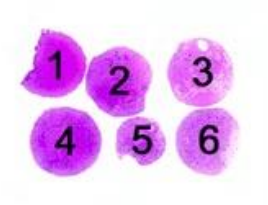
Assessment Run B1 2006

Estrogen Receptor alpha (ER)



The slide to be stained for estrogen receptor alpha (ER) comprised:
1. Uterine cervix, 2. Lobular Breast carcinoma, ER status 80 – 100 % positivity
3. Fibrocystic disease, 4 - 6. Ductal Breast carcinoma with following ER status
4: negative, 5: 40 – 60 % and 6: 80 – 100 % positivity as verified in 4 reference IHC laboratories. All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an ER staining as optimal included:

- A strong and distinct nuclear staining of both the columnar and squamous epithelial cells as well as the stromal cells (with the exception of endothelial cells and smooth muscle cells) in the uterine cervix.
- A strong and distinct nuclear staining of the epithelial cells in the fibrocystic disease.
- A strong and distinct nuclear staining of the ductal breast carcinomas no. 5 and 6 and the breast lobular carcinoma in accordance with the ER status.
- No staining of the ductal breast carcinoma no. 4 (but a week and focal positivity of the stromal cells).
- No or only a weak cytoplasmic reaction of cells with strong nuclear staining.

68 laboratories submitted stains. At the assessment 25 achieved optimal marks (37 %), 26 good (38 %), 7 borderline (10 %) and 10 (15 %) poor marks.

The following mAbs were used:
mAb clone **6F11** (Novocastra, n=15; Ventana, n=14).
mAb clone **1D5** (Dako n=25; Immunotech, n=2; NeoMarkers, n=1).
rmAb clone **SP1** (NeoMarkers, n=8).
mAb clones **1D5 + ER-2-123** (Dako ER/PR pharmDx, n=2).
mAb clones **6F11+1D5** (NeoMarkers, n=1).

Optimal staining for ER in this assessment was obtained with the mAbs clone **6F11** (10 out of 29), clone **1D5** (10 out of 28), clone **SP1** (4 out of 8) and the cocktail clones **1D5 + ER-2-123** (1 out of 2).

All 25 optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

With mAb clone **6F11** all protocols resulting in an optimal staining were based on **HIER** using either **Tris-EDTA/EGTA pH 9** (7 out of 13 laboratories using this obtained an optimal mark), or **CC1** (Cell Conditioning 1 - Ventana, 3 out of 12 laboratories using this obtained an optimal mark). Clone 6F11 was typically diluted in the range of 1:35 – 1:200 depending on the total sensitivity of the protocol employed. Using these settings 7 out of 8 (88%) obtained a sufficient staining. **6F11** could also be used as a ready-to-use (RTU) Ab. 9 out of 13 using 6F11 as RTU obtained a sufficient staining (69%).

With mAb clone **1D5** all protocols giving an optimal staining were based on HIER using Tris-EDTA/EGTA pH 9 (10 out of 24 using this obtained optimal marks). Clone 1D5 was typically diluted in the range of 1:50 – 1:100. Using these settings 16 out of 19 (84%) obtained a sufficient staining.

With rabbit mAb (rmAb) clone **SP1**, all optimal protocols were based on **HIER** using either **CC1** (3 out of 3 laboratories using this obtained an optimal mark) or **Tris-EDTA/EGTA pH 9** (1 out of 3 using this obtained an optimal mark). Clone SP1 was typically used in the range of 1:50-1:100. Using these settings 5 out of 5 (100%) obtained a sufficient staining.

With mAb clones **1D5 + ER-2-123**, the optimal protocol was performed according to the protocol of the ER-PharmDx kit (Dako) based on **HIER** in Target Retrieval Solution and a RTU Ab. 1 out of 2 using this kit obtained an optimal mark, the other a good mark.

The most frequent causes of insufficient staining were:
- Excessive false positive cytoplasmic staining due to endogenous biotin (9/17)

- Insufficient heat induced epitope retrieval (citrate pH 6 and/or too short heating time) (5/17)
- Too low concentration of the primary antibody (3/17)

In this assessment the most prevalent feature of the insufficient results was a false positive cytoplasmic reaction of the lobular breast carcinoma compromising the interpretation of the nuclear reaction of ER. This was seen in protocols based on the use of a biotin based detection system and performing HIER in an alkaline buffer. It is highly recommended to either block the endogenous biotin or change the detection system to a polymer based system. It is **not** recommendable to change HIER system to a less efficient buffer (such as Citrate pH 6) to reduce the biotin reaction, as this change also will decrease the sensitivity of the ER detection. Another prevalent feature of the insufficient results was – as in the previous ER assessments – a false negative reaction of ER in the tested specimens, especially seen in the lobular carcinoma. This was typically seen in protocols using HIER in Citrate pH 6 or too short HIER time.

As found in the previous runs the uterine cervix can be used as an appropriate control for the evaluation of the sensitivity of the ER staining. In the optimal protocols almost all epithelial cells throughout the cell layers showed a distinct nuclear reaction compared to the protocols giving insufficient results in which only the basal epithelial cells were demonstrated.

This was the 4th NordiQC assessment of ER and the first in the new breast module B1. Compared to the previous assessment (run 13 2005), the proportion of insufficient results increased from 16 % to 25 %. However, including only laboratories participating in both runs (n=57), almost the same proportion of insufficient results was seen (25% and 23%, respectively). The insufficient results in the present run were mainly due the excessive false positive cytoplasmic reaction of the breast lobular carcinoma and/or false negative staining reactions in the same tumour.

Conclusion
The mAb clones **6F11**, **1D5**, **1D5 + ER-2-123** and the rmAb clone **SP1** are all well functioning ER Abs.

HIER in an alkaline buffer (e.g., Tris-EDTA/EGTA pH 9 or CC1) is highly recommended for optimal demonstration of ER with these clones.
Endogenous biotin should be suppressed, or a biotin free detection system employed.

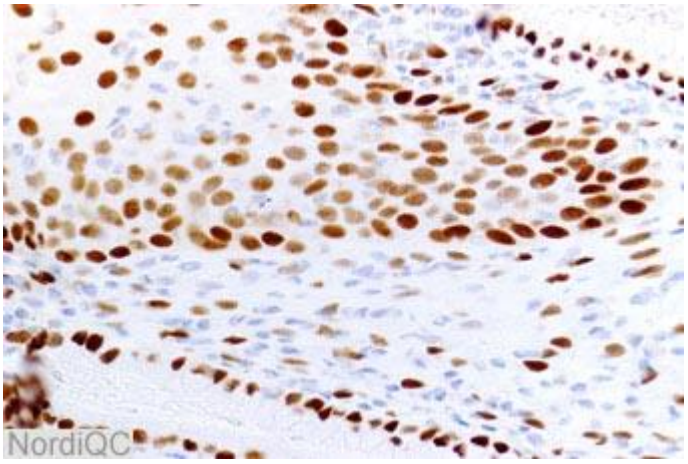


Fig. 1a
Optimal ER staining of the uterine cervix. Both stromal cells and columnar and squamous epithelial cells are positive, almost all nuclei are strongly stained.

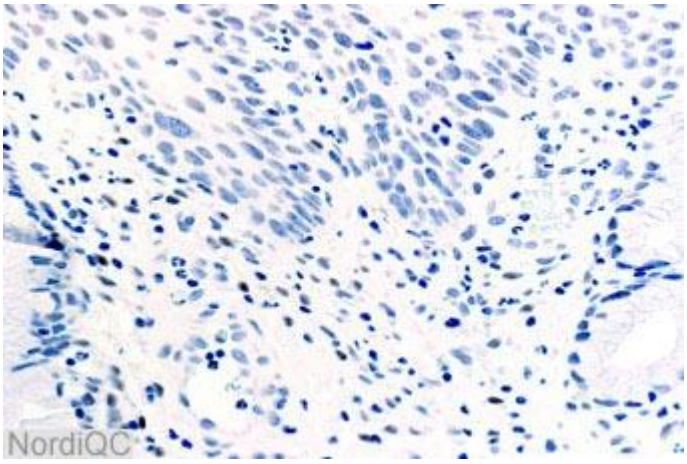


Fig. 1b
Insufficient ER staining of the uterine cervix Only a few stromal and columnar epithelial cells are faintly positive.

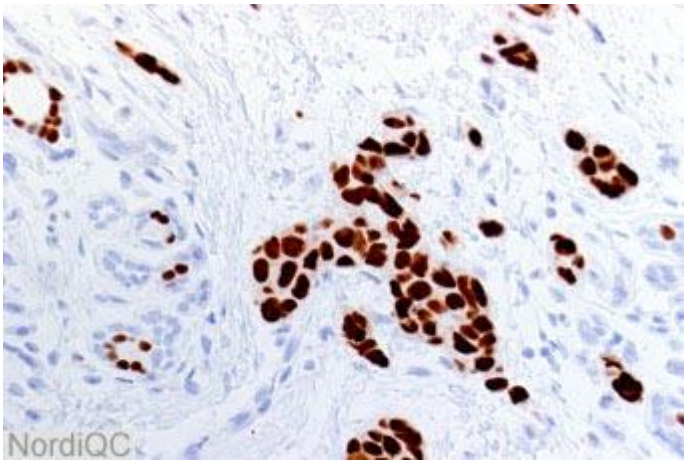


Fig. 2a
Optimal ER staining of the ductal breast carcinoma with 80 – 100 % cells positive. All nuclei are strongly stained with a weak cytoplasmic reaction. Same protocol as in Fig. 1a.

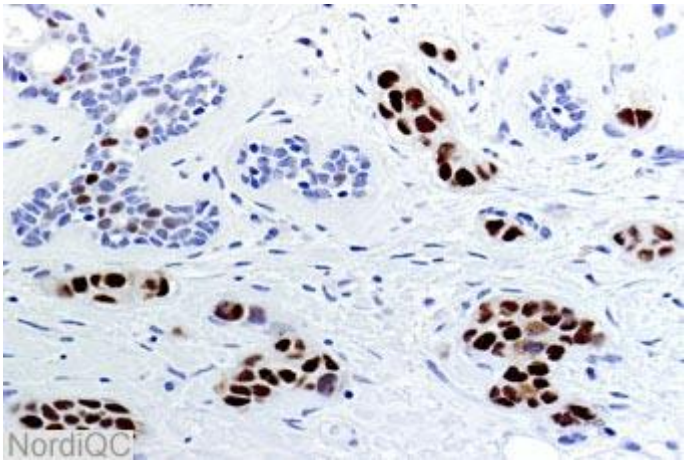


Fig. 2b
ER staining of the ductal breast carcinoma with 80 – 100 % cells positive using an insufficient protocol. Almost all nuclei are stained. However, compare with Figs. 1b and 3b – same protocol.

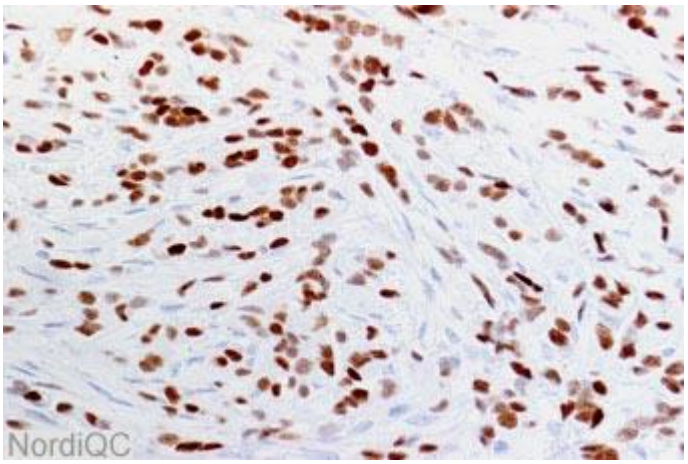


Fig. 3a
Optimal ER staining of the lobular breast carcinoma with 80 – 100 % cells positive. All nuclei are strongly stained. Same protocol as in Figs. 1a and 2a.

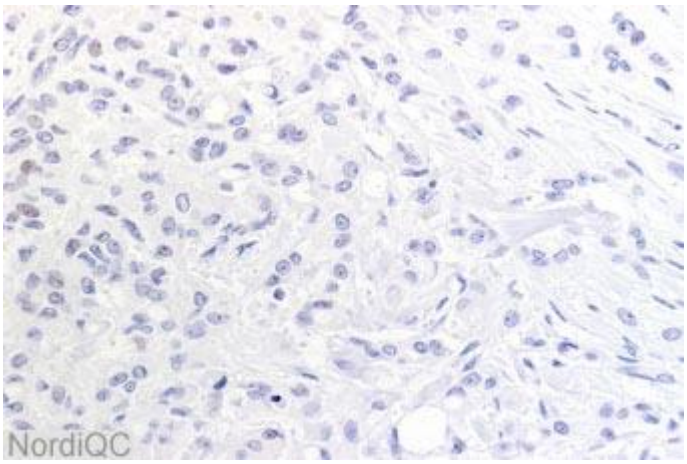


Fig. 3b
Insufficient ER staining of the lobular breast carcinoma with 80 – 100 % cells positive. Only few nuclei are faintly stained. Same protocol as in Figs. 1b and 2b.

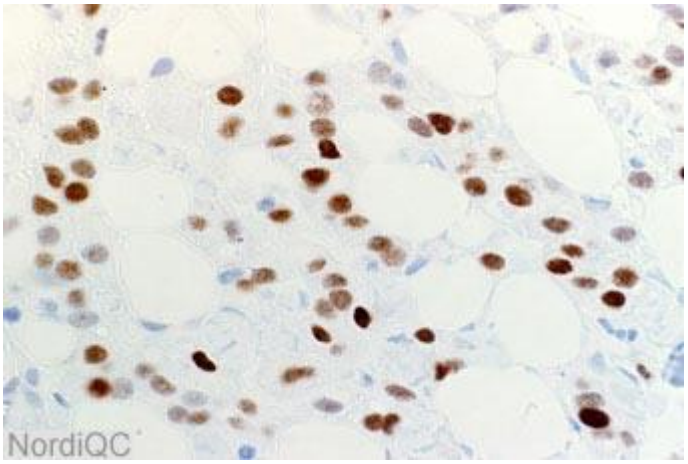


Fig. 4a
High magnification of the optimal ER staining of the lobular breast carcinoma with 80 – 100 % cells positive. The nuclei are strongly stained, no cytoplasmic reaction is seen. Same protocol as in Figs. 1a - 3a.
Compare the optimal result with the staining in Fig. 4b.

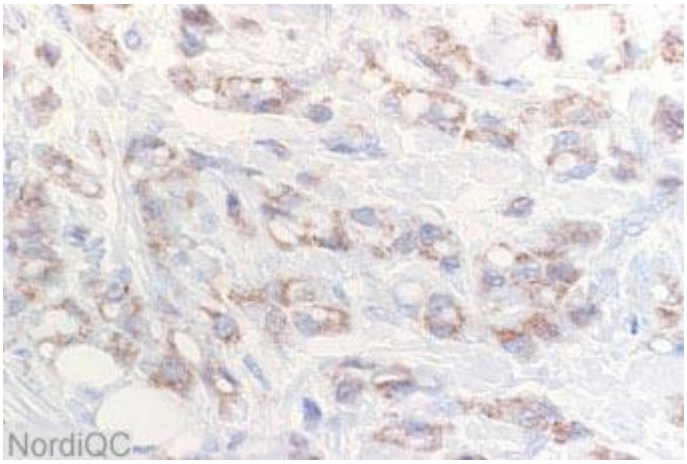


Fig. 4b
This reaction pattern was the most frequent cause for an insufficient ER staining. The lobular breast carcinoma with 80 – 100 % cells positive show a moderate to strong (false positive) cytoplasmic staining and no nuclear reaction. This pattern was typically due to the combination of HIER in an alkaline buffer, a primary Ab in a too low concentration, and a biotin based detection system.

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