Assessment Run B33 2022

Estrogen receptor (ER)

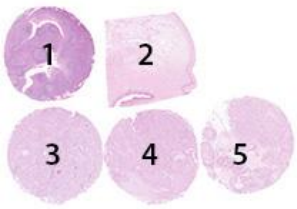
Purpose

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	80-90%	Weak to moderate
4.	Breast carcinoma	90-100%	Moderate to strong
5.	Breast carcinoma	0%	Negative



* 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] 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.

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

- A moderate to strong, distinct nuclear staining of virtually all the columnar epithelial cells, 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.
- An at least weak to moderate distinct nuclear staining in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 3 and 4.
- No nuclear staining in the neoplastic cells in the breast carcinoma no. 5.
- 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 no. 3 and 4 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, excessive counterstaining or impaired morphology.

- An ER IHC result was assessed as **borderline** if ≥ 1% and < 10% of the neoplastic cells in one of the breast carcinomas no. 3 and 4 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 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 no. 3 and 4 or false positive staining (≥ 1%) was seen in the breast carcinoma no. 5. Poor signal-to-noise ratio as described above could also result in a grade of **poor** where interpretation was severely hampered.

Participation

Number of laboratories registered for ER, B33	433
Number of laboratories returning slides	407 (94%)

At the date of assessment, 94% 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.

Results

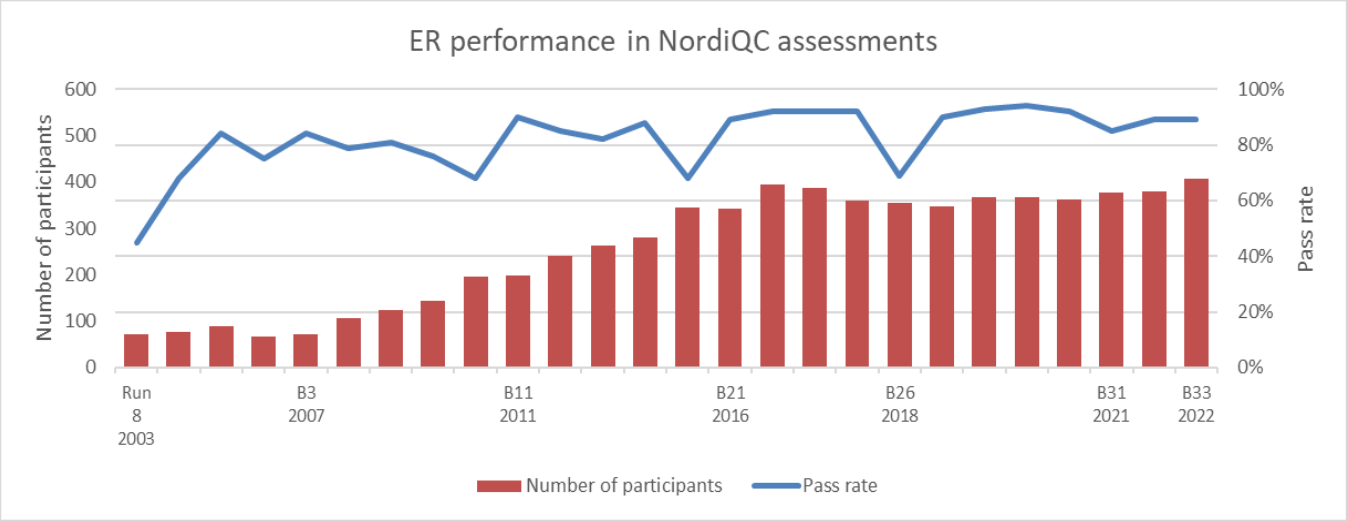
407 laboratories participated in this assessment. 363 of 407 (89%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 3).

The most frequent causes of insufficient staining reactions were:
- Insufficient Heat Induced Epitope Retrieval (HIER) in an acidic buffer
- Use of detection systems with low sensitivity
- Unexplained / technical issues during staining process

Performance history

In this run the pass rate of 89% (proportion of sufficient results) was identical to the level seen in run B32 and the pass rate has been stable at a high and satisfactory level in runs between 2016-2022, with the exception of run B26 (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER from 2003 - 2022**



Fluctuations in pass rates, as seen in run B26 and to a lesser extent in run B31, is likely caused by more challenging material circulated compared to other runs. In order to secure the consistency of the material circulated, NordiQC evaluates the material with two reference standard methods and monitor the ER expression levels throughout all TMAs used in the assessment. Fluctuation in pass rates can also be influenced by many new participants, but in this run 10% (n=40) more participants were registered compared to run B32 and still same pass rate was obtained.

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 (86%) used Ready-To-Use (RTU) systems, with the majority of these (227 of 350, 65%) using the Ventana/Roche platform. "Plug and play" RTU assays performed on fully automated platforms gave an overall pass rate of 96% across the two major manufacturers (Ventana/Roche BenchMark 96% and Dako/Agilent Omnis: 97%).
The commonest feature 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 counterstaining, or sections showing poor signal-to-noise ratios and/or impaired morphology, leading to difficulties in scoring. Three insufficient results were caused by false positive staining reaction.

Uterine cervix and tonsil are recommended as positive tissue controls for ER. In uterine cervix, virtually all squamous and columnar epithelial cells must show a moderate to strong and distinct nuclear staining

reaction. Endothelial cells and lymphocytes must be negative in this tissue. Tonsil is particularly recommended as a tool to monitor the level of analytical sensitivity for the demonstration of ER. Dispersed follicular dendritic cells[2] in germinal centers and squamous epithelial cells must show an at least weak, distinct nuclear staining reaction. In addition, tonsil can be used as negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

Table 1. **Antibodies and assessment marks for ER, B33**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11**	18	Leica Biosystems	5	9	3	1	78%	28%
rmAb clone **EP1**	13 2	Dako/Agilent Cell Marque	6	7	2	0	87%	40%
rmAb clone **SP1**	9 5 1 1 1 2 1 1	Thermo Scientific Cell Marque Abcam Epredia Biocare Zytomed Diagnostic Biosystems DCS	14	1	4	2	71%	67%
rmAb clone **BP6026**	1	BioLynx	0	1	0	0	-	-
rmAb clone **IHC561**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **ZR147**	1	Zeta Corporation	0	0	1	0	-	-
Ready-To-Use antibodies							Suff.[1]	OR[2]
mAb clone **1D5** **IR657**	1	Dako/Agilent	1	0	0	0	-	-
mAb clone **6F11** **PA0009/PA0151 (VRPS[3])**	3	Leica Biosystems	0	1	2	0	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	10	Leica Biosystems	4	3	3	0	70%	40%
rmAb **EP1** **IR/IS084 (VRPS[3])**	3	Dako/Agilent	1	1	1	0	67%	33%
rmAb **EP1** **IR/IS084 (LMPS[4])**	34	Dako/Agilent	22	11	1	0	97%	65%
rmAb **EP1** **GA084 (VRPS[3])**	33	Dako/Agilent	29	3	1	0	97%	88%
rmAb **EP1** **GA084 (LMPS[4])**	27	Dako/Agilent	15	9	0	3	89%	56%
rmAb **EP1** **AN710-5M**	1	BioGenex	0	0	0	1	-	-
rmAb **EP1** **8361-C010**	2	Sakura Finetek	1	1	0	0	-	-
rmAb clone **SP1** **790-4324/4325 (VRPS[3])**	50	Ventana/Roche	24	24	2	0	96%	48%
rmAb clone **SP1** **790-4324/4325 (LMPS[4])**	175	Ventana/Roche	93	67	11	4	91%	53%
rmAb clone **SP1** **249R-17/18**	5	Cell Marque	1	2	1	1	-	-
rmAb clone **SP1** **KIT-0012**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **SP1** **MAD-000306QD/V**	3	Master Diagnostica Vitro SA	1	2	0	0	-	-
rmAb clone **SP1** **RMPD001**	1	Diagnostic BioSystems	1	0	0	0	-	-
Total	407		221	142	32	12		
Proportion			54%	35%	8%	3%	89%	

1) Proportion of sufficient results (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).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s) or other platforms.

Detailed analysis of ER: Run B33
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: Five optimal results were obtained, based on HIER using Cell Conditioning 1 (CC1, Ventana/Roche) (2/4)* or Bond Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica Biosystems) (3/9) as retrieval buffer. The mAb was diluted in the range of 1:25-1:200 and combined most commonly with a 3-layer detection system. Using these protocol settings, 14 of 18 (78%) of laboratories produced a sufficient staining result (optimal and good). Four laboratories were able to achieve sufficient staining using HIER in a low pH buffer (4/5), none being optimal.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (5/11) or BERS2 pH 9.0 (Leica Biosystems) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:100 and combined with a 2-layer or 3-layer detection system. Using these protocol settings, 12 of 14 (86%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Protocols with optimal results were typically based on HIER using CC1 (Ventana/Roche) (6/9), TRS pH 9 (Dako/Agilent) (3/3), BERS2 pH 9.0 (Leica Biosystems) (2/3) or Tris-EDTA pH 9 (3/6) 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, 15 of 20 (75%) 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**		**Dako/Agilent Omnis**		**Ventana BenchMark XT/Ultra/GX**		**Leica Bond III / Max**	
	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**	-	-	2/4	-	3/9 (33%)	0/4
rmAb clone **EP1**	2/6 (33%)	0/1	3/5 (60%)	-	0/2	-	1/1	-
rmAb clone **SP1**	-	-	3/3	-	6/9 (67%)	-	2/3	-

* 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).

Ready-To-Use antibodies and corresponding systems
mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica Biosystems, Bond III/Bond Max:
Protocols with optimal results were based on HIER using BERS2 (high pH) for 20 min., 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient staining result (optimal or good).

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), 20-40 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) or EnVision FLEX+, (K8009/K8019) as detection system. Using these protocol settings, 19 of 21 (90%) laboratories produced a sufficient staining result.
12 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), 10-27 min. incubation of the primary Ab and EnVision FLEX (GV800) with or without rabbit linker (GV800+GV809) as detection system. Using these protocol settings, 52 of 54 (96%) laboratories produced a sufficient staining result.
1 laboratory used product no. GA084 on another platform and was not included in the description above.

rmAb clone **SP1**, product no. **790-4324/4325**, Ventana/Roche, BenchMark GX, XT, ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 16-95 min. (mode = 64 min.), 12-64 min. incubation of the primary Ab (mode range = 16-32 min.) and UltraView (760-500) with or without UltraView Amplification kit (760-080), iView (760-091) or OptiView (760-700) without amplification as detection system. Using these protocol settings, 202 of 219 (92%) laboratories produced a sufficient staining result.
3 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 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 rmAb EP1 **IR084/IS084**	2/3	1/3	21/22 (95%)	13/22 (59%)
Dako Omnis rmAb EP1 **GA084**	32/33 (97%)	29/33 (88%)	23/26 (88%)	14/26 (54%)
Leica Bond mAb 6F11 **PA009/PA0151**	1/3	0/3	7/10 (70%)	4/10 (40%)
VMS Ultra/XT/GX rmAb SP1 **790-4324/4325**	48/50 (96%)	24/50 (48%)	157/172 (91%)	90/172 (52%)

* 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 and detection kit. 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 control material is essential to ensure an appropriate lower limit of sensitivity for demonstration of ER.

As seen in previous NordiQC runs for ER, in this assessment the most common feature of an insufficient staining result was inadequate analytical sensitivity, resulting in a weak or false negative staining reaction, with reduced detection of the ER epitope. This was seen in 84% of the insufficient results (37 of 44). Weak or false negative staining reaction was further complicated by excessive counterstain in 18% (8 of 44) of the insufficient results and in 7% (3 of 44) a false positive nuclear staining reaction was noted in the ER negative breast tumour. In this assessment, false positive staining was linked to clone EP1 (GA084) on the Dako Omnis, mostly with use of rabbit linker. Poor morphology, generally resulting from aggressive HIER, complicated interpretation in 11% (5 of 44) and poor signal-to-noise ratio and excessive background were each seen in 4.5% of insufficient staining results.

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 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. 3), in which an at least weak nuclear staining reaction of 80-90% of the neoplastic cells was expected, was much more challenging.

Ready-To-Use (RTU) Abs were used by 86% (350 of 407) of the participants. 91% (319 of 350) of these laboratories obtained a sufficient staining result, 56% optimal (195 of 350).

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% of the participants and gave an overall pass rate of 92%. 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 (LPMS) adjusting incubation time of the primary Ab, HIER time and detection system as shown in Table 3. In this assessment, VRPS were used by only 23% of the laboratories and provided a slightly higher overall pass rate compared to LMPS as shown in Tables 1 and 3. Use of OptiView detection was a successful protocol modification, resulting in an optimal staining result for 84% (26 of 31) of users, compared to 48% for the manufacturer's protocol (see Table 3). Protocols based on OptiView detection gave a pass rate of 100% (31/31 users). Use of UltraView amplification in addition to the base detection system gave a pass rate of 95% (18 of 19) and an optimal rate of 84% (16 of 19).

The Dako/Agilent RTU system GA084 for Omnis, based on rmAb clone EP1 was used by 15% of the participants and gave an overall pass rate of 93%. The proportion of sufficient and optimal results obtained by the VRPS was significantly increased compared to LPMS as 88% of labs using VRPS achieving optimal results versus only 54% of those using LPMS (see Table 3). The modified protocols either increased incubation time of the primary Ab and/or added a rabbit linker for the detection system.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb EP1 was used by 9% of the participants and provided an overall pass rate of 95%. As shown in Table 3, 88% (22 of 25) of the laboratories modified the protocol settings and obtained a relatively significant higher pass rate and increased proportion of optimal results compared to laboratories using the RTU system according to the Dako recommended protocol settings. The most common and successful modification observed was use of FLEX+ with rabbit linker as detection system. A modification including rabbit linker was used by 15 laboratories: 100% obtained a sufficient mark (15/15) and 80% (12/15) being optimal staining results.

The Leica RTU system PA0009/PA0151 for BOND based on mAb 6F11, was used by 3% of the laboratories and gave an overall pass rate of 62%. 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 three participants, with only one participant achieving sufficient results. Laboratories using a protocol modification increasing analytical sensitivity by using HIER in BERS2 (high pH) obtained a pass rate of 86% (6 of 7), 57% optimal (4 of 7). However high pH retrieval should be used with caution with this clone due to the concerning number of false positive staining reactions noted by this modification in run B28. Protocol modifications increasing incubation time of the primary antibody alone did not yield any optimal results and resulted in weak staining in 1 of 2 laboratories employing this method.

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 14% (57 of 407) of the participants. 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, with the highest rate of optimal results seen with rmAb clone SP1 on the Ventana platform (6 of 9, 67%). However, overall, the rmAb clone EP1 produced the highest pass rate, with 13 of 15 (87%) laboratories using this clone attaining a mark of good or optimal. Sufficient results were obtained with all three Abs on the main IHC platforms (Dako/Agilent, Leica Biosystems and Ventana/Roche), see Tables 1 and 2. The overall pass rate for all laboratories using concentrated antibody formats in this run was 77% (44 of 57), with 46% (26 of 57) 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 run B28 and B15) were found to be the common core elements for an optimal performance.

In this run, both 2- and 3-layer detection systems performed relatively evenly: 53% (30 of 57) of the laboratories using concentrated antibody formats used a 3-layer detection system, with 46% (26 of 57) using a 2-layer system. Laboratories using a 3-layer system obtained a pass rate of 73%, with 43% optimal, compared to 2-layer detection system, with a pass rate of 81%, 50% optimal.

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 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.

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

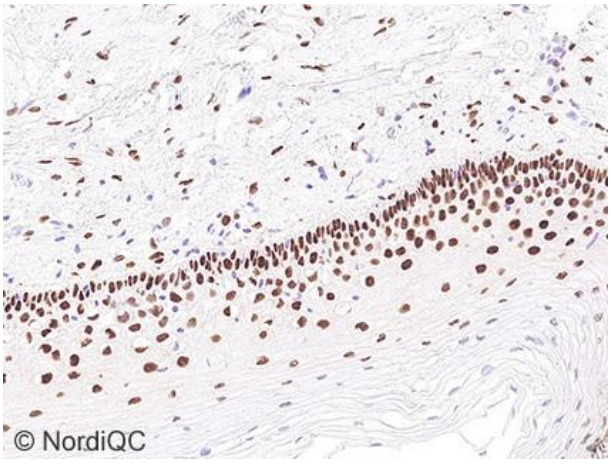


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, using HIER in CC1 (pH 8.5), visualized by UltraView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a-5a, same protocol.

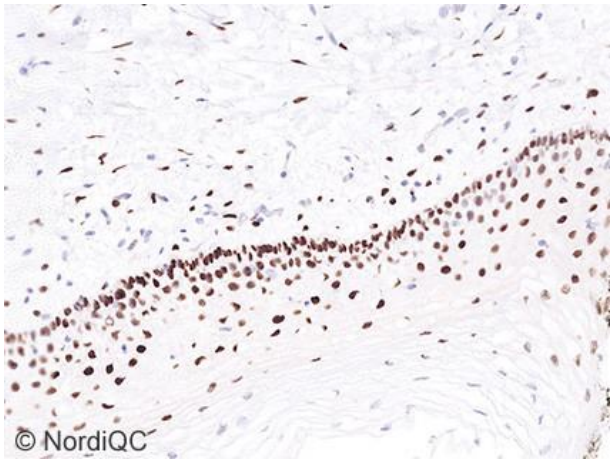


Fig. 1b
ER staining of the uterine cervix using the rmAb clone SP1 as concentrate, using HIER in an alkaline buffer and visualized by a 2-step detection system – same field as in Fig. 1a.
The intensity of the ER staining reaction is reduced compared to the level seen in Fig. 1a, but still the expected proportion of cells are demonstrated.
However, also compare with Figs. 2b- 4b, same protocol.

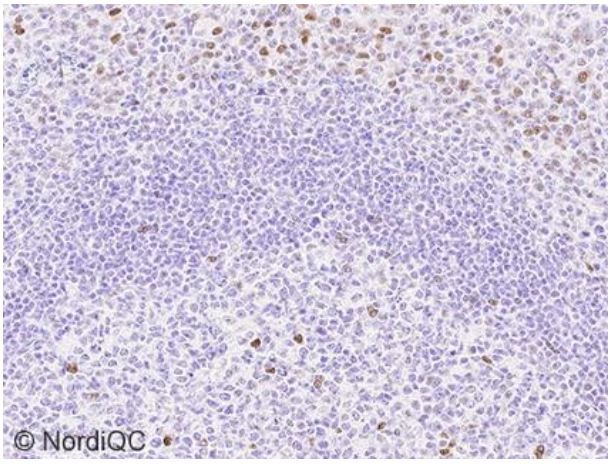


Fig. 2a
Optimal ER staining of the tonsil using same protocol settings as in Fig. 1a.
A moderate, distinct nuclear staining reaction is seen in dispersed follicular dendritic cells in the germinal center and squamous epithelial cells.
No nuclear staining reaction is seen in the mantle zone B-cells and in general a high signal-to-noise ratio is seen

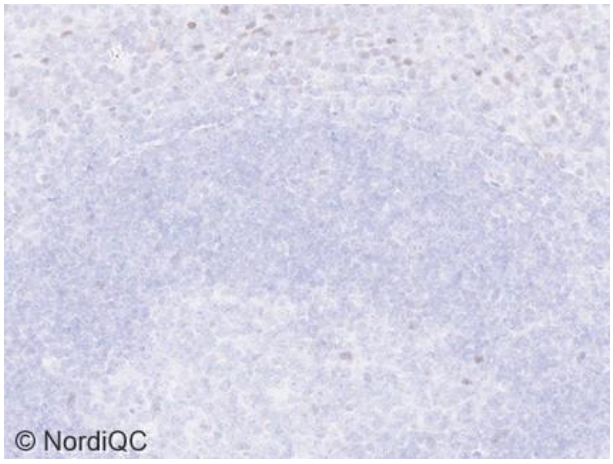


Fig. 2b
Insufficient ER staining of the tonsil using same protocol settings as in Fig. 1b.
Only a faint equivocal staining reaction is observed in few follicular dendritic cells in the germinal center and squamous epithelial cells.
Compare with Fig. 2a – same field.
Tonsil was found to be superior as positive tissue control compared to uterine cervix in order to monitor the accuracy for low limit ER demonstration.

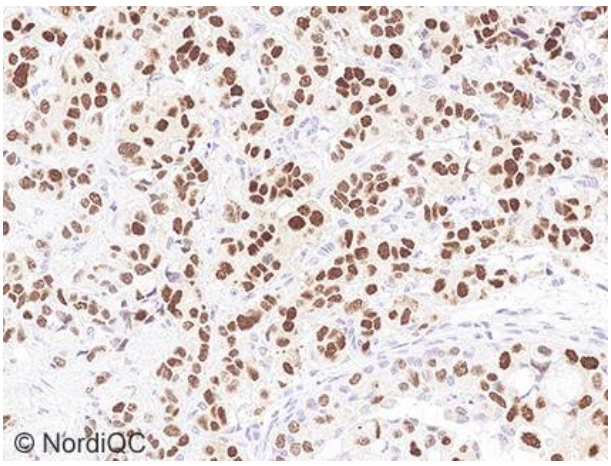


Fig. 3a
Optimal ER staining of the breast carcinoma, tissue core no. 4, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a-2a.
The neoplastic cells display a strong and distinct nuclear staining reaction.

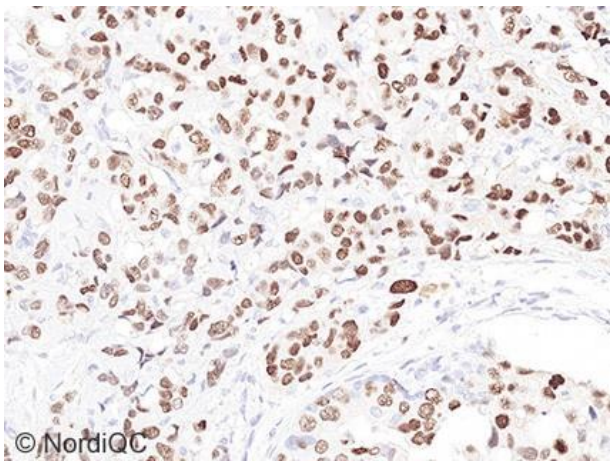


Fig. 3b
ER staining of the breast carcinoma, tissue core no. 4, with expected 90-100% cells being positive using same protocol as in Figs. 1b-2b – same field as in Fig. 3a.
The staining intensity of positive cells is reduced compared to the optimal result in Fig. 3a. However, the protocol still provides the expected proportion of positive cells, as the ER expression level is high in the neoplastic cells

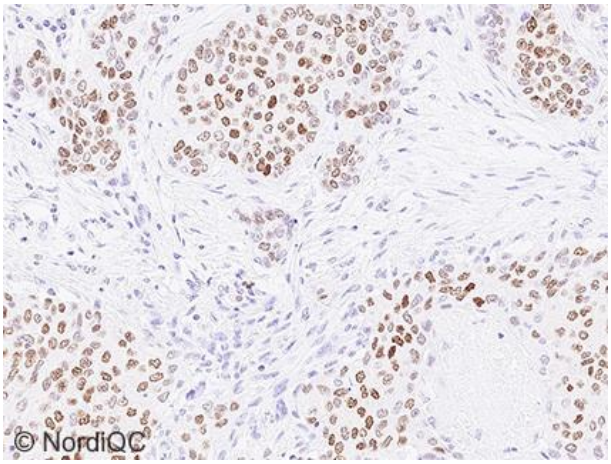


Fig. 4a
Optimal ER staining of the breast carcinoma, tissue core no. 3, with 80-90% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a-3a.
The vast majority of the neoplastic cells display a weak and distinct nuclear staining reaction.

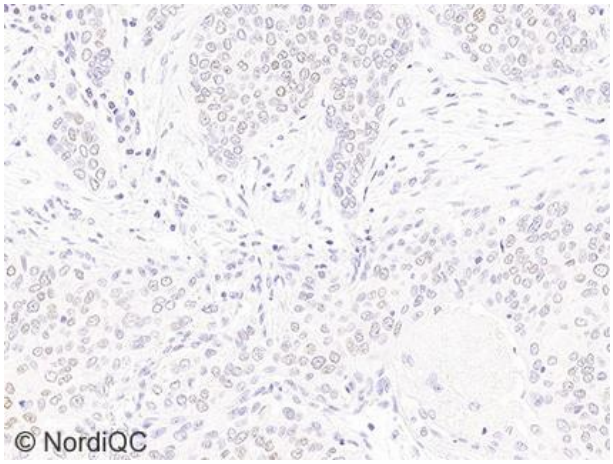


Fig. 4b
Insufficient and virtually false negative ER staining of the breast carcinoma, tissue core no. 3, with expected 80-90% cells being positive using same protocol as in Figs. 1b-3b.
Only dispersed neoplastic cells show an equivocal positive staining result.

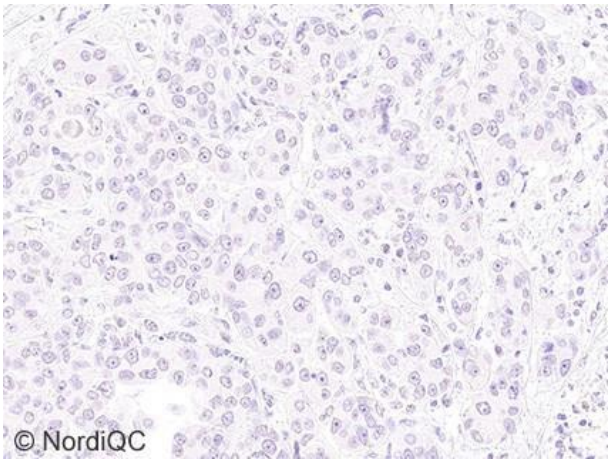


Fig. 5a
Optimal ER staining of the breast carcinoma, tissue core no. 5, expected to be negative using same protocol as in Figs. 1a-4a.
No nuclear staining reaction is seen in the neoplastic cells and a high signal-to-noise ratio is observed facilitating the read-out.
Only, dispersed stromal cells show a weak positive nuclear staining reaction. This reaction is expected due to low-level ER expression in these cells and observed when using IHC protocols with high technical sensitivity.

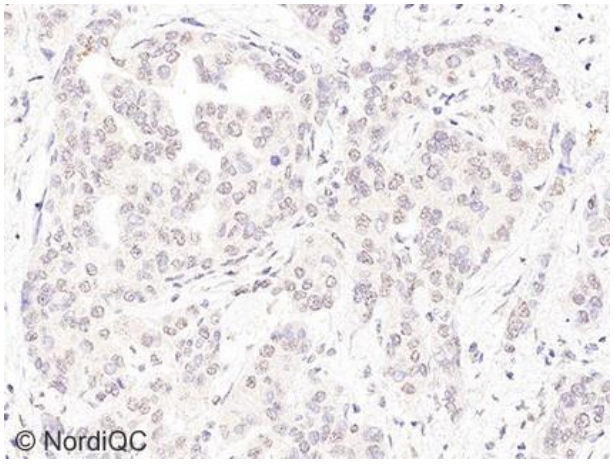


Fig. 5b
Insufficient and false positive ER staining of the breast carcinoma, tissue core no. 5, expected to be negative.
The protocol based on the rmAb clone EP1 as RTU (Dako/Agilent GA084,) using HIER in an alkaline buffer, a 3-step polymer-based detection system and performed on Dako Omnis.
An aberrant diffuse positive nuclear staining reaction is seen in>1% of the neoplastic cells. This pattern was seen in few protocols applying FLEX+ as detection system instead of FLEX as recommended by Dako/Agilent for the Omnis RTU format of EP1.

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