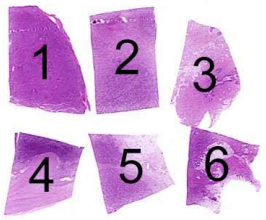
Assessment Run 10 2004

Progesterone Receptor (PR)



The slide to be stained for progesterone receptor (PR) comprised:
1. Meningioma, 2. Ductal breast carcinoma with 10 - 30 % PR+ neoplastic cells*, 3. Breast fibrocystic disease, 4. Endometrial stromal sarcoma, 5. Lobular breast carcinoma, PR-negative, 6. Ductal breast carcinoma with 80 – 100 % PR+ neoplastic cells*.
*) as determined in four reference laboratories.

Criteria for assessing a PR staining as optimal included:
A distinct nuclear staining reaction for PR in an neoplastic cells in the two ductal breast carcinomas and in the meningioma and the endometrial stromal sarcoma the majority of the neoplastic cells should be labelled. The normal epithelial cells of the glands in the breast fibrocystic disease should focally display a positive nuclear reaction.
A weak cytoplasmic reaction of cells with strong nuclear staining was accepted, as was staining of necrotic tissue.

79 laboratories submitted stains. At the assessment 36 achieved optimal staining (46 %), 18 good (23 %), 17 borderline (22 %) and 7 poor staining (9 %).

The following mAbs were used:
clone PgR 636 (DakoCytomation, n=35)
clone 16 (Novocastra, n=12; Ventana, n=9)
clone 1A6 (Novocastra, n=6; Ventana, n=2; DakoCytomation, n=1)
clone PR88 (BioGenex, n=1)
clone hPRa 2 + hPRa 3 1 (NeoMarkers, n=1)
clone 16 + SAN27 (Novocastra, n=1)

In this assessment optimal stains could be obtained with the clones PgR 636 (29/35), 16 (5/19), 1A6 (1/9) & PR88 (1/1). In the optimal protocols (36) all used HIER (MWO: 29, pressure cooker: 5, water bath 2) with Tris-EDTA/EGTA pH 9 (n=32), Citrate pH 6 (n=2) or Target Retrieval Solution pH 6 (DakoCytomation, n=1) as the heating buffer.

Using clone PgR 636 optimal staining was obtained with a concentration in the range of 1:100–500, using clone 16 (Novocastra), the concentration was in the range of 1:50–500 (both ranges depending on the total sensitivity of the protocol). 1A6 (Ventana) was used as a Ready-To-Use Ab.

The most prevalent feature of the insufficient results (24/797) was a false negative reaction of the neoplastic cells of the ductal breast carcinoma with 10–30 % positivity whereas almost all laboratories were able to detect PR in the ductal carcinoma with 80-100 % positivity and in the normal glands in the breast fibrocystic disease.

The neoplastic cells of the meningioma and especially the endometrial sarcoma were also most frequently negative in the insufficient stains.

The most frequent causes of insufficient stains (often in combination) were:
- Insufficient HIER, too short efficient heating time (<15 min), especially when Citrate pH 6 was used as the heating buffer.
- Too low concentration of the primary antibody.

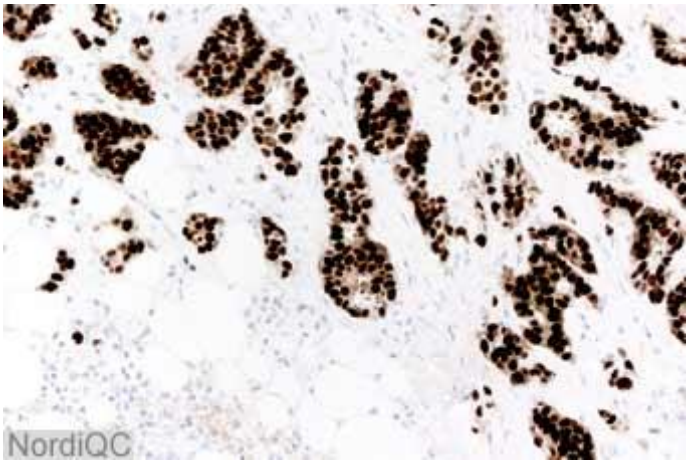


Fig. 1a
Optimal PR staining (mAb clone PgR 636) of the ductal breast carcinoma with the high expression of ER. All nuclei are strongly stained with a weak cytoplasmic reaction.

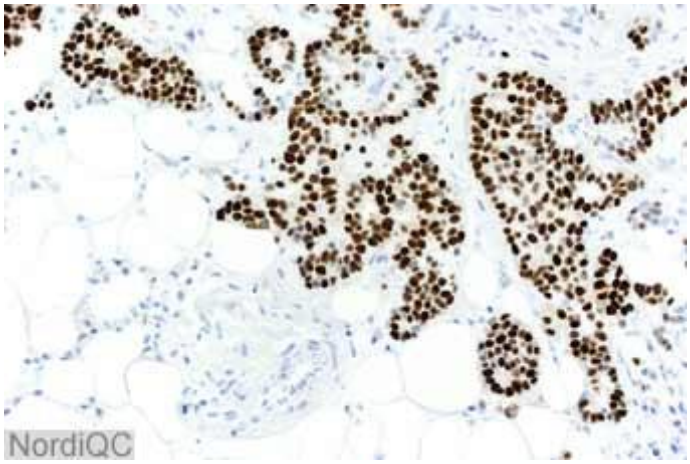


Fig. 1b
Insufficient PR staining of the ductal breast carcinoma with the high expression of ER. Almost all nuclei are stained. However compare with fig. 2b – same protocol.

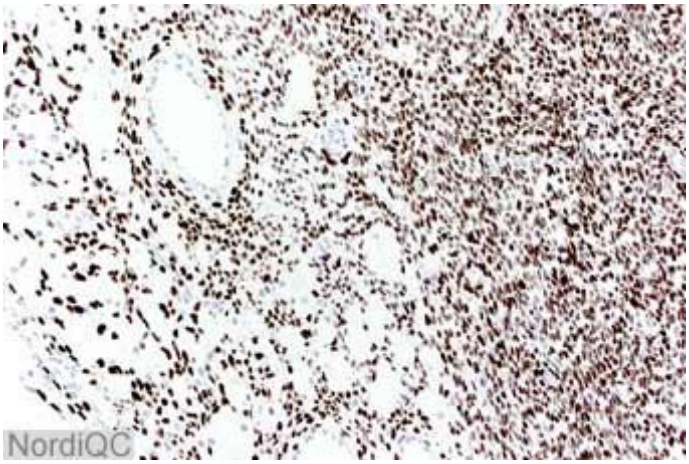


Fig. 2a
Optimal PR staining (mAb clone PgR 636) of the endometrial stroma sarcoma. All nuclei are strongly stained.

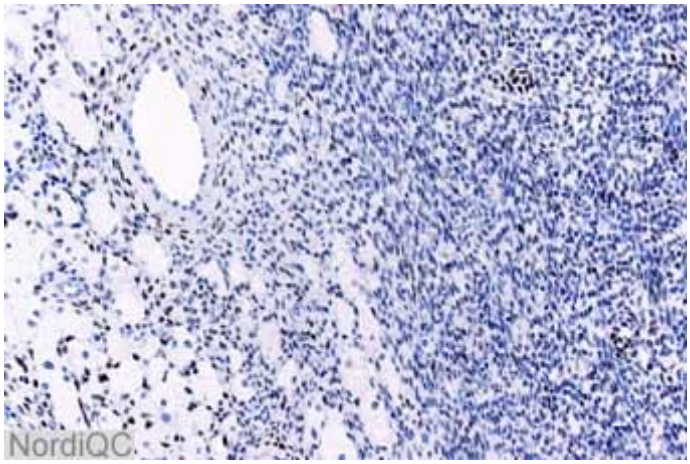


Fig. 2b
Insufficient PR staining of the enodometrial stroma sarcoma. All nuclei are virtually negative.

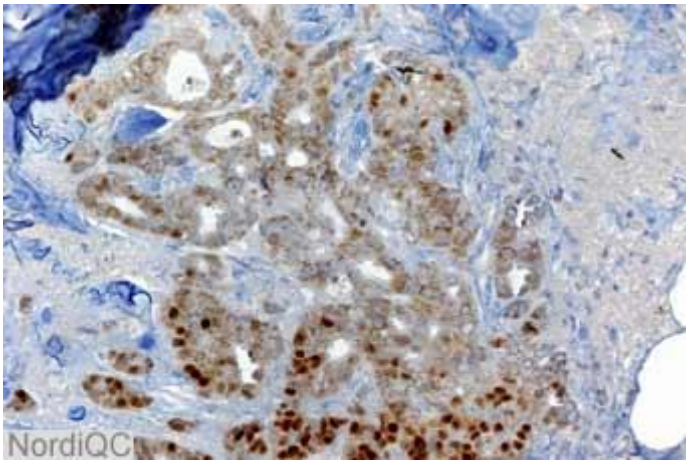


Fig. 3a
Insufficient PR staining of the ductal breast carcinoma with the high expression of PR. Only focally the nuclei are stained. At the same time the neoplastic cells show a strong false positive cytoplasmic staining, probably due to a reaction of endogenous biotin combined with a too low primary Ab concentration.

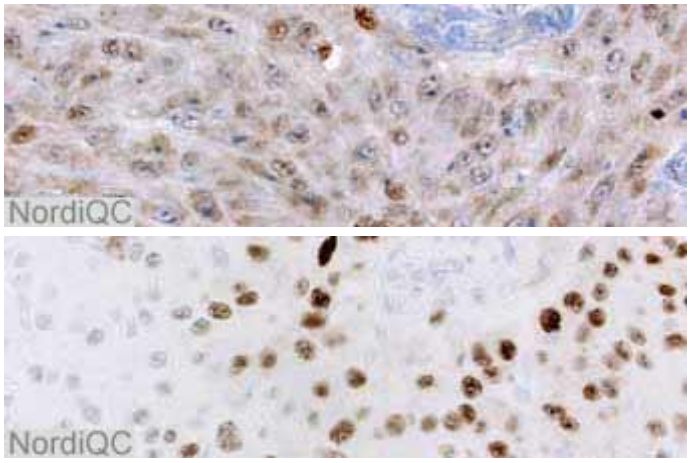


Fig. 4a (top)
Insufficient PR staining of the meningioma (same protocol as in Fig. 3a). Only few nuclei are stained. At the same time the neoplastic cells show a strong false positive cytoplasmic staining.
Fig 4b (bottom)
Optimal PR staining of the menigioma (same field as in Fig. 4a) revealing a distinct nuclear staining with only a weak cytoplasmic staining.

SN/MV/LE 29-3-2004