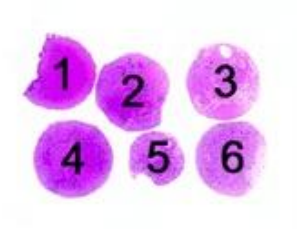
Assessment Run B3 2007

Estrogen Receptor alpha (ER)

The slide to be stained for ER comprised:
1. Uterine cervix, 2. Lobular Breast carcinoma with 80 - 100% positivity, 3. Breast fibrocystic disease, 4-6. Ductal Breast carcinoma with the following ER status 4: negative, 5: 40 – 60 % and 6: 80 – 100 % positivity as defined in four reference laboratories.
(the block is identical to the multitissue block used in run B1 2006).
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing an ER staining as optimal included:

- A strong, distinct nuclear staining of both the columnar and squamous epithelial cells and most stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- A strong, distinct nuclear staining of the epithelial cells in the fibrocystic disease.
- A strong, distinct nuclear staining of the appropriate proportion of neoplastic cells in the ductal breast carcinomas no. 5 and 6 and in the breast lobular carcinoma.
- No nuclear staining in the ductal breast carcinoma no. 4 but a week, focal positivity of the stromal cells.
- No more than a weak cytoplasmic reaction in cells with strong nuclear staining.

73 laboratories participated in the assessment. 38 achieved optimal marks (52 %), 23 good (32 %), 11 borderline (15 %) and 1 (1 %) poor marks.

The following antibody clones were used:
rmAb clone **SP1** (NeoMarkers, n=13; Ventana, n=11; Diagnostic Biosystems; n=1).
mAb clone **1D5** (Dako n=24; Immunotech, n=1).
mAb clone **6F11** (Novocastra, n=12; Ventana, n=8; Monosan, n=1).
mAb clones **1D5 + ER-2-123** (Dako ER/PR pharmDx, n=2).

Optimal staining for ER in this assessment was obtained with the rmAb clone **SP1** (16 out of 25, 64%), the mAb clone **1D5** (7 out of 25, 28%) and the mAb clone **6F11** (15 out of 21, 71%). All 38 optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

SP1: all protocols resulting in an optimal staining were based on HIER using either Tris**-**EDTA/EGTA pH 9 (8 out of 10 laboratories using this obtained an optimal mark), Cell Conditioning1 (Benchmark, Ventana, 6 out of 11 laboratories using this obtained an optimal mark), EDTA/EGTA pH 8 (only one laboratory used this buffer and got the an optimal mark) or Citrate buffer pH 6 (1 out of 2 laboratories using this obtained an optimal mark). The Ab was typically used in the range of 1:25 - 1:100 or applied as a Ready-To-Use (RTU) Ab. Using these settings 23 out of 25 (92 %) obtained a sufficient staining.

1D5: all protocols resulting in an optimal staining were based on HIER using Tris-EDTA/EGTA pH 9 (7 out of 14 using this obtained optimal marks). The Ab was typically diluted in the range of 1:50 – 1:100. Using these settings 16 out of 20 (80 %) obtained a sufficient staining.

6F11: all protocols resulting in an optimal staining were based on HIER using either Tris-EDTA/EGTA pH 9 (8 out of 9 laboratories using this obtained an optimal mark), Cell Conditioning1 (Benchmark, Ventana, 5 out of 9 laboratories using this obtained an optimal mark), Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems, only one laboratory used this buffer and got an optimal mark) or Citrate buffer pH 6 (only one laboratory used this buffer and got an optimal mark). The Ab was typically used in the range of 1:50 – 1:300 depending on the total sensitivity of the protocol employed, or applied as a RTU Ab. Using these settings 18 out of 20 (90 %) obtained a sufficient staining.

The most frequent causes of insufficient staining were:
- Insufficient HIER (citrate pH 6 for the clone 1D5 and/or too short heating time)
- Too low concentration of the primary antibody
- Use of a biotin based detection system.

The tissues circulated in this run were the same as used in run B1 2006 allowing a direct comparison. In both runs false negatives as well as false positives are seen. In the current run the prevalent feature was a false negative reaction especially the lobular carcinoma, typically seen in protocols using HIER in Citrate pH 6 or too short HIER time. The false positive reaction was typically seen in the lobular breast carcinoma (compromising the interpretation of the specific nuclear reaction of ER) in protocols based on a biotin based detection system in combination with HIER espc. in an alkaline buffer. However compared to run B1, the number of laboratories obtaining false positive reactions was reduced from 9 to 4, in parallel with the declining number of laboratories using biotin based detection systems.
This was the 5th NordiQC assessment of ER. The proportion of sufficient results has increased relatively constant through the individual assessments. Many factors contribute to the improvement but especially the focus on 1. HIER, 2. Ab. concentration and 3. Use of non-biotin based detection systems seems to be the main parameters for an optimal demonstration of ER. If these parameters are individually adjusted for the Ab, the choice of clone is of less importance. However the rmAb SP1 seems to be slightly more robust than both 1D5 and 6F11. Using the individual protocol settings as listed to obtain an optimal staining for each of the 3 clones, 28 out of 30 laboratories (93%) using SP1 obtained a sufficient result for ER in run B1 and B3, compared to 34 out of 41 laboratories using 6F11 (83%) and 32 out of 39 laboratories (82%) using 1D5.

The uterine cervix seems to be an appropriate control for the evaluation of the sensitivity of the ER staining. In an optimal protocol almost all squamous epithelial cells shall show a distinct nuclear reaction and also the majority of stromal cells shall be demonstrated.

Conclusion
The mAb clones 6F11, 1D5 and the rmAb SP1 are all well performing Abs for ER. 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.

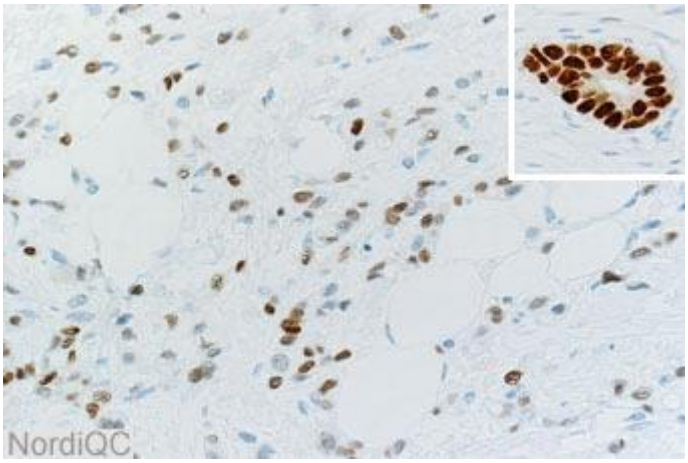


Fig. 1a
Optimal ER staining of the lobular breast carcinoma with the rmAb clone SP1. The majority of the tumour cell nuclei are strongly stained. Same protocol as in Fig. 1b.
Insert: High magnification of the ER staining in the ductal breast carcinoma with 80 - 100% positivity using the same protocol.

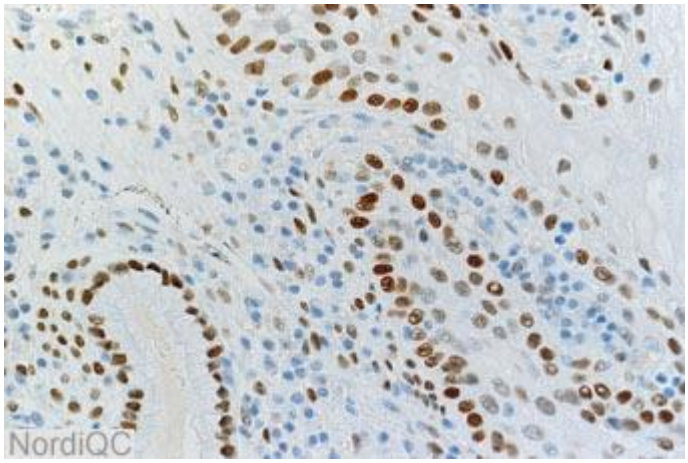


Fig. 1b
Optimal ER staining of the uterine cervix. Virtually all columnar and squamous epithelial cells show a distinct nuclear staining. The smooth muscle cells are also stained, while endothelial and lymphoid cells are negative.

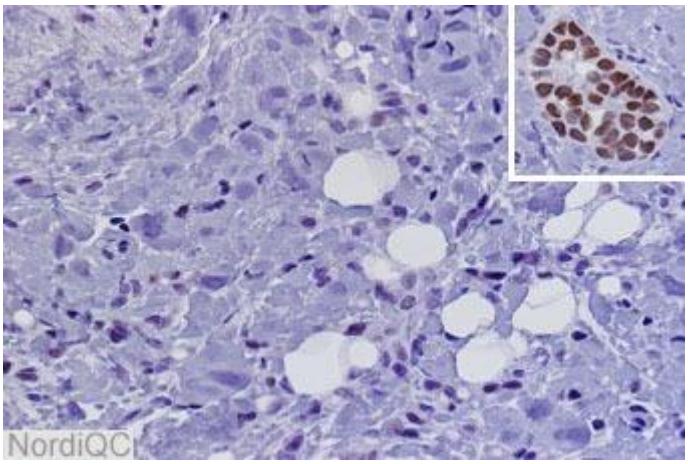


Fig. 2a
Insufficient ER staining of the lobular breast carcinoma. All nuclei are weakly stained and an excessive counterstain complicates the interpretation. (Also compare with Fig. 2b, same protocol). Insert: High magnification of the ER staining in the ductal breast carcinoma with 80 - 100% positivity using same protocol.

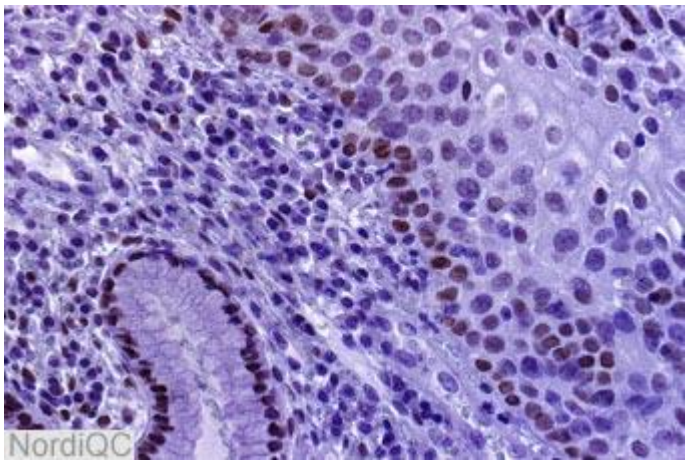


Fig. 2b
Insufficient ER staining of the uterine cervix (same field as in Fig. 1b). Only a subset of epithelial cells shows a nuclear reaction.

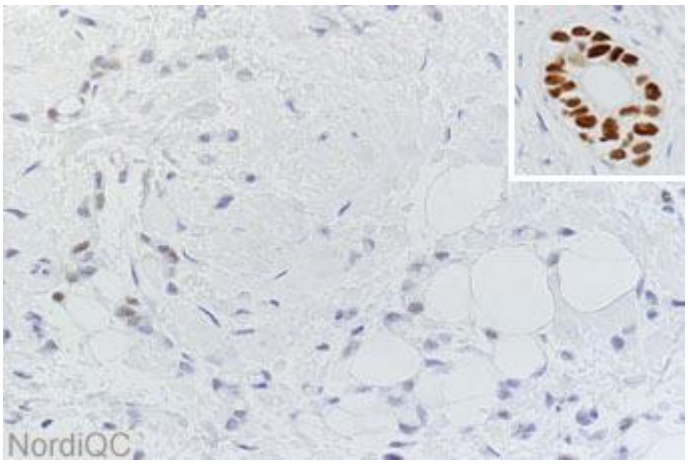


Fig. 3a
Insufficient ER staining of the lobular breast carcinoma. All nuclei are virtually negative. Also compare with Fig. 3b, same protocol). The mAb clone 1D5 has been used in a too low concentration.
Insert: High magnification of the ER staining in the ductal breast carcinoma with 80 - 100% positivity using the same protocol.

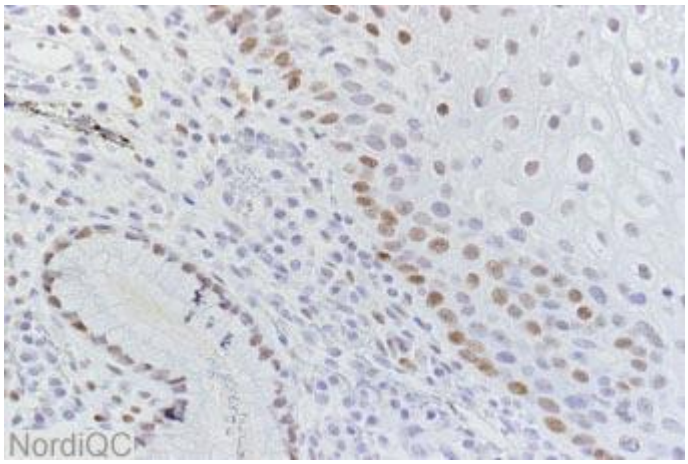
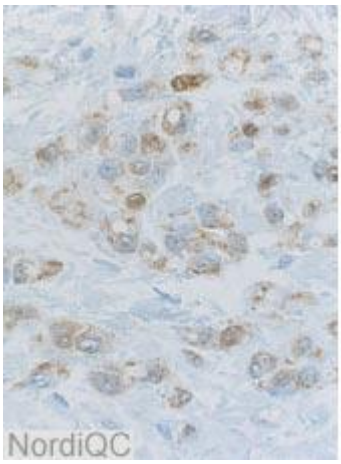


Fig. 3b
Insufficient ER staining of the uterine cervix (same field as in Fig. 1b). Only a subset of epithelial cells shows a nuclear reaction.



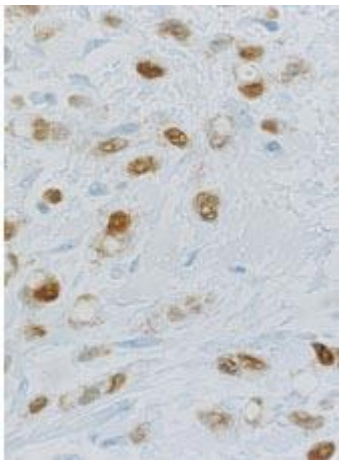


Fig. 4a
Left: Insufficient staining of the lobular breast carcinoma. There is a moderate to strong cytoplasmic staining and only minimal nuclear reaction. This pattern is most likely caused by HIER in an alkaline buffer in combination with a too dilute primary Ab and a biotin based detection system.
Right: Staining scored as sufficient (good). Same protocol as in Fig. 4a left, but using the mAb in a higher concentration. A positive nuclear reaction can be detected despite the cytoplasmic reaction due to endogenous biotin.

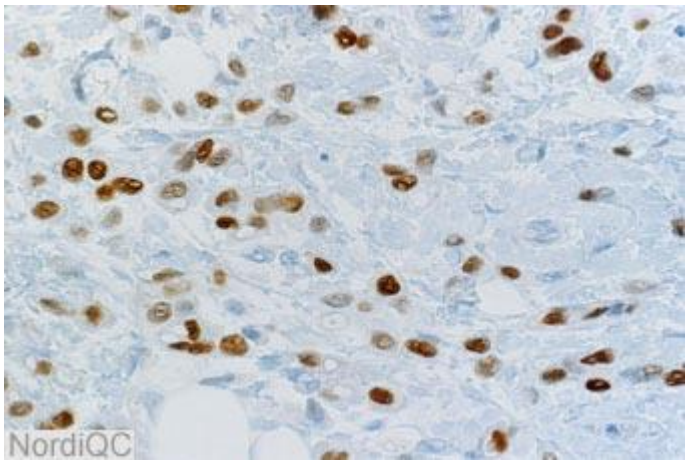


Fig. 4b
High magnification of the optimal ER staining of the lobular breast carcinoma. The nuclei are strongly stained and no cytoplasmic reaction is seen. Same protocol as in fig. 1a – 1b using the rmAb SP1 with HIER in an alkaline buffer and a multimer/polymer based detection system.
Compare the optimal result with the staining in fig. 4a.

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