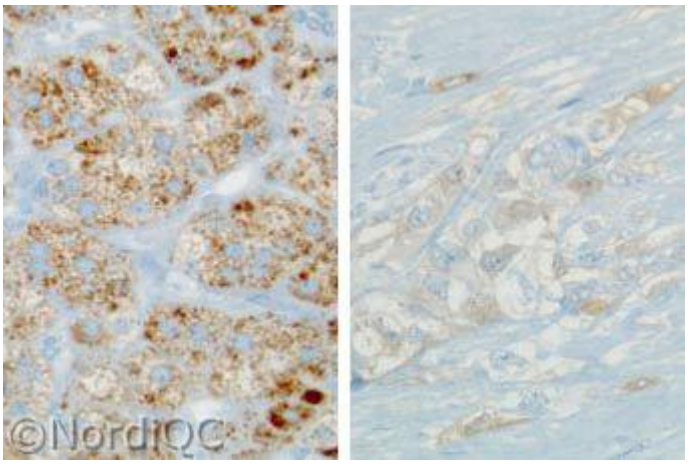



Fig. 3a
Insufficient CR staining using a too low concentration of the primary Ab combined with HIER in an alkaline buffer and a biotin based detection system.
Left: The adrenal cortical epithelial cells show a distinct cytoplasmic staining, but no nuclear reaction. The positive reaction is false positive and due to reaction of endogenous biotin.
Right: The neoplastic cells of the biphasic mesothelioma show a weak diffuse reaction only.

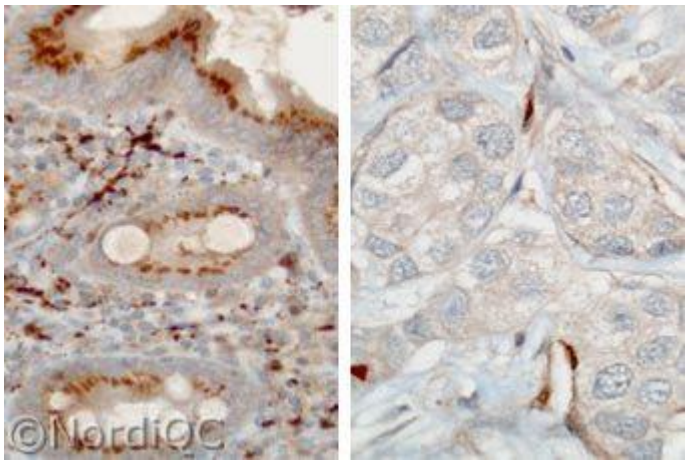



Fig. 3b
Insufficient 3179CR staining with the pAb 760-2700 used in combination with too sensitive protocol settings.
Left: The peripheral nerves and macrophages show a distinct cytoplasmic and nuclear staining but also a cytoplasmic cross reaction of the appendiceal enterocytes is seen.
Right: The neoplastic cells of the ductal breast carcinoma show a diffuse cytoplasmic reaction.

SN/MV/LE 3-7-2008