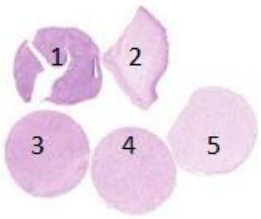
## Assessment Run B37 2024

# Estrogen receptor (ER)

**Purpose**
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests performed by the NordiQC participants for demonstration of estrogen receptor (ER) expression in breast carcinomas. IHC, based on the rmAb clones SP1 and EP1, performed in a NordiQC reference laboratory served as reference standard methods and were used to identify breast carcinomas with the dynamic, diagnostic and critical relevant expression levels of ER. The obtained score in NordiQC is indicative of the performance of the IHC tests, but due to the limited number and composition of samples internal validation and extended quality control (e.g. regularly measurement of ER results) is needed.

**Material**
The slide to be stained for ER comprised:

| No. | Tissue | ER-positivity* | ER-intensity* |
|---|---|---|---|
| 1. | Tonsil | 1-5% | Weak to moderate |
| 2. | Uterine cervix | 80-90% | Moderate to strong |
| 3. | Breast carcinoma | 0% | Negative |
| 4. | Breast carcinoma | 90-100% | Moderate to strong |
| 5. | Breast carcinoma | 50-80% | Weak to moderate |




* ER-status and staining pattern as characterized by NordiQC reference laboratories using the rmAb clones EP1 and SP1.

All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Allison et al.[1]

Criteria for assessing an ER staining as **optimal** included:

- A moderate to strong, distinct nuclear staining of virtually all columnar epithelial cells (if present), most squamous epithelial and stromal cells (with the exception of endothelial cells and lymphoid cells) in the uterine cervix.
- An at least weak to moderate nuclear staining reaction in scattered follicular dendritic cells/T-cells and squamous epithelial cells in the tonsil easily identified at low magnification (5x).
- An at least weak to moderate distinct nuclear staining in the appropriate proportion of the neoplastic cells in the breast carcinomas, tissue cores no. 4 and 5.
- No nuclear staining in the neoplastic cells in breast carcinoma, tissue core no. 3.
- No more than a weak cytoplasmic reaction in cells with a strong nuclear staining reaction.

- An ER IHC result was classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas, tissue cores no. 4 and 5 showed an at least weak nuclear staining reaction but in a significantly reduced proportion compared to the reference range. An at least weak to moderate nuclear staining reaction in the majority of the uterine columnar and squamous epithelial cells and in the dispersed cells expected to be positive in the tonsil.

  An IHC result was also assessed as **good**, if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, background staining, excessive or inselective counterstaining or impaired morphology.

- An ER IHC result was assessed as **borderline** if ≥1% and <10% of the neoplastic cells in one or both of the breast carcinomas, tissue cores no. 4 and 5, showed a nuclear staining reaction. A negative staining reaction of the cells expected to be demonstrated in tonsil/uterine cervix can also be marked as **borderline.**

  An IHC result could also be assessed as **borderline**, if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive/inselective counterstaining or impaired morphology, to the extent where interpretation was compromised.

- An IHC result was assessed as **poor** if a false negative staining (<1%) was seen in one of the breast carcinomas, tissue cores no. 4 and 5, or false positive staining (≥1%) was seen in the breast carcinoma, tissue core no. 3. Poor signal-to-noise ratio or poor morphology as described above could also result in a grade of **poor** where interpretation was severely hampered.

**KEY POINTS FOR ER IHC ASSAYS**

- Tonsil is highly recommendable to monitor ability of low-level ER demonstration
- Efficient HIER should be performed in an alkaline buffer.
- rmAb clone **EP1** provided highest pass rate both as RTU system and concentrated format
- Counterstaining must be balanced to identify ER reaction and cellular architecture

**Participation**

| | |
|---|---|
| Number of laboratories registered for ER, B37 | 448 |
| Number of laboratories returning slides | 428 (96%) |

At the date of assessment, 428 (96%) of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report. Two slides received at the time of the assessment were unable to be assessed (due to damaged coverslip and an inadequate quantity of tissue remaining on the slide) and these are excluded from this analysis.

**Results**

426 laboratories were assessed in this assessment. 361 of 426 (85%) achieved a sufficient mark (optimal or good) see Tabel 1a. Tables 1b and 1c summarizes antibodies (Abs) used and assessment marks given (see page 4 and 5).

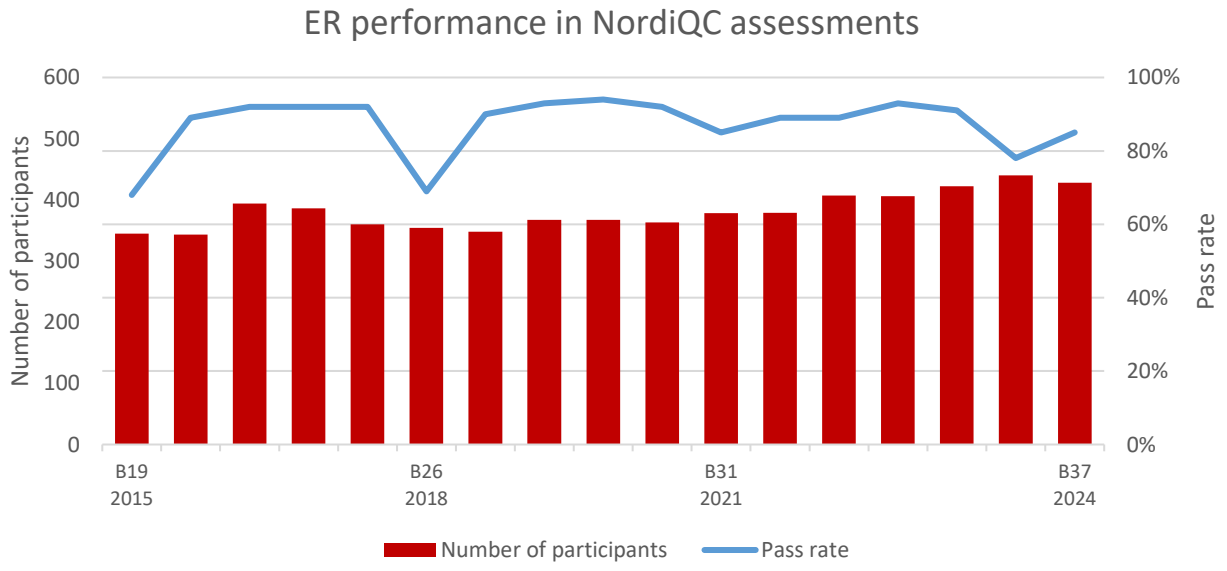
The most common staining faults reported were weak staining and excessive counterstaining hindering interpretation, and the most frequent causes of insufficient staining reactions were:

- Unexplained inferior performance of the Ventana/Roche IHC RTU system based on rmAb clone SP1
- Use of detection systems with low analytical sensitivity (weak staining)
- Insufficient Heat Induced Epitope Retrieval (HIER) time or HIER in acidic buffer (weak staining)
- Excessive HIER or primary Ab incubation time (scoring hindered by excessive background or poor signal/noise ratio)

**Performance history**

In this run a pass rate of 85% was seen and in the range of the mean level of 87% obtained since run B19 2015. Although the pass rate has been relatively stable at a high and satisfactory level in in the period between 2015-2023, few exceptions of reduced performance has been observed in runs B19, B26 and B36 (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER from 2015 - 2024**



Fluctuations in pass rates (e.g. as seen in three runs B19, B26 and B36), can be caused by the circulation of more challenging material. In order to ensure the consistency of the material circulated, NordiQC evaluates the material with two reference standard methods and monitors the ER expression levels throughout all TMAs used in the assessment. Fluctuation in pass rates may also be influenced by new participants and change of protocol settings by existing participants. In this run, a continued reduction in pass rate was in particular attributed to an inferior performance using the most common combination of primary antibody and staining platform, RTU format of rmAb clone SP1 (790-4324/790-4325) on the Ventana BenchMark platform group. As for Run B35 and B36, over half of the participants used this combination (B35 = 61%; B36 = 58%; B37 = 55%). However, the pass rate for this group fell from 95% in run B35 to 78% in Run B36, rising to 84% in this run. For the Ventana/Roche RTU system, the insufficient results were mainly characterized by reduced analytical sensitivity. At present no single parameter causing the low pass rate e.g. certain lot no of SP1 790-4324/4325 has been identified

**Controls**
In concordance with previous NordiQC runs, uterine cervix and tonsil were found to be valuable positive and negative tissue controls for ER staining: In the uterine cervix, optimal results were characterized by a moderate to strong, distinct nuclear staining reaction in virtually all epithelial cells throughout the squamous epithelium and in the glands. In the stromal compartment, moderate to strong nuclear staining reaction was seen in most cells except endothelial and lymphatic cells.

Use of tonsil as a control tissue is particularly recommended as a tool to monitor analytical sensitivity for the demonstration of ER, and this tissue is superior to uterine cervix in this context. It was observed, that dispersed cells (most likely follicular dendritic cells[2]) in germinal centers and squamous epithelial cells were distinctively demonstrated in virtually all protocols providing an optimal result. If the follicular dendritic cells were negative or weakly demonstrated, a reduced proportion of ER positive cells were seen in the other tissues and, most critically, an unsatisfactory weak or even false negative staining pattern was seen in breast carcinomas, tissue cores no. 3 and 4. In addition, tonsil can be used as supplementary negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

To validate the specificity of the IHC protocol further, an ER negative breast carcinoma must be included as primary negative tissue control, in which only remnants of normal epithelial and stromal cells should be ER positive, serving as internal positive tissue control. Positive staining reaction of the stromal cells in breast tissue indicates that the IHC protocol provides a high analytical sensitivity for ER, whereas the analytical sensitivity cannot reliably be evaluated in normal epithelial cells in breast as they typically express moderate to high levels of ER.

**Conclusion**
In this assessment, the rabbit monoclonal antibodies (rmAb) clones **SP1** and **EP1** and the mouse monoclonal Ab (mAb) clone **6F11** could all be used to provide an optimal result for demonstration of ER. The majority of participants (91%, 386 of 426) used Ready-To-Use (RTU) systems, with the majority of these (247 of 386, 64%) using the Ventana/Roche platform. Both RTU and concentrated primary antibody formats could be used successfully: the pass rate for participants using RTU antibodies was 86% (331/386), versus 75% (30/40) for concentrated formats. "Plug and play" RTU assays (where a RTU format was used on its intended fully automated platform) gave an overall pass rate of 89% across the two major manufacturers platforms (Ventana/Roche BenchMark: 84% and Dako/Agilent Omnis: 95%). In this run, the most robust performance was seen using clone EP1 for Omnis used as a true "plug and play" assay, with a pass rate of 95% and an optimal rate of 64%.

The commonest failing, accounting for 89% (58/65) of insufficient results in this assessment, was low analytical sensitivity giving a weak or false negative staining reaction. Low analytical sensitivity and weak demonstration of ER was often further complicated by excessive or "inselective" counterstaining (where nuclei were difficult to distinguish from cytoplasm), excessive background or poor signal-to-noise ratios, leading to difficulties in scoring.

Table 1a. **Overall results for ER, run B37**

| | n | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|
| Concentrated antibodies | 40 | 13 | 17 | 4 | 6 | 75% | 33% |
| Ready-To-Use antibodies | 386 | 136 | 195 | 48 | 7 | 86% | 35% |
| Total | 426 | 149 | 212 | 52 | 13 | | |
| Proportion | | 35% | 50% | 12% | 3% | 85% | |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for ER, run B37**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **6F11** | 15 | Leica Biosystems | 4 | 8 | 1 | 2 | 80% | 27% |
| rmAb clone **EP1** | 4<br>1 | Dako/Agilent<br>CellMarque | 2 | 3 | 0 | 0 | 100% | 40% |
| rmAb clone **SP1** | 11<br>3<br>1<br>1<br>1<br>1 | Thermo Sci./ePredia<br>Cell Marque<br>Zytomed<br>AbCam<br>BioCare<br>DCS | 6 | 5 | 3 | 4 | 61% | 33% |
| rmAb clone **QR013** | 1 | Quartett | 0 | 1 | 0 | 0 | - | - |
| rmAb clone **BP6139** | 1 | Biolynx Biotech. | 1 | 0 | 0 | 0 | - | - |
| Total | 40 | | 13 | 17 | 4 | 6 | | |
| Proportion | | | 33% | 42% | 10% | 15% | 75% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for ER, run 37**

| Ready-To-Use antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **6F11 PA0009/PA0151 (VRPS[3])** | 3 | Leica Biosystems | 0 | 0 | 2 | 1 | - | - |
| mAb clone **6F11 PA0009/PA0151 (LMPS[4])** | 15 | Leica Biosystems | 2 | 7 | 5 | 1 | 60% | 13% |
| rmAb **EP1 IR/IS084 (VRPS[3])** | 2 | Dako/Agilent | 1 | 1 | 0 | 0 | - | - |
| rmAb **EP1 IR/IS084 (LMPS[4])** | 23 | Dako/Agilent | 12 | 10 | 1 | 0 | 96% | 52% |
| rmAb **EP1 GA084 (VRPS[3])** | 44 | Dako/Agilent | 28 | 14 | 2 | 0 | 95% | 64% |
| rmAb **EP1 GA084 (LMPS[4])** | 32 | Dako/Agilent | 16 | 15 | 0 | 1 | 97% | 50% |
| rmAb **EP1 8361-C010** | 3 | Sakura Finetek | 1 | 2 | 0 | 0 | - | - |
| rmAb clone **SP1 790-4324/4325 (VRPS[3])*** | 61 | Ventana/Roche | 5 | 46 | 9 | 1 | 84% | 8% |
| rmAb clone **SP1 790-4324/4325 (LMPS[4])*** | 186 | Ventana/Roche | 62 | 95 | 27 | 2 | 84% | 33% |
| rmAb clone **SP1 249R-17/18** | 3 | Cell Marque | 1 | 0 | 1 | 1 | - | - |
| rmAb clone **SP1 MAD-000306QD/V MAD-000306QD-7/N** | 3 | Master Diagnostica Vitro SA | 1 | 1 | 1 | 0 | - | - |
| rmAb clone **SP1 RMPD001** | 1 | Diagnostic BioSystems | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP1 GT205602** | 1 | Gene Tech | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP1 BRB053** | 3 | Zytomed Systems | 1 | 2 | 0 | 0 | - | - |
| rmAb clone **SP1 ALR 301 G7** | 1 | BioCare Medical | 0 | 1 | 0 | 0 | - | - |
| Ab clone **658G3A2 PA212** | 1 | Abcarta | 0 | 1 | 0 | 0 | - | - |
| Ab clone **C6H7 CEM-0081** | 1 | Celnovte | 1 | 0 | 0 | 0 | - | - |
| Ab clone **DA155 DMRD0191** | 1 | Shenzhen Dartmon Biotech. | 1 | 0 | 0 | 0 | - | - |
| Ab clone **MXR030 RMA-1065** | 2 | Fuxhou Maixin | 2 | 0 | 0 | 0 | - | - |
| Total | 386 | | 136 | 195 | 48 | 7 | | |
| Proportion | | | 35% | 51% | 12% | 2% | 86% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Product used on another platform than developed for

### Detailed analysis of ER: Run B37

The following protocol parameters were central to obtain optimal staining:

### Concentrated antibodies

mAb clone **6F11**: Four of 15 laboratories obtained optimal results: all were based on high pH HIER, using Cell Conditioning 1 (CC1, Ventana/Roche) (1/2)* or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) pH 9.0 (3/11) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 and combined with a 2- or 3-layer detection system. Using these protocol settings, 9 of 10 (90%) laboratories produced a sufficient staining result (optimal or good), and 4 of 10 laboratories produced an optimal result. Low pH HIER (Bond Epitope Retrieval Solution 1, Leica Biosystems) pH 6.0 was employed by one laboratory, without a sufficient result.

* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Two of 5 laboratories obtained an optimal result, using a protocol based on high pH HIER, using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (2/2) as retrieval buffer. The rmAb was diluted 1:50-100 and combined with a 2-layer detection system. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Six of 18 laboratories obtained optimal results. Protocols with optimal results were all based on high pH HIER, using CC1 (Ventana/Roche) (4/7) or TRS pH 9 (Dako/Agilent) (2/3) as retrieval buffer. The rmAb was typically diluted in the range of 1:30-1:200 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 11 of 16 (69%) laboratories produced a sufficient staining result.

Table 2 summarizes the overall proportion of optimal staining results when using the three most frequently used concentrated Abs on the most commonly used IHC staining platforms.

Table 2. **Optimal results for ER using concentrated antibodies on the main IHC systems***

| Concentrated antibodies | **Dako/Agilent Autostainer[1]** | | **Dako/Agilent Omnis** | | **Ventana/Roche BenchMark[2]** | | **Leica Biosystems Bond[3]** | |
|---|---|---|---|---|---|---|---|---|
| | TRS High pH 9.0 | TRS Low pH 6.1 | TRS High pH 9.0 | TRS Low pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | BERS2 pH 9.0 | BERS1 pH 6.0 |
| mAb clone **6F11** | - | - | 0/1** | - | 1/2 | - | 3/11 (27%) | 0/1 |
| rmAb clone **EP1** | 1/1 | - | 1/2 | - | 0/1 | - | 0/1 | - |
| rmAb clone **SP1** | - | - | 2/3 | - | 4/7 (57%) | - | 0/4 | - |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer).
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra plus
3) Bond III, Prime

**Ready-To-Use antibodies and corresponding systems**

mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica Biosystems Bond III/ Bond Prime:
Two optimal results were obtained, using the following protocols: HIER in BERS2 (high pH) for 30 and 40 min., 20 and 60 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Of the 18 laboratories using this antibody, 10/13 (77%) were able to achieve sufficient results using a protocol based on HIER using BERS2 (20-40 min.), 15-60 min. incubation of primary Ab and Bond Polymer Refine Detection. Five laboratories used a protocol using HIER with BERS1 (low pH), but none achieved a sufficient result.

rmAb clone **EP1**, product no. **IR084/IS084**, Dako/Agilent, Dako Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 97-98°C; mode = 20 min.), 20-40 min. incubation of the primary Ab (mode = 20 min.) and EnVision FLEX (K8000/SM802, K8010/DM822) or EnVision FLEX+ (K8002/SM802) as detection system, with or without Rabbit Linker (K8009, K8019). Of the laboratories using these protocol settings, 15 of 16 (94%) produced a sufficient staining result.
*5 laboratories used product no IR084/IS084 on other platforms. These were not included in the description above.*

rmAb clone **EP1**, product no. **GA084**, Dako/Agilent, Dako Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 20-30 min. at 97°C, mode = 30 min.), 10-27 min. incubation of the primary Ab (mode = 10 min.) and EnVision FLEX (GV800/GV823) with or without rabbit linker (GV809) as detection system. Of the laboratories using these protocol settings, 65 of 67 (97%) produced a sufficient staining result.
*7 laboratories used product no. GA084 on another platform and are not included in the description above.*

rmAb clone **SP1**, product no. **790-4324/4325**, Ventana/Roche, BenchMark XT/ULTRA/ULTRA Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 4-92 min. (mode = 64 min.), 16-44 min. incubation of the primary Ab (modes = 16, 32 min.) and UltraView (760-500) with or without UltraView Amplification kit (760-080), UltraView Alkaline Phosphatase red (760-501) or OptiView (760-700) as detection system. Using these protocol settings, 184 of 220 (84%) laboratories produced a sufficient staining result.
*14 laboratories used product no 790-4324/4325 on other platforms. These were not included in the description above.*

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocol assays performed on the specific IHC platform(s) indicated on the datasheet are included.

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified RTU protocols**

| RTU systems | **Vendor recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako AS48<br>rmAb EP1<br>**IR084/IS084** | 2/2 | 1/2 | 17/18 (94%) | 8/18 (44%) |
| Dako Omnis<br>rmAb EP1<br>**GA084** | 42/44 (95%) | 28/44 (64%) | 25/25 (100%) | 14/32 (56%) |
| Leica Bond III<br>mAb 6F11<br>**PA009/PA0151** | 0/3 | 0/3 | 7/10 (70%) | 1/10 (10%) |
| VMS Ultra/XT<br>rmAb SP1<br>**790-4324/4325** | 51/61 (84%) | 5/61 (8%) | 144/172 (84%) | 53/172 (31%) |

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time, detection kit and use of amplification. Only protocols performed on the specified vendor IHC stainer are included.

**Comments**

Since Run B30, the assessment criteria continue to be centered on the tissue controls, tonsil and uterine cervix, in concordance to the ASCO/CAP 2020 recommendation on ER IHC testing. The results of previous NordiQC ER assessments, along with the ASCO/CAP guidelines (2020) strongly suggest that the use of tonsil as positive control material is essential to ensure an appropriate lower limit of sensitivity for demonstration of ER.

As in previous NordiQC runs for ER, the most common feature of an insufficient staining result on this assessment was inadequate analytical sensitivity, resulting in a weak or false negative staining reaction, with reduced detection of the ER epitope. This was seen in 89% of the insufficient results (58/65). A weak or false negative staining reaction was further complicated by excessive or "inselective" counterstain in 22% (14/65) of the insufficient results. Clinically relevant false positive staining reaction, where >1% of cells in the negative breast tumour (tissue core no. 3) stained unequivocally, was seen in one of the insufficient results. Poor signal-to-noise ratio and excessive background were seen in 6% and 3% of insufficient staining results respectively.

Virtually all laboratories were able to demonstrate ER in the high-level ER-expressing breast carcinoma (tissue core no. 4), in which 90-100% of the neoplastic cells were expected to be demonstrated and as characterized by the NordiQC reference standard methods, the cells showed a moderate to strong intensity. Demonstration of ER in the heterogeneous mid-level ER-expressing breast carcinoma (tissue core no. 5), in which an at least weak nuclear staining reaction of 50-80% of the neoplastic cells was expected, was much more challenging.

In this run, in common with run B36, an increased number of sufficient results assessed as good (50% of all results, 212/426) was observed in comparison to Run B35 and previous runs, and the majority (82%, 174/212) of these were characterized by reduced analytical sensitivity manifesting as significantly fewer cells staining positive for ER than expected. Other features included a poor-signal-to noise ratio, excessive background reactions and/or excessive or "inselective" nuclear counterstaining. The observation on both excessive background and excessive nuclear counterstaining was primarily related to staining on the Omnis platform (Dako/Agilent), as discussed in Runs B35 and 36, as well as the Leica Bond platform. "Inselective" counterstaining, where nuclei were difficult to resolve, was again primarily related to staining on the Ventana BenchMark platform group.

Ready-To-Use (RTU) Abs were used by 91% (386/426) of the participants. 86% (331/386) of these laboratories obtained a sufficient staining result, 35% optimal (136/386).

The Ventana/Roche RTU system, **790-4324/4325** for BenchMark based on the rmAb clone **SP1** was in this assessment the most widely applied assay being used by 55% (233/426) of all the participants and gave an overall pass rate of 84% (195/233), 25% optimal. Laboratory modified protocols (LMPS) were used by the majority (74%, 172/233) of participants using this system. Optimal results could be obtained both by the vendor recommended protocol settings (VRPS) (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView or iView as detection kit) and by laboratory modified protocol settings (LMPS) adjusting incubation time of the primary Ab, HIER time, detection systems and use of amplification as shown in Table 3. In this assessment, VRPS were used by only 26% (61/233) of the laboratories and provided an identical pass rate compared to LMPS as shown in Tables 1c and 3, although the optimal rate was somewhat higher with LMPS. Increasing the incubation time in primary antibody to 32 min. was the most successful single modification to the VRPS, with 100% (23/23) laboratories using this protocol

achieving adequate results, 35% optimal. Reduced HIER time to e.g. 24-36 min. in CC1 was found to be less successful and should be avoided (see Figs. 1-4).
Use of OptiView as a substitute for UltraView detection as the sole modification to the VRPS gave a pass rate of 89% (8/9), 56% optimal. Protocol modifications using OptiView detection (with or without alteration of primary incubation and HIER time) were largely successful, resulting in a similar pass rate of 85% (35/41 users) versus 84% for the manufacturer's protocol, but with an optimal score rate of 54% (22/41 users) compared to 8% using VRPS (see Table 3). Use of UltraView amplification in addition to the base detection system gave a pass rate of 100% (17/17) and an optimal rate of 53% (9/17).

The Dako/Agilent RTU system **GA084** for Omnis, based on rmAb clone **EP1** was used by 16% of the participants and gave an overall pass rate of 97%, 61% optimal. The proportion of sufficient results were similar for both VRPS (95%) and LMPS (100%), where the VRPS provided 64% optimal results compared to 56% for laboratories applying LPMS. Five laboratories used a modified protocol including rabbit linker and 8 laboratories increased the primary antibody incubation time to 20-27 minutes, with both groups obtaining a pass rate of 100%.

The Dako/Agilent RTU system **IR084/IS084** for Autostainer, also based on the rmAb **EP1** was used by 5% of the participants and provided an overall pass rate of 95%, 45% optimal. As shown in Table 3, 90% (18/20) of the laboratories modified the protocol settings and obtained a similar pass rate to laboratories using the RTU system according to the Dako/Agilent recommendations. The commonest and most successful modification included use of a rabbit linker and was used by 11 laboratories: 100% of these passed, 54% (6/11) optimal.

The Leica RTU system **PA0009/PA0151** for BOND based on mAb **6F11,** was used by 3% (13/426) of the participants and gave an overall pass rate of 54%, 8% optimal. In this assessment, VRPS based on HIER in BERS1 (low pH) for 20 min., 15 min. incubation of the primary Ab and Bond Refine as detection system was used by two participants, with neither achieving sufficient results. Laboratories using a protocol modification increasing analytical sensitivity by using HIER in BERS2 (high pH) for 20 min. without extending the incubation time in primary antibody obtained a pass rate of 75% (3/4), although no optimal results were obtained. One participant extended the HIER time in BERS2 to 30 minutes, increased the primary Ab incubation time to 20 min. and received an optimal result (1/1). In this run, false positive staining of the negative tumour (breast carcinoma no. 3) was not seen with high pH retrieval, as previously noted sporadically from run to run, even with extended retrieval times of up to 40 min. However, 40 min. HIER in BERS2 without extension of the primary antibody incubation time resulted in excessive background compromising interpretation (1/1).

In general, it must be emphasized that modifications of vendor recommended protocol settings for the RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process for the end-users. As seen in this and previous assessments, modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

Concentrated antibody formats with laboratory-developed (LD) assays were used by 9% (40 of 426) of the participants, in common with run B36 and a reduction from 11% in run B35. The three most commonly applied Abs mAb clone **6F11**, rmAb clones **EP1** and **SP1** used in a LD assay could all provide an optimal result on the tissues supplied in this run. Overall, the rmAb clone SP1 was the most commonly used concentrated antibody, with 33% (6/18) of laboratories attaining an optimal result across all platforms. Sufficient results were obtained with all three Abs on the main IHC platforms (Dako/Agilent, Leica Biosystems and Ventana/Roche), see Tables 1b and 2. The most robust antibody clone in this run was EP1, with an overall pass rate of 100%. The overall pass rate for all laboratories using concentrated antibody formats in this run was 75% (30/40), with 33% (13/40) obtaining optimal results.

Irrespective of the clone applied, careful calibration of the primary Ab concentration in combination with efficient HIER, preferably in an alkaline buffer (except for mAb clone 6F11 as discussed in runs B35, B28 and B15) were found to be the common core elements for an optimal performance.

In this run, 2-layer detection systems performed slightly more successfully than 3-layer detection systems: the majority of the laboratories using concentrated antibody formats used a 3-layer detection system (68%, 27/40), with 32% (13/40) using a 2-layer system. Laboratories using a 3-layer system obtained a pass rate of 70%, with 26% optimal, compared to 2-layer detection system, with a pass rate of 85%, 46% optimal.

1. Kimberly H. Allison, M. Elizabeth H. Hammond, Mitchell Dowsett, Shannon E. McKernin, Lisa A. Carey, Patrick L. Fitzgibbons, Daniel F. Hayes, Sunil R. Lakhani, Mariana Chavez-MacGregor, Jane Perlmutter, Charles M. Perou, Meredith M. Regan, David L. Rimm, W. Fraser Symmans, Emina E. Torlakovic, Leticia Varella, Giuseppe Viale, Tracey F. Weisberg, Lisa M. McShane, and Antonio C. Wolff.
Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. Arch Pathol Lab Med. 2020 May;144(5):545-563

2. Sapino A, Cassoni P, Ferrero E, Bongiovanni M, Righi L, Fortunati N, Crafa P, Chiarle R, Bussolati G.
Estrogen receptor alpha is a novel marker expressed by follicular dendritic cells in lymph nodes and tumor-associated lymphoid infiltrates. Am J Pathol. 2003 Oct;163(4):1313-20. PubMed PMID: 14507640

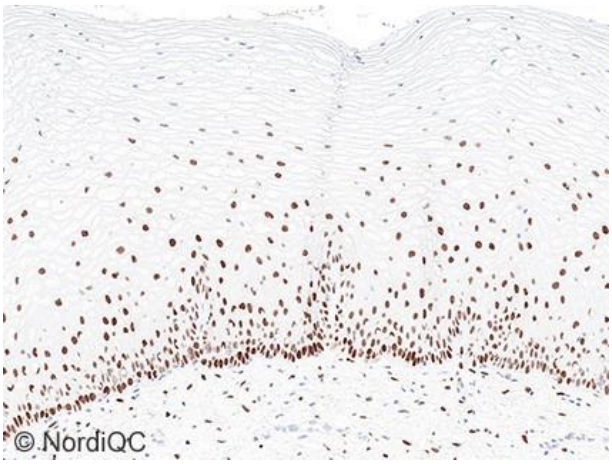



Fig. 1a
Optimal ER staining reaction of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, by vendor recommended protocol settings using HIER in CC1 (pH 8.5) for 64 min., incubation time 16 min. in primary Ab and visualized by UltraView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a – 4a, same protocol

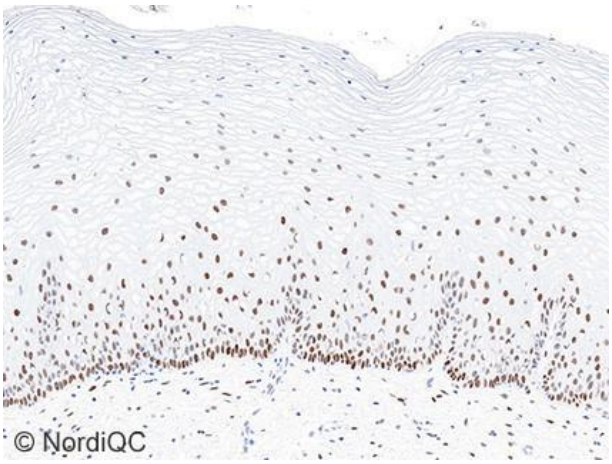



Fig 1b
ER staining reaction of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche by reduced HIER time for 32 min. compared to the vendor recommended protocol – same field as in Fig. 1a.
Also compare with Figs. 2b – 4b, same protocol,

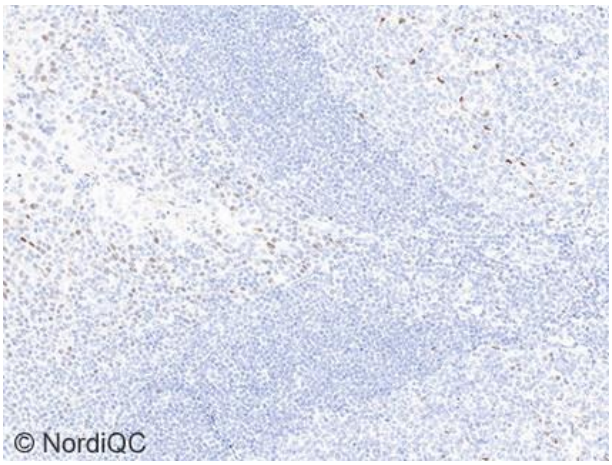



Fig. 2a
Optimal ER staining reaction of the tonsil using the same protoicol as in Fig. 1a.
A distinct nuclear staining reaction in both dispersed follicular dendritic cells/T-cells in the germinal center and many squamous epithelial cells can be identified at even low magnification (10x).
.

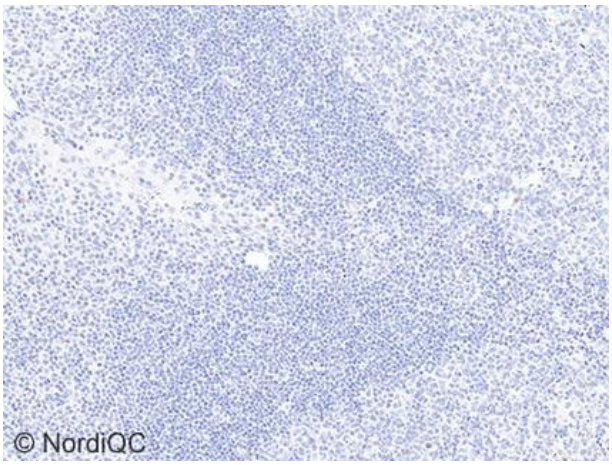



Fig. 2b.
Insufficient ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
Only a faint equivocal staining reaction in few follicular dendritic cells/T-cells in the germinal center and squamous epithelial cells is observed.

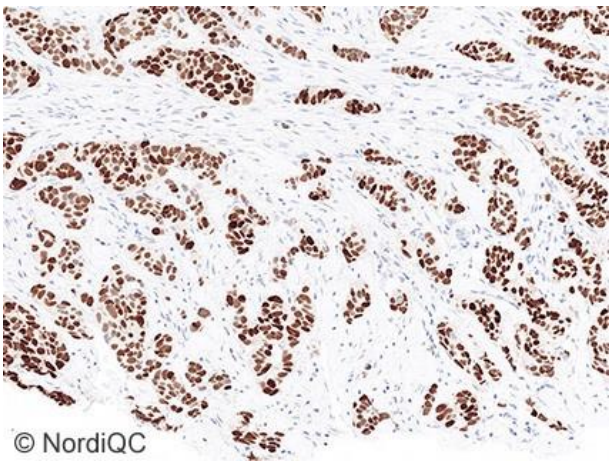



Fig. 3a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 4, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a – 2a.
The neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

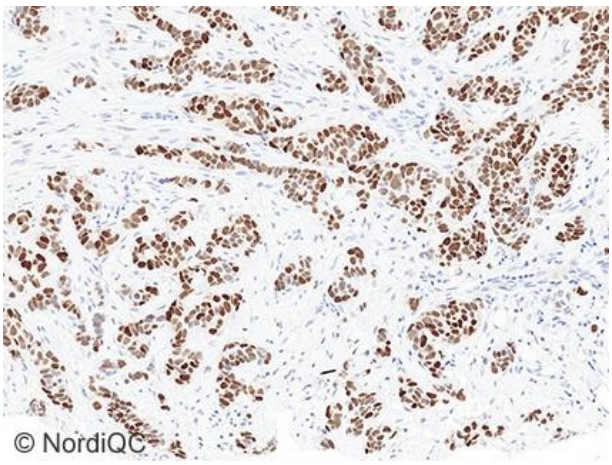



Fig. 3b.
ER staining reaction of the breast carcinoma, tissue core no. 4, with 90-100% cells being positive using the same protocol as in Figs. 1b – 2b.
The neoplastic cells display a clear positive staining reaction for ER.
However also compare with Fig. 4b, same protocol.

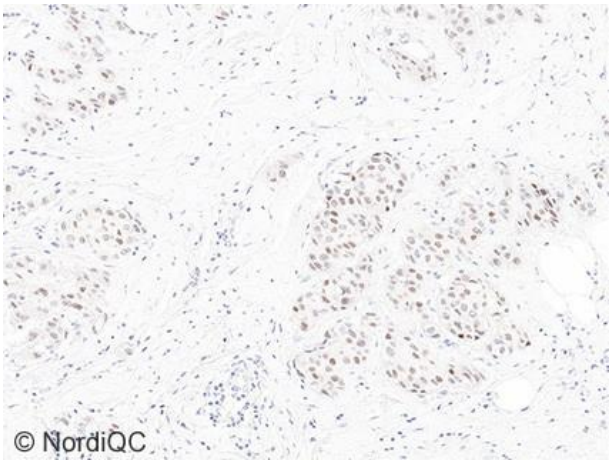



Fig. 4a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 5, with 50-80% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a – 3a.
The majority of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

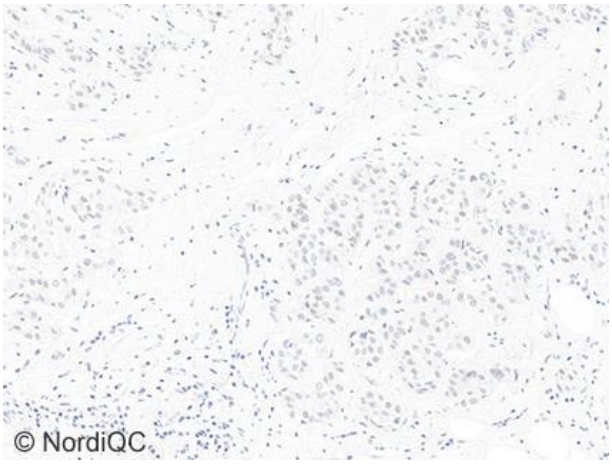



Fig. 4b
Insufficient (borderline) ER staining reaction of the breast carcinoma, tissue core no. 5, with 50-80% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1b – 4b.
Only dispersed neoplastic cells show a weak and equivocal nuclear staining reaction for ER.

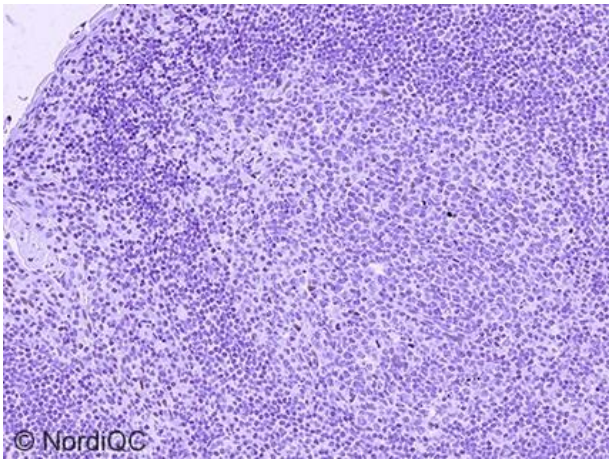



Fig. 5a
Insufficient ER staining reaction of the tonsil using the rmAb SP1 by a protocol providing a combination of reduced analytical sensitivity and excessive counterstaining compromising the evaluation of the ER IHC assay quality.
The intense counterstaining makes it virtually impossible to evaluate if the immunohistochemical critical assay performance controls (squamous epithelial cells and follicular dendritic cells/T-cells in germinal centres) are positive or negative. Also compare with Fig. 5b, same protocol.

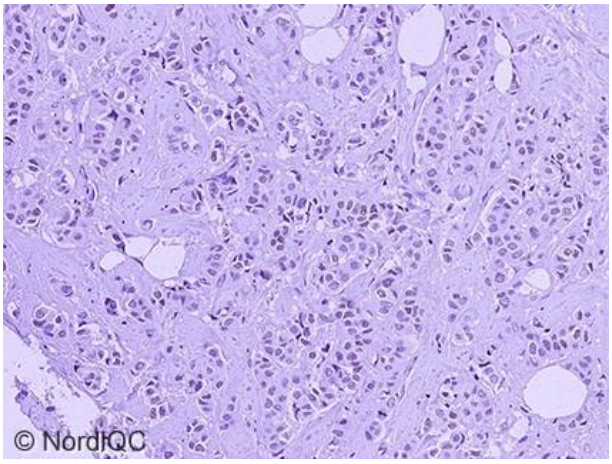



Fig. 5b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 5, with 50-80% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Fig. 5a.
The read-out and determination of ER positivity in the neoplastic cells is significantly hampered due to the intense counterstaining.

NG/LE/SN 19.04.2024