Assessment Run 16 2006

α-methylacyl-CoA racemase (AMACR, P504S)

The slide to be stained for AMACR comprised:
1. Prostate hyperplasia, 2-3. Prostate adenocarcinoma.
All specimens were fixed in 10 % NBF.

Criteria for assessing an AMACR staining as optimal included:

- A moderate to strong cytoplasmic staining of the two prostate adenocarcinomas
- A negative or week focal cytoplasmic staining of the hyperplastic prostate glands
- A negative or week cytoplasmic staining of the stromal cells.

65 laboratories submitted stains. At the assessment 39 achieved optimal marks (60 %), 19 good (29 %), 6 borderline (9 %) and 1 (2 %) poor marks.

The following Abs were used:
mAb clone **13H4** (Dako, n=47; Biologo, n=4; Zeta Corporation, n=1)
mAb clone **P504-S** (Immunologic, n=1)
pAb **CP200 & PP200** (BioCare, n=9; PP200: cocktail of P504S + p63)
pAb **ab12498** (Abcam, n=1)
pAb **RB9407** (NeoMarkers, n=1)
pAb **RP134** (Diagnostic Biosystems, n=1)

Optimal staining for AMACR in this assessment was obtained with the rmAb clone **13H4** (33 out of 52 (63%)), and the pAbs **CP200/PP200**, (5 out of 9) and **ab12498** (1 out of 1).

All optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

Using the clone **13H4**, HIER in Tris-EDTA/EGTA pH 9 (30 out of 41), EDTA/EGTA pH8 (1 out of 3) and CC1/Ventana (2 out of 6) gave an optimal staining. The clone **13H4** was used in the range of 1:40 – 1:300 depending on the total sensitivity of the protocol employed.

Using the pAb **CP200/PP200** optimal staining was obtained with HIER in CC1/Ventana (2 out of 2), Tris-EDTA/EGTA pH9 (2 out of 3) and Borg/BioCare (1 out of 2). The pAb **CP200/PP200** was used in the range of 1:100 – 1:200 depending on the total sensitivity of the protocol employed, or as a ready-to-use Ab.

The pAb **ab12498** gave an optimal staining using a dilution of 1:100 (overnight 4ºC) and HIER in Citrate pH 6.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody

In the assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the epithelial cells in the prostate carcinomas probably due to a too low concentration of the primary Ab. In a few cases the staining was too strong, which gave a distinct cytoplasmic granular reaction in the normal epithelial cells in the prostate hyperplasia imitating the reactivity expected in carcinoma. In case of a very strong reaction in the epithelial cells of the prostate carcinoma, the smooth muscle cells typically showed a moderate positive staining.
They main issue in the immunohistochemical demonstration of AMACR seems to be a correct calibration of the primary Ab resulting in a negative or only weak and focal reaction in normal prostate epithelial cells and a strong granular reaction of the majority of the neoplastic cells in a prostate adenocarcinoma.

Conclusion
- The mAb clone 13H4, the pAb CP200 (used alone or combined with p63 as in RP200; both from Biocare) and the pAb ab12498 seem to be appropriate markers for AMACR

- HIER is recommended for optimal performance of all the Abs.

As control for AMACR a multitissue block containing both normal prostate, prostate in situ neoplasia (PIN) and prostate adenocarcinoma is appropriate.

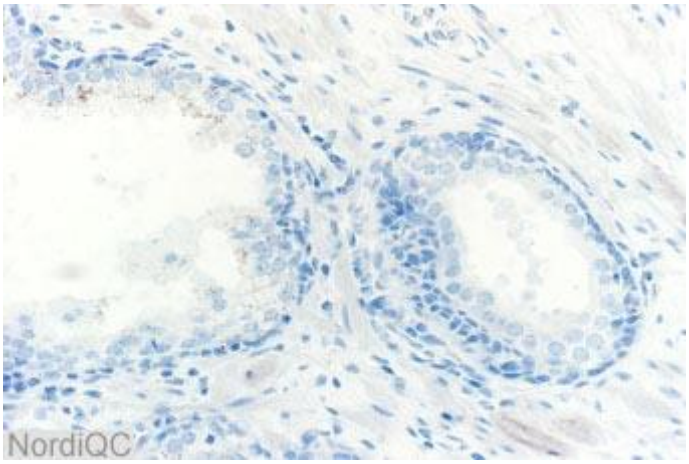

Fig. 1a
Optimal staining for AMACR of the prostate hyperplasia. The majority of the epithelial cells are negative and only a few cells show a weak granular staining reaction of the cytoplasm.

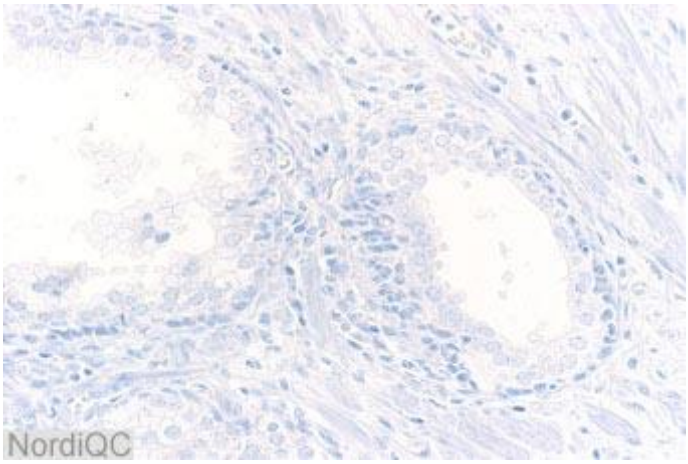

Fig. 1b
Staining for AMACR of the prostate hyperplasia using an insufficient protocol (same field as in Fig. 1a.). The prostatic epithelial cells are all negative. However, compare with Fig. 2b – same protocol.

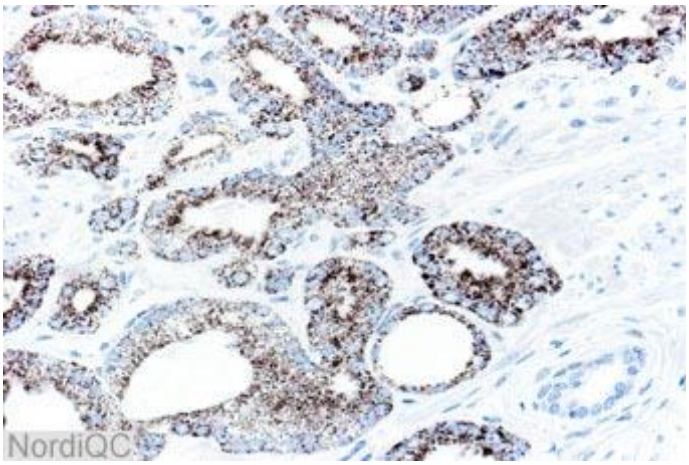

Fig. 2a
Optimal staining for AMACR of the prostate adenocarcinoma. Virtually all neoplastic cells show a strong granular cytoplasmic reaction (same protocol used in Fig. 1a). In the right corner a residual normal gland serves as negative control.

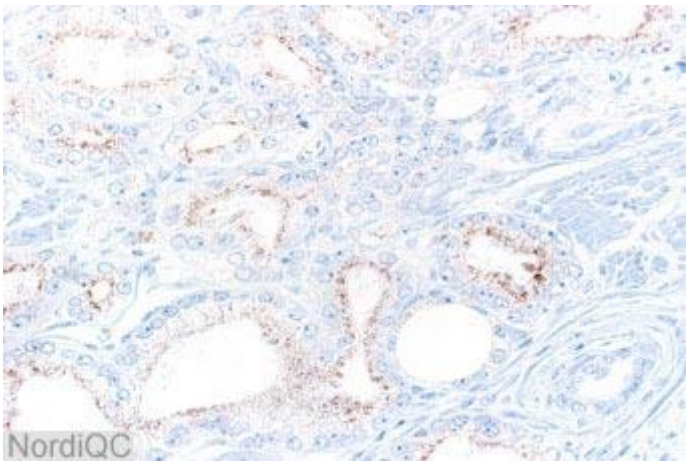

Fig. 2b
Insufficient staining for AMACR of the prostate adenocarcinoma (same field as in Fig 2a). The neoplastic cells are negative or only weakly positive (same protocol used in Fig. 1b).

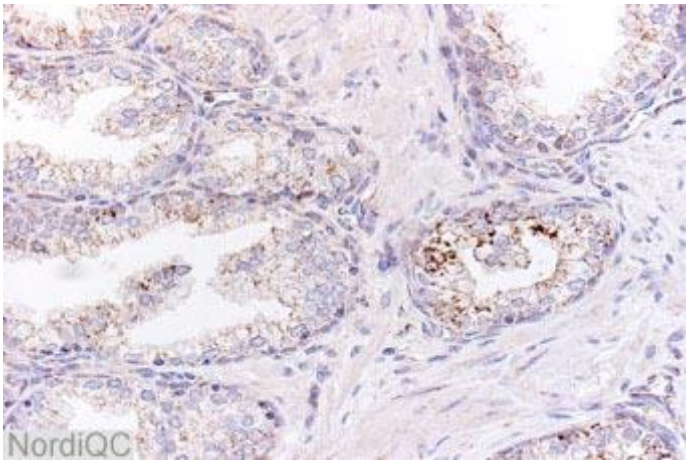

Fig. 3a
Insufficient staining for AMACR of the prostate hyperplasia. The majority of the epithelial cells show a granular cytoplasmic reaction. This is probably due too a too high concentration of the primary Ab.

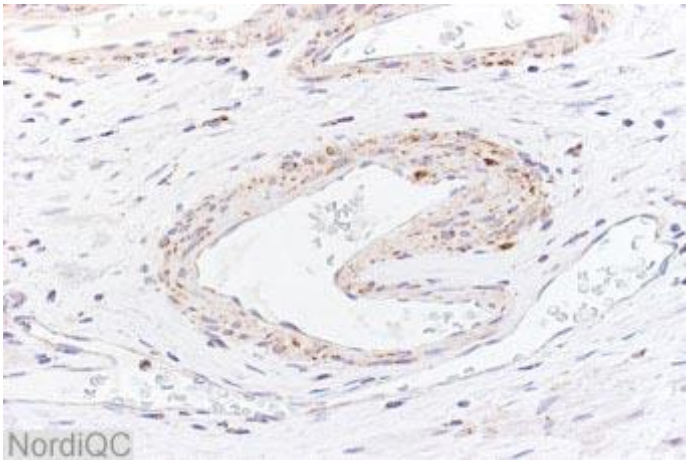

Fig. 3b
Insufficient staining for p504s of the prostate (same protocol used in Fig. 3a). The smooth muscle cells in vessels show a moderate positive staining reaction. This was frequently seen in protocols using a too high concentration of the primary Ab.

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