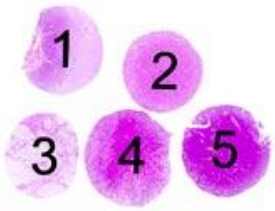
Assessment Run 19 2007

Calretinin (CR)

The slide to be stained for Calretinin (CR) comprised:
1. Appendix, 2. Kidney, 3. Malignant mesothelioma, epithelioid, 4. Malignant mesothelioma, biphasic, 5. Ovarian granulosa cell tumour.
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 strong, distinct cytoplasmic and nuclear staining of the majority of the neoplastic cells of the epithelioid mesothelioma, whereas at least focally the neoplastic cells of the biphasic mesothelioma should be demonstrated.
- A moderate, distinct cytoplasmic and nuclear staining of the luteinized cells in the granulosa cell tumour and a strong reaction in the stromal component.
- A negative or only a focal staining of the tubular epithelium in the kidney.

87 laboratories participated in the assessment. At the assessment 16 achieved optimal marks (18 %), 33 good (38 %), 28 borderline (32 %) and 10 (12 %) poor marks.

The following Abs were used:
mAb clone **DAKCalret1** (Dako, n=38)
mAb clone **5A5** (Novocastra, n=17)
mAb clone **2E7** (ImmunVision, n=1)
mAb clone **CAL6** (Novocastra, n=1)
rmAb clone **SP13** (NeoMarkers, n=2)
pAb **18-0211** (Zymed, n=16)
pAb **760-2700** (Ventana, n=7)
pAb **7699/4** (Swant, n=3)
Unknown Ab (n=1)

Optimal staining for **CR** in this assessment was obtained with the mAb clones **DAKCalret1** (8/38) and **5A5** (3/17), and the pAbs **18-0211** (4/16) and **7699/4** (1/3).
All optimal protocols, irrespective of the Ab, were based on heat induced epitope retrieval (HIER) using **Tris-EDTA/EGTA pH 9**.
DAKCalret1 was used in the range of 1:25 – 1:500 depending on the total sensitivity of the protocol employed, or as a Ready-To-Use (RTU) Ab. Using these protocol settings 25 out of 34 laboratories (74%) produced a sufficient staining (optimal or good).
5A5 was used in the dilution 1:40. Using these protocol setting 4 out of 6 laboratories (67%) obtained a sufficient staining.
18-0211 was used in the range of 1:100- 1:1,500 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 6 (83%) laboratories produced a sufficient staining.
7699/4 was used in the dilution 1:2,000. 1 out of 3 laboratories (33%) using this pAb obtained a sufficient staining marked as optimal.

Comparing run 17 and run 19, the following pass rates are seen with Abs used by at least 3 participants:

	Run 17 2006		**Run 19 2007**		**Total**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols analyzed	Sufficient
MAb clone DakCalret1	32	20	38	28	70	48 (68%)
MAb clone 5A5	21	11	17	9	38	20 (53%)
PAb 18-0211	17	14	16	9	33	23 (70%)
PAb 760-2700	4	2	7	0	11	2 (18%)

PAb-7699/4	4	1	3	1	7	2 (29%)

When only protocols with HIER in an alkaline buffer as Tris-EDTA/EGTA pH 9 or equivalent as CC1, Ventana, BERS-2 Vision BioSystem and TRS S2367, Dako are included, the following pass rates are seen:

	Run 17 2006		**Run 19 2007**		**Total**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols analyzed	Sufficient
MAb clone DakCalret1	29	19	35	27	64	46 (72%)
MAb clone 5A5	16	11	15	9	31	20 (65%)
PAb 18-0211	16	14	12	8	28	22 (79%)
PAb 760-2700	4	2	6	0	10	2 (20%)
PAb-7699/4	4	1	3	1	7	2 (29%)

From the tables the overall pass rate increases compared to the general result obtained in run 17 and 19. Of particular interest, the pass rate is markedly higher using the mAb clone DakCalret1, 5A5 and the pAb 18-0211 with HIER in an alkaline buffer: In total 88 out of 123 protocols (72 %) were assessed as sufficient.

The most frequent causes of an insufficient staining were:
- Less successful primary Ab
- Insufficient HIER (typically with Citrate pH 6 as the heating buffer)
- Too low concentration of the primary Ab.

In this assessment (and in concordance with the CR assessment in run 17) the prevalent feature of an insufficient staining was a too weak or false negative staining of the granulosa cell tumour. The stromal component in the tumour was almost always positive, but only in the correctly calibrated protocols the neoplastic luteinized granulosa cells were demonstrated. The two mesotheliomas showed different levels of CR expression, as the epithelioid tumour was strongly positive - CR being demonstrated by almost all laboratories - whereas the biphasic mesothelioma expressing limited amounts of CR - only demonstrated by the laboratories with correctly calibrated protocols.

As observed in run 17 appendix can be used as control for CR. However to serve as a reliable quality indicator for the immunohistochemical demonstration of CR, the axons and ganglion cells in the Aurbach's plexus in the appendix and the fat cells must show an intense reaction, while smooth muscle cells and epithelial cells shall remain negative.

In the CR assessment run 17 (2006) 82 laboratories participated out of which 44% (36 laboratories) obtained an insufficient mark. Each of these laboratories was given specific recommendation to improve their protocol. 29 laboratories, which obtained an insufficient result in run 17, submitted a new CR stain in run 19. 18 of these followed the recommendation, of which 12 improved to good or optimal (67 %). 11 laboratories did not follow the recommendation, and only 1 of these (9 %) obtained a sufficient staining in run 19.

However, the proportion of insufficient results has not been reduced from run 17 as 44% of the results still are insufficient. The main reasons seem to be a high proportion of laboratories using less successful antibodies and difficulties in calibrating and setting the Ab concentration of otherwise successful antibodies. This emphasizes the importance of identifying a robust control for CR. Currently appendix is the preferred choice.

Conclusion
The mAb clones **DAKCalret1**, **5A5** and the pAb **18-0211** are recommendable Abs for CR. HIER in an alkaline buffer is highly recommended for optimal performance with all 3 Abs. Appendix can be used as positive control: The nerves must be as strongly stained as possible, while no staining of the epithelial and smooth muscle cells shall be seen.

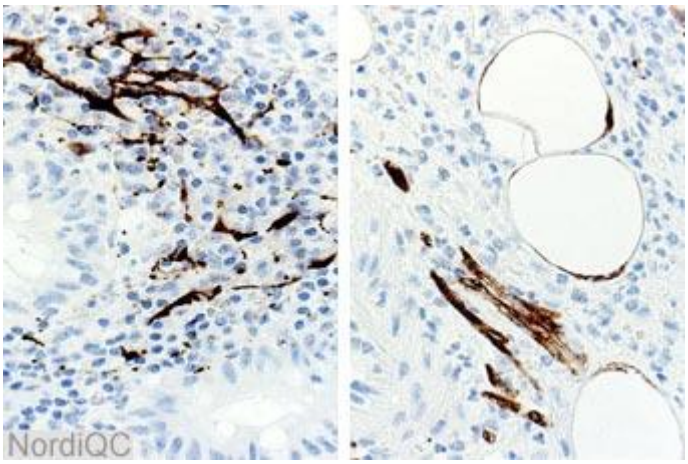


Fig. 1a
Optimal staining for Calretinin in the appendix.
Left: The peripheral nerves and macrophages show a strong distinct cytoplasmic and nuclear staining while no staining is seen in the epithelial cells.
Right: The adventitial fat cells show a strong distinct reaction.

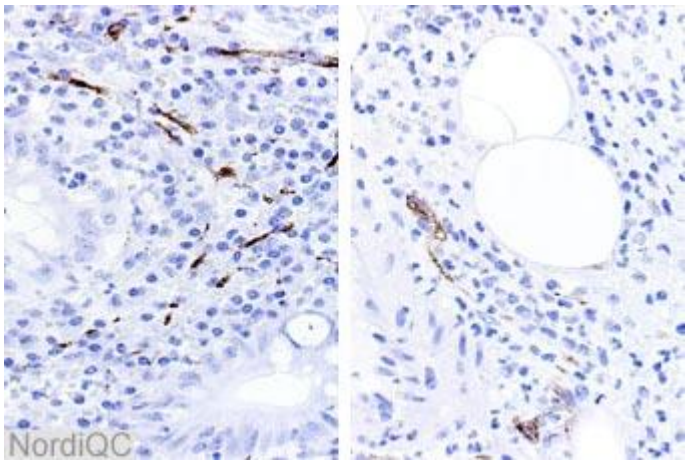


Fig. 1b
Staining for Calretinin in the appendix using an insufficient protocol (same fields as in fig 1a).
Left: The cells expected to stain are demonstrated. However, compare with Fig. 2b – same protocol.
Right: The fat cells are negative and only the peripheral nerves show a moderate reaction.

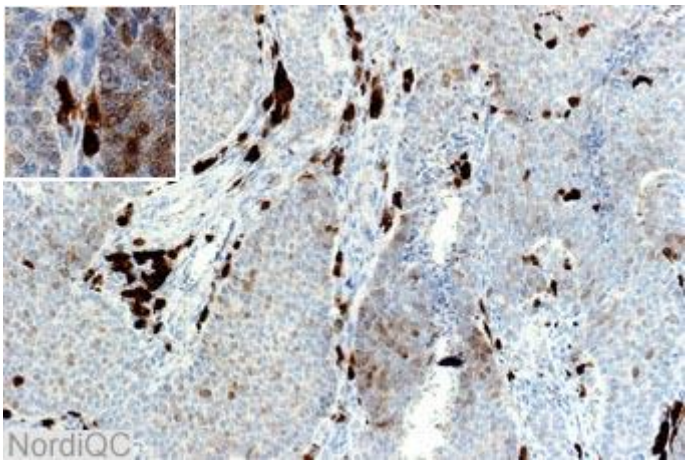


Fig. 2a
Optimal staining for Calretinin of the granulosa cell tumour. The majority of the neoplastic cells show a moderate cytoplasmic and nuclear staining. The stromal theca cells show an intense reaction. Same protocol as in fig 1a.
Insert: high magnification of the neoplastic cells and the theca cells.

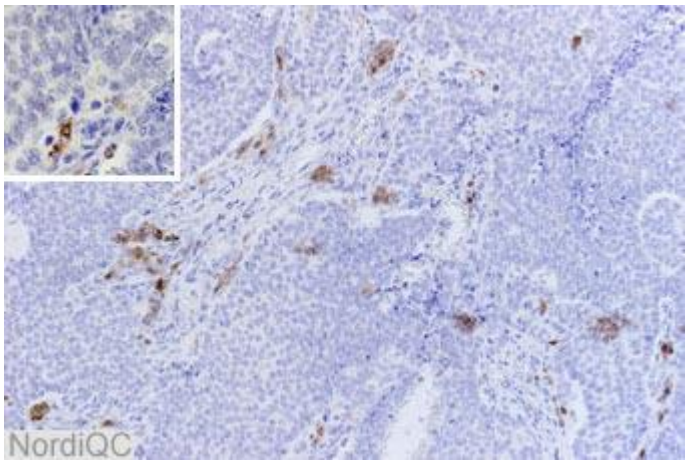


Fig. 2b
Insufficient staining for Calretinin of the granulosa cell tumour (same field as in Fig 2a). The neoplastic cells are virtually negative and only the stromal theca cells are stained. Same protocol as in fig 1b.
Insert: high magnification of the neoplastic cells and the theca cells.

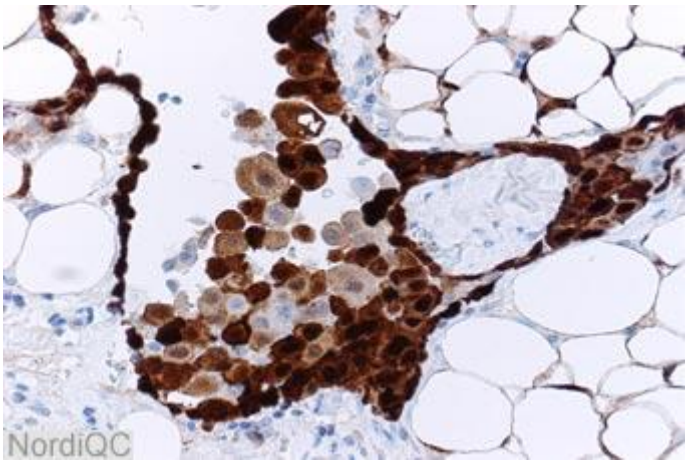


Fig. 3a
Optimal staining for Calretinin in the epithelial malignant mesothelioma. Virtually all the neoplastic cells show a strong and distinct staining. Note also the distinct reaction of the fat cells. No reaction is seen in the connective tissue (same protocol used in Fig. 1 and 2a).

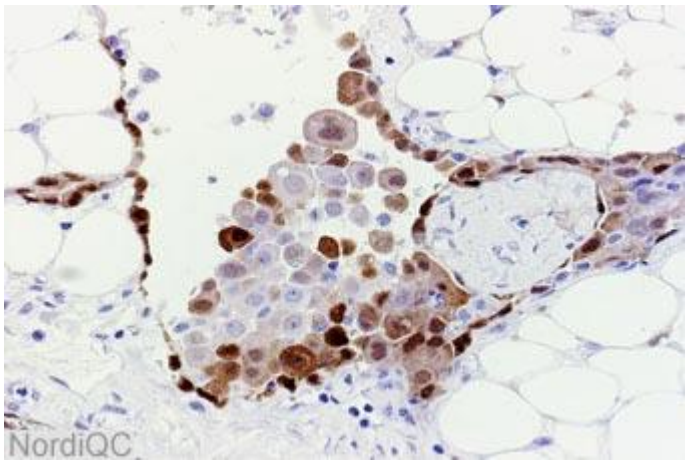


Fig. 3b
Insufficient staining for Calretinin in the epithelial malignant mesothelioma (same field as in Fig. 3a). The majority of the neoplastic cells show a moderate staining, while the fat cells are negative (same protocol used in Fig. 1 and 2b).

SN/MV/LE 2-4-2007