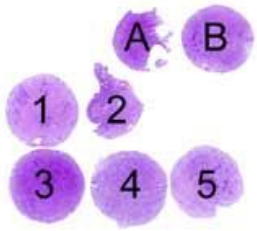
Assessment Run 13 2005

Estrogen Receptor alpha (ER)



The slide to be stained for estrogen receptor alpha (ER) comprised:
1. Breast fibrocystic disease, 2. Uterine cervix, 3 - 5. Ductal breast carcinoma with following ER status 3: negative (very few cells positive), 4: 40 – 60 % of cells positive, 5: 80 – 100 % of cells positive. Due to differences in the ER expression in tumour 4 throughout the sections from the multiblocks two specimens were added: A. breast lobular carcinoma with 30 – 50 % positivity and B. breast ductal carcinoma with 40 – 60 % positivity. The ER status was verified by 4 reference IHC laboratories. All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an ER staining as optimal included:
A distinct nuclear reaction in all cells expected to stain in the fibrocystic disease, ductal carcinomas and lobular carcinoma. A weak cytoplasmic reaction in cells with strong nuclear staining was accepted. In the uterine cervix virtually all epithelial and stromal cells (with the exception of endothelial cells and smooth muscle cells) should be positive.

89 laboratories participated in the assessment. At the assessment 33 achieved optimal (37 %), 42 good (47 %), 13 borderline (15 %) and 1 (1%) poor marks.

The following antibody clones were used:
mAb clone **1D5** (DakoCytomation, n=39; Immunotech, n=1; Zymed, n=1).
mAb clone **6F11** (Novocastra, n=24; Ventana, n=14).
mAb clone **SP1** (NeoMarkers, n=9).
mAb clone **6F11+1D5** (NeoMarkers, n=1).

Optimal stainings could be obtained with the mAbs 1D5 (10 out of 41), 6F11 (18 out of 38 were optimal), and SP1 (5 out of 9).

In the optimal protocols all used HIER. Using mAb clone 1D5 all 10 protocols were based on HIER in Tris-EDTA/EGTA pH 9. Using mAb clone 6F11 the majority were based on Tris-EDTA/EGTA pH 9, but also CC1 Ventana Benchmark and EDTA pH 8 could give optimal results. Using mAb clone SP1, optimal results were obtained with Tris-EDTA/EGTA pH 9, TRS pH 9,9 DakoCytomation and CC1 Ventana Benchmark.

In the optimal staining mAb clone 1D5 was used in the range of 1:25 – 1:75, mAb clone 6F11 in the range of 1:20 – 1:100, and mAb clone SP1 in the range of 1:50 - 300 (all ranges depending on the total sensitivity of the IHC protocols applied).

The probable causes of the insufficient staining were similar to the causes identified in the previous assessment (run 8 and run 10):
- Insufficient HIER, too short and especially too short efficient heating time (i.e. <15 min. in a MWO)
- HIER with Citrate buffer pH 6
- Too dilute concentration of the primary antibody concentration.

The most prevalent feature of the insufficient results was a false negative reaction of the lobular breast carcinoma and a too weak reaction of the ductal carcinomas with 40 – 60 % positivity, as well as a too weak staining of the uterine cervix. Almost all laboratories were able to detect ER in the ductal carcinoma with 80 - 100 % positivity.

This was the 3th assessment of ER in NordiQC. Comparing the overall results, the laboratories in the 3 runs have reduced the proportion of insufficient staining from 55 % in run 8, to 32 % in run 10 and to 16 % in this run. The two main recommendations giving to the laboratories in run 8 and 10 achieving an insufficient staining were:
1) to optimize HIER (prolong heating time and/or substitute Citrate pH 6 with Tris/EDTA pH 9), and
2) increase the primary antibody concentration.

In run 8, 25 laboratories were recommended to change their protocol accordingly to the two above mentioned

recommendations. 13 laboratories changed their protocol accordingly and of these 10 improved their result in run 10.
In run 10, 22 laboratories were given the same recommendations and in run 13, 16 followed the advice of which 14 improved their result from insufficient to good or optimal. 6 out of 22 laboratories did not change their protocol. Of these, 2 improved their score from insufficient to good, while the remaining 4 still had an insufficient staining.

Grouped together, 47 recommendations have been given. 29 laboratories followed the recommendations and 24 of these (83%) improved their staining from insufficient to good/optimal. 18 laboratories did not follow the recommendations and 5 of these (27 %) improved their staining.

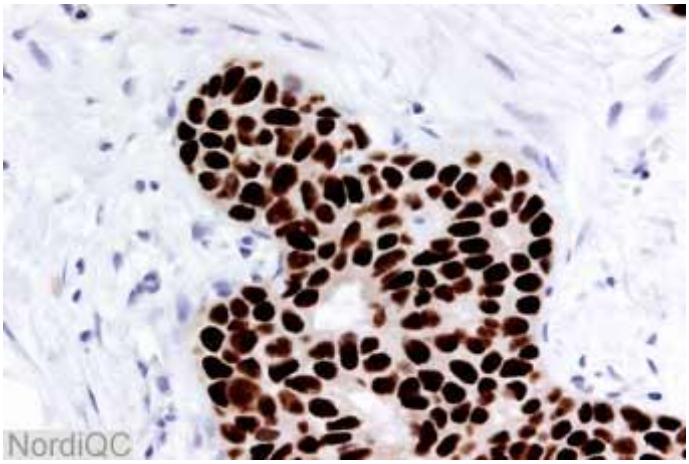


Fig. 1a
Optimal ER staining of the ductal breast carcinoma with 80 – 100 % cells positive. All nuclei are strongly stained with a weak cytoplasmic reaction.

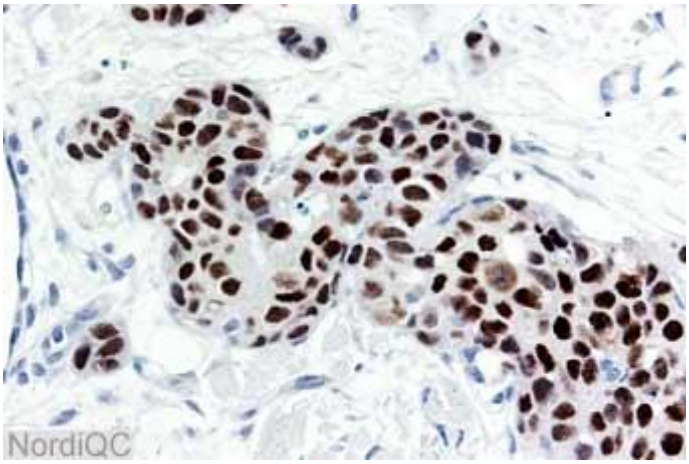


Fig. 1b
ER staining of the ductal breast carcinoma with 80 – 100 % cells positive (same field as in in Fig. 1a) using an insufficient protocol. Almost all nuclei are stained. However, compare with fig. 2b. – same protocol.

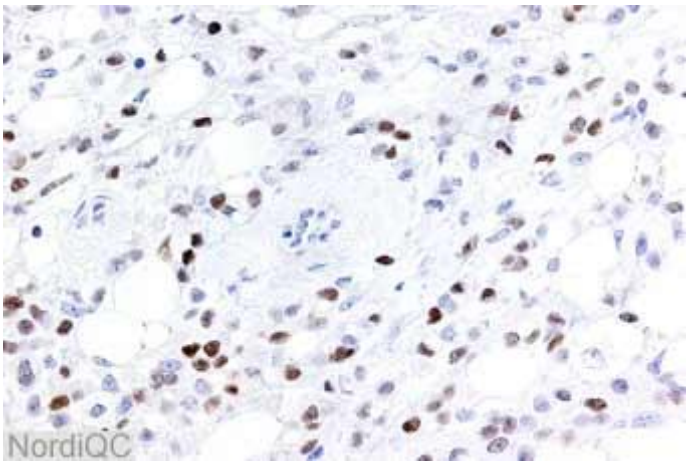


Fig. 2a
Optimal ER staining of the lobular breast carcinoma with 30 – 50 % cells positive.

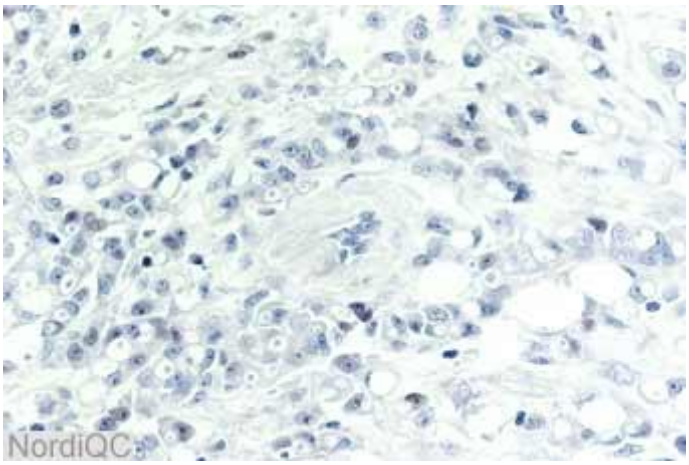


Fig. 2b
Insufficient ER staining of the lobular breast carcinoma with 30 – 50 % cells positive (same field as in Fig. 2a). The neoplastic cells are all negative (same protocol as fig. 1b.).

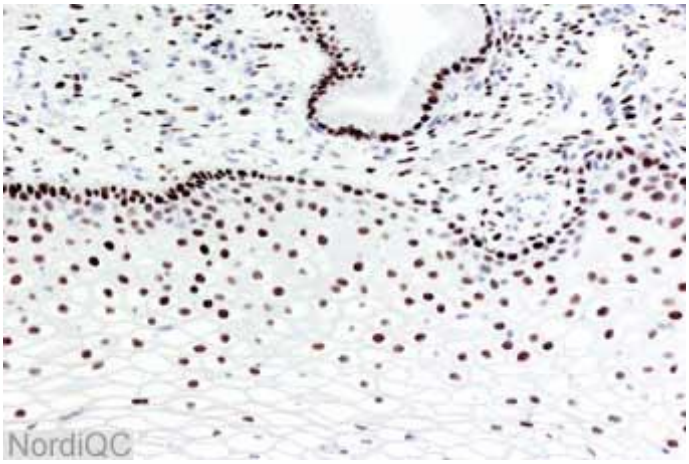


Fig. 3a
Optimal ER staining of the uterine cervix.
Almost all nuclei are strongly stained. Both stromal cells and columnar and squamous epithelial cells are labelled.

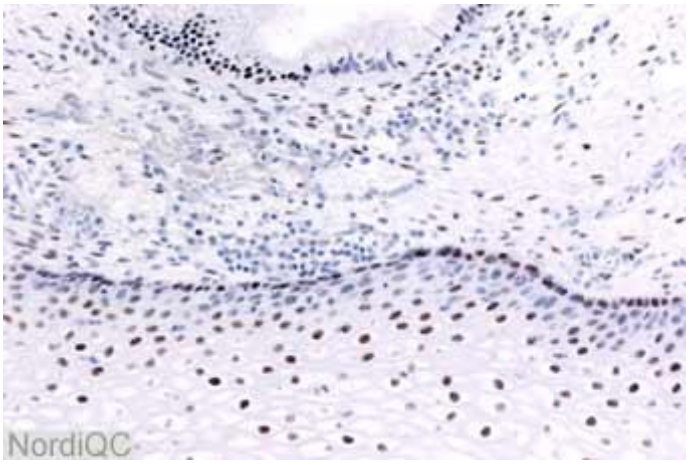


Fig. 3a
Insufficient ER staining of the uterine cervix (same field as in Fig. 3a; protocol as in figs. 1b. and 2b.). Many cells, particularly stromal cells, are unstained.

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