Assessment Run B4 2007

Progesterone Receptor (PR)

The slide to be stained for progesterone receptor (PR) comprised:
1. Uterine cervix, 2. Ductal breast carcinoma, PR negative, 3. Ductal breast carcinoma, PR 40-60 % positive, 4. Ductal breast carcinoma, PR 80–100 % positive. (The block was identical to the block used in B2 2006).
The positivity of the 3 ductal breast carcinomas was verified in 4 reference IHC laboratories.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PR staining as optimal included:

- A strong and distinct nuclear staining of the columnar epithelial cells, the basal squamous epithelial cells and the stromal cells in the uterine cervix.
- A moderate to strong and distinct nuclear staining of the ductal breast carcinomas no. 3 and 4 in accordance with the PR status.
- No nuclear staining of the PR negative ductal breast carcinoma no. 2 – only epithelial cells in remnants of normal glands should show a positive reaction.

95 laboratories submitted stains. At the assessment 54 achieved optimal marks (57 %), 22 good (23 %), 4 borderline (4 %) and 15 poor marks (16 %).

The following Abs were used:
mAb clone **PgR 636** (Dako, n=41)
mAb clone **16** (Novocastra, n=13; Monosan n=1)
mAb clone **1A6** (Novocastra, n=4; Ventana n=1)
mAb clone **PgR** 1294 (Dako, n=2)
mAb clone **PR 88** (BioGenex, n=1)
mAb clone **PR-1** (Klinipath, n=1)
mAb clone **hPRa 2 + hPRa 3** (NeoMarkers, n=1)
rmAb clone **1E2** (Ventana, n=23)
rmAb clone **SP2** (NeoMarkers, n=6; Diagnostic BioSystems, n=1)

Optimal staining for **PR** in this assessment was obtained with the mAb clone **PgR 636** (24 out of 41), mAb clone **16** (6 out of 14), the rmAb clone **1E2** (17 out of 23) and the rmAb clone **SP2** (3 out of 7).

Using the mAb clone **PgR 636** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (18/29)*, Citrate pH 6 (2/6), Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (2/2), EDTA/EGTA pH8 (1/1) or EnVision™ FLEX, High pH (Dako) (1/1) as HIER buffer. The mAb was typically diluted in the range of 1:50 – 1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 32 out of 37 (86 %) laboratories produced a sufficient staining marked as optimal.
* (number of optimal results/number of laboratories using this buffer)

Using the mAb clone **16** the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (4/6)*, Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (1/1), 1mM EDTA pH 9 (1/1) as HIER buffer. The mAb was typically diluted in the range of 1:100 – 1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 8 (75 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Using the rmAb clone **1E2** the protocols giving an optimal result were all based on HIER using Cell Conditioning 1 (BenchMark, Ventana) (14/19)*, EDTA/EGTA pH8 (2/3) or Cell Conditioning 2 (BenchMark, Ventana) (1/1) as HIER buffer. The rmAb was used as a Ready-To-Use antibody. Using these protocol settings 22 out of 23 (96 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Using the rmAb clone **SP2** the protocols giving an optimal result were all based on HIER using Cell Conditioning 1 (BenchMark, Ventana) (1/3)*, Tris-EDTA/EGTA pH 9 (1/2) or Target Retrieval Solution pH 6,1 (TRS, Dako) (1/1) as HIER buffer. The rmAb was typically diluted in the range of 1:100 – 1:350 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 6 (75 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Less successful primary antibody
- Insufficient epitope retrieval – too short efficient HIER time

In this assessment (and in concordance with the observation in the previous PR assessment, run B2, 2006) almost all laboratories were able to demonstrate PR in the ductal breast carcinoma no. 4 with 80-100 % positivity and a strong staining intensity, whereas the prevalent feature of the insufficient staining was a too weak or entirely false negative staining of the ductal breast carcinoma no. 3 with 40-60 % positivity and a moderate staining intensity.
A too weak or false negative staining was seen in 89 % of the insufficient results (17 out of 19), while in 11 % (2 out of 19) a too strong staining and false positive PR staining reaction was seen.

This was the third NordiQC assessment of PR. The proportion of sufficient results has increased relatively constant through the individual assessments – from 68% in run 10 2004, to 74% in run B2 2006 to 80% in the present run. Multiple factors contribute to the improvement, but especially the focus on the choice of antibody and appropriate control as normal cervix uteri for PR seem to be the central parameters for an optimal demonstration of PR.

The rmAb clone 1E2 and the mAb clone PGR 636 seem to be the most robust clones for the PR demonstration, as respectively 93% and 80% of all submitted stains based on these clones were assessed as sufficient in run B2 and B4.

	Run B2 2006		**Run B4 2007**		**Total**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols analyzed	Sufficient
MAb clone **1A6**	6	4	5	3	11	7 (64%)
MAb clone **16**	21	17	14	8	35	25 (71%)
MAb clone **PGR 636**	39	31	41	33	80	64 (80%)
RmAb **1E2**	5	4	23	22	28	26 (93%)
RmAb **SP2**	4	2	7	4	11	6 (55%)

Table 1. Number of protocols submitted in the last two PR assessments, the number of sufficient results and Abs used.

Also the specific tailored recommendations to the laboratories in run 10 and B2 achieving an insufficient staining seem to contribute to the improvement of the pass rate. The three main recommendations given were following:
1) Consider change of primary antibody
2) Increase the primary Ab concentration
3) Optimize HIER, i.e., prolong heating time and/or substitute Citrate pH 6 with an alkaline buffer (Tris/EDTA pH 9 or equivalent).

Among laboratories participating in all three runs, 37 recommendations have been given. The results are indicated in Table 2.

	Followed recommendations*	
	Yes	No
Number of laboratories advised	30	7
Number of laboratories improved	25 (83%)	0 (0%)

* 9 laboratories changed their entire system – all 9 improved their mark from insufficient.
Table 2. Improvement of results from insufficient to sufficient as consequence of recommendations given to 37 laboratories participating in all three PR assessments.

As described previously the uterine cervix seems to be an appropriate control for the evaluation of the sensitivity of the PR staining. In an optimal protocol almost all the columnar epithelial cells, the basal squamous epithelial cells and the stromal cells shall show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction.

Conclusion
In particular the mAb clones PGR 636 and 16 and the rmAb clone 1E3 are all well performing and robust Abs for PR. HIER is mandatory. For optimal demonstration an alkaline buffer is preferable. The concentration of the Ab must be carefully calibrated on an appropriate control such as the uterine cervix.

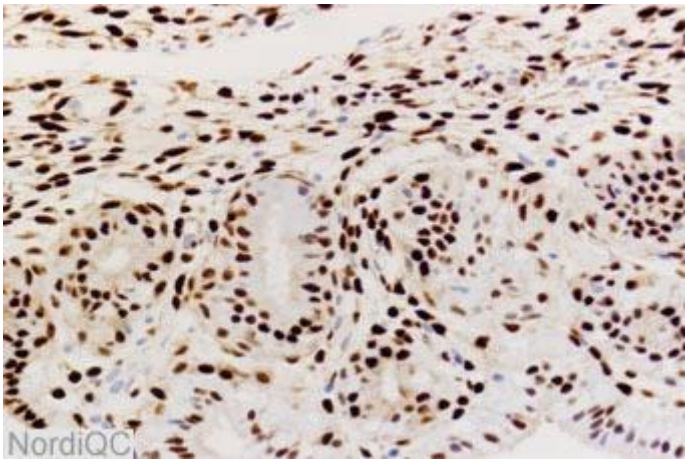

Fig. 1a
Optimal staining for PR of the cervix using the mAb clone 1E2. The stromal cells show a strong nuclear staining and the columnar epithelial cells a moderate to strong nuclear staining. A weak reaction is seen in the cytoplasma of the PR positive cells, while the background is clean.

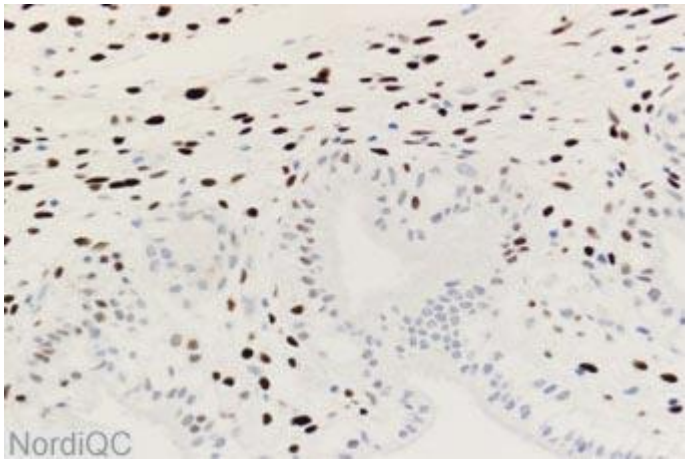

Fig. 1b
Insufficient staining for PR of the cervix, same field as in Fig. 1a using a too low sensitive protocol. The stromal cells show a strong nuclear staining, but only scattered columnar epithelial cells are demonstrated. Compare with Fig. 3b – same protocol.

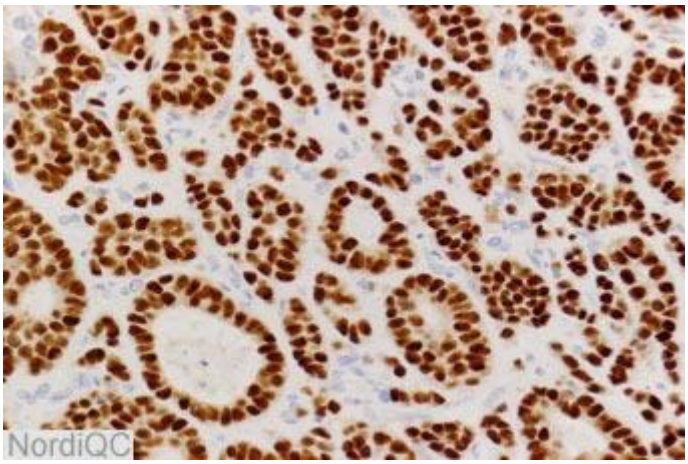

Fig. 2a
Optimal staining for PR of the ductal breast carcinoma no. 4 in which almost 100% of the neoplastic cells show a nuclear reaction. Same protocol as in Fig.1a.

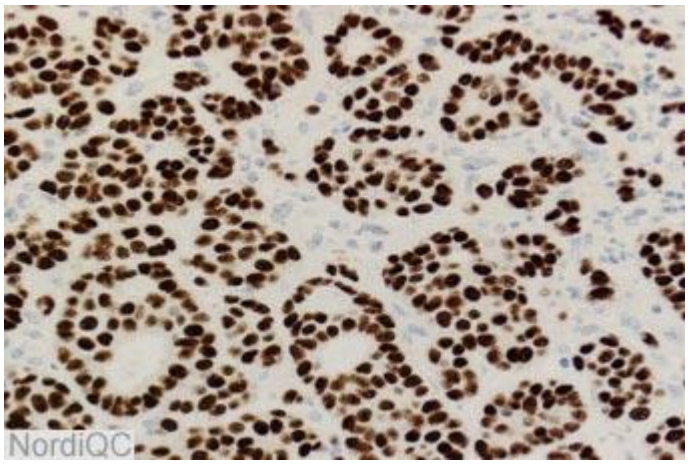

Fig. 2b
Staining for PR of the ductal breast carcinoma no. 4 in which almost 100% of the neoplastic cells show a nuclear reaction. Same protocol as in Fig.1b. However also compare with Fig. 3 b.

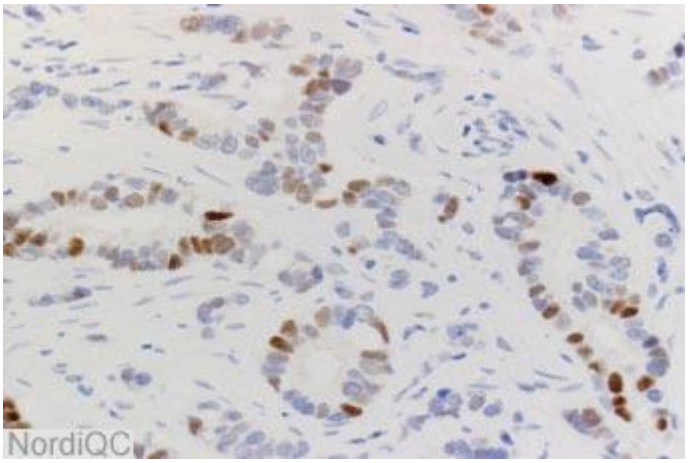

Fig. 3a
Optimal staining for PR of the ductal breast carcinoma no. 3 in which 40-60 % of the neoplastic cells show a nuclear reaction. Same protocol as in Fig.1a.

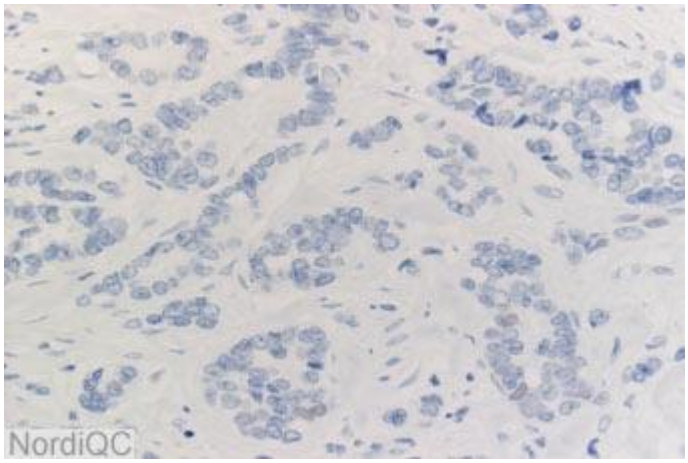

Fig. 3b
Insufficient staining for PR of the ductal breast carcinoma no. 3. The proportion of the positive cells is <10 % and thus false negative, same protocol as in Fig. 1b.

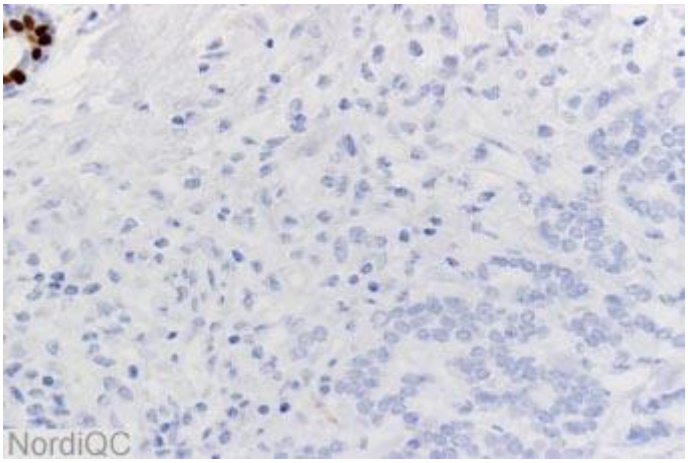

Fig. 4a
Optimal staining for PR of the PR negative ductal breast carcinoma no. 2 using the rmAb SP2 in a correctly calibrated protocol. The neoplastic cells are negative and only the normal epithelial glands show a distinct nuclear reaction (left).

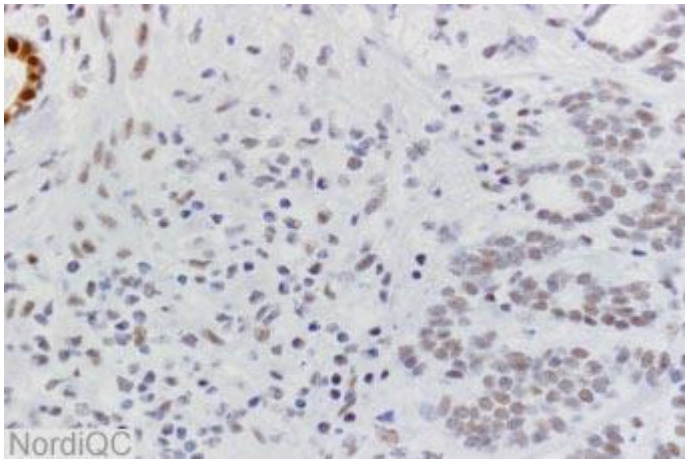

Fig. 4b
Insufficient staining for PR of the PR negative ductal breast carcinoma no. 2 using the rmAb SP2 in a too high concentration. The majority of the neoplastic cells show a false positive nuclear reaction. Compare with Fig.4a, same field.

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