Assessment Run B32 2021

Estrogen receptor (ER)

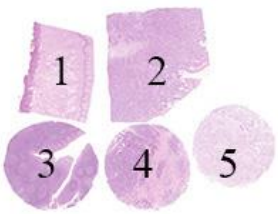
Purpose

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.	Uterine cervix	80-90%	Moderate to strong
2.	Breast carcinoma	90-100%	Moderate to strong
3.	Tonsil	1-5%	Weak to moderate
4.	Breast carcinoma	Negative	-
5.	Breast carcinoma	50-90%	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 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. 2 and 5.
- No nuclear staining in the neoplastic cells in the breast carcinoma no. 4.
- 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. 2 and 5 showed an at least weak nuclear staining reaction but significantly less than 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. 2 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 was assessed as **poor** if a false negative staining (< 1%) was seen in one of the breast carcinomas no. 2 and 5 or false positive staining (≥ 1%) was seen in the breast carcinoma no. 4.

An IHC result can also be assessed as **borderline/poor** related to technical artefacts, e.g. poor signal-to-noise ratio, excessive counterstaining, impaired morphology and/or excessive staining reaction hampering the interpretation.

Participation

Number of laboratories registered for ER, B32	417
Number of laboratories returning slides	380 (91%)

At the date of assessment, 91% 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.

One laboratory was excluded from the assessment. This laboratory used PR on the ER slide.

Results

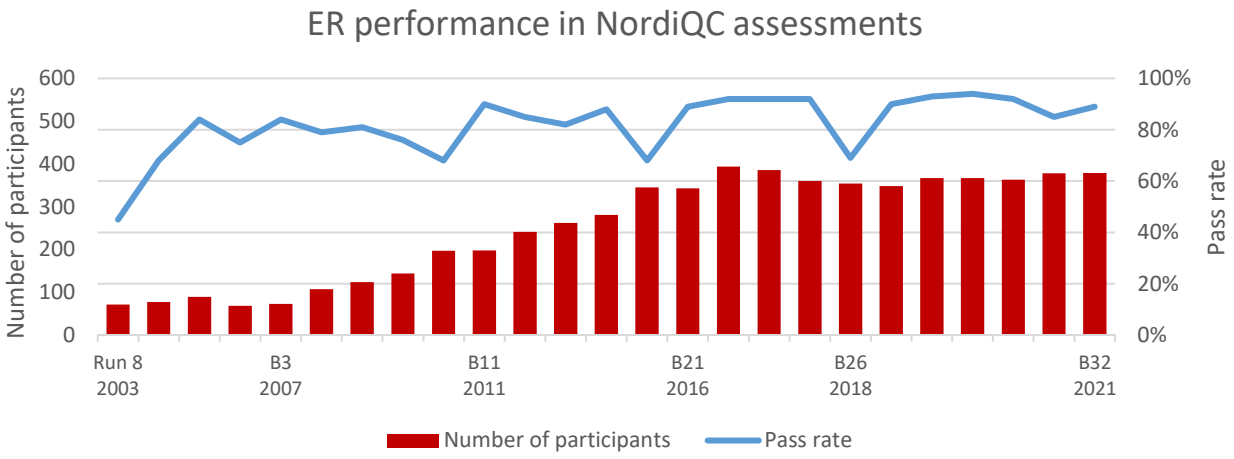
379 laboratories participated in this assessment. 338 of 379 (89%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 3).

The most frequent causes of insufficient staining reactions were:
- Insufficient Heat Induced Epitope Retrieval (HIER) – too short efficient time and/or use of low pH buffer
- Too low concentration of the primary Ab
- Use of detection systems with low sensitivity

Performance history

This was the 25th NordiQC assessment of ER. The pass rate (proportion of sufficient results) has been stable at a high and satisfactory level in runs between 2016-2021, with the exception of run B26 (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER during 25 NordiQC runs**



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.

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 (88%) used Ready-To-Use (RTU) systems, with the majority of these (63%) using the Ventana platform. "Plug and play" RTU assays provided an overall pass rate of 94% across the major manufacturers (Ventana Benchmark: 98% pass rate, Dako Omnis: 97%, Dako Autostainer: 100%). In this assessment, the commonest feature of insufficient results was low analytical sensitivity giving a weak or false negative staining reaction. In addition, low analytical sensitivity and weak demonstration of ER was frequently also complicated by excessive counterstaining, leading to difficulties in the evaluation of the ER status. One insufficient result was caused by a false positive staining reaction of the breast carcinoma tissue core no. 4 expected to be ER negative.

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, B32**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11**	13	Leica Biosystems	1	5	5	2	46%	8%
rmAb clone **EP1**	11 2	Dako/Agilent Cell Marque	2	7	4	0	69%	15%
rmAb clone **SP1**	9 5 1 1 1	Thermo Scientific Cell Marque Abcam DCS Biocare	13	3	1	0	94%	76%
rmAb clone **BP6026**	1	BioLynx	0	1	0	0	-	-
rmAb clone **IHC423**	1	GenomeMe	1	0	0	0	-	-
Ready-To-Use antibodies							Suff.[1]	OR[2]
mAb clone **6F11** **PA0009/PA0151 (VRPS[3])**	3	Leica Biosystems	0	0	0	3	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	12	Leica Biosystems	2	7	1	2	75%	17%
mAb clone **6F11** **PDM048-10MM**	1	Diagnostic BioSystems	1	0	0	0	-	-
rmAb **EP1** **IR/IS084 (VRPS[3])**	4	Dako/Agilent	0	4	0	0	100%	0%
rmAb **EP1** **IR/IS084 (LMPS[4])**	32	Dako/Agilent	9	14	6	3	72%	28%
rmAb **EP1** **GA084 (VRPS[3])**	31	Dako/Agilent	12	18	1	0	97%	39%
rmAb **EP1** **GA084 (LMPS[4])**	25	Dako/Agilent	9	14	2	0	92%	36%
rmAb **EP1** **AN710-5M**	1	BioGenex	0	0	0	1	-	-
rmAb **EP1** **8361-C010**	2	Sakura Finetek	0	2	0	0	-	-
rmAb clone **SP1** **790-4324/5 (VRPS[3])**	46	Ventana/Roche	35	10	1	0	98%	76%
rmAb clone **SP1** **790-4324/5 (LMPS[4])**	166	Ventana/Roche	120	41	5	0	97%	72%
rmAb clone **SP1** **249R-17/18**	6	Cell Marque	5	0	0	1	-	-
rmAb clone **SP1** **BRB053**	1	Zytomed Systems	0	0	1	0	-	-
rmAb clone **SP1** **MAD-000306QD/V**	3	Master Diagnostica Vitro SA	0	1	2	0	-	-
rmAb clone **SP1** **RMPD001**	1	Diagnostic BioSystems	0	1	0	0	-	-
Total	379		210	128	29	12		
Proportion			55%	34%	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, B32
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: Only one protocol producing optimal results was seen, based on HIER using Cell Conditioning 1 (CC1, Ventana/Roche) (1/3)* as retrieval buffer. The mAb was diluted 1:50 and combined with a 3-layer detection system. However, a sufficient result (optimal or good) could be obtained using a primary antibody dilution of 1:25-1:200, high pH retrieval buffer and a three-layer detection system (6/10).
* (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) (1/3) or CC1 (Ventana/Roche) (1/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:35-1:50 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Protocols with optimal results were typically based on HIER using CC1 (Ventana/Roche) (6/8), TRS pH 9 (Dako/Agilent) (2/2), Bond Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica) (3/4) or Tris-EDTA pH 9 (2/2) 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 16 (94%) 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/Roche BenchMark XT/Ultra/GX**		**Leica Biosystems 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**	-	-	-	-	1/3	-	0/8	0/2
rmAb clone **EP1**	0/7**	-	1/3	-	1/2	-	0/1	-
rmAb clone **SP1**	-	-	2/2	-	6/8 (75%)	-	3/4	-

* 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).

Ready-To-Use antibodies and corresponding systems
mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica Biosystems, Bond III/Max:
Protocols with optimal results were based on HIER using BERS2 (high pH) for 30 min., 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP1**, product no. **IR084/IS084**, Dako/Agilent, 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+ with rabbit linker (K8009/K8019) as detection system. Using these protocol settings, 18 of 21 (86%) laboratories produced a sufficient staining result.
9 laboratories used product no IR084/IS084 on other platforms. These were not included in the description above.

mAb clone **EP1**, product no. **GA084**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 20-30 min. at 97°C), 10-20 min. incubation of the primary Ab and EnVision FLEX (GV800) or EnVision FLEX+ with rabbit linker (GV800+GV809) as detection system. Using these protocol settings, 48 of 50 (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 16-76 min.; most common time = 64 min), 4-64 min. incubation of the primary Ab (most common range = 16-32 min.) and UltraView (760-500) with or without UltraView Amplification kit (760-080), iView (760-091) or OptiView (760-700) with or without amplification kit (760-099) as detection system. Using these protocol settings, 198 of 204 (97%) 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**	4/4 (100%)	0/4 (0%)	17/23 (74%)	5/23 (22%)
Dako Omnis rmAb EP1 **GA084**	30/31 (97%)	12/31 (39%)	22/24 (92%)	8/24 (33%)
Leica Bond mAb 6F11 **PA009/PA0151**	0/3	0/3	8/11 (73%)	2/11 (18%)
VMS Ultra/XT/GX rmAb SP1 **790-4324/4325**	45/46 (98%)	35/46 (76%)	158/163 (97%)	117/163 (72%)

* 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 centred on the tissue controls, tonsil and uterine cervix, in concordance to the ASCO/CAP 2020 recommendation on ER IHC testing.
According to both previous NordiQC results and the recommendations from ASCO/CAP 2020, use of tonsil is recommended as essential to ensure an appropriate lower limit of analytical sensitivity for demonstration of ER. If both breast carcinomas (tissue cores no. 2 and 5) showed the expected staining reaction both regarding intensity and proportion of cells demonstrated, but the tissue controls were too weak or a reduced proportion of positive cells were seen in these controls, the overall result was assessed as "good" or "borderline" depending on the overall performance.

In this assessment and as seen in previous NordiQC runs for ER, the most common feature of an insufficient staining result was inadequate protocol sensitivity, resulting in a weak or false negative staining reaction, with reduced detection of the ER epitope. This pattern was seen in 95% of the insufficient results (39 of 41). Weak or false negative staining was further complicated by excessive counterstain in 21% (16/41) of the insufficient results and in 2.5% (1 of 41) a false positive nuclear staining reaction was noted in the negative breast tumour. In the remaining 2.5% (1 of 41), poor signal-to-noise ratio was seen.

Virtually all laboratories were able to demonstrate ER in the high-level ER-expressing breast carcinoma (tissue core no. 2), 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. 5), in which an at least weak nuclear staining reaction of 50-90% of the neoplastic cells was expected, was much more challenging.

Ready-To-Use (RTU) Abs were used by 88% (334 of 379) of the participants. 91% (305 of 334) of these laboratories obtained a sufficient staining result, 58% optimal (193 of 334).

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 56% of the participants and gave an overall pass rate of 97%. Optimal results could be obtained both by the vendor recommended protocol settings (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView or iView as detection kit) and by laboratory modified protocols adjusting incubation time of the primary Ab, HIER time and detection system as shown in Table 3. In this assessment, vendor recommended protocol settings

were used by only 22% of the laboratories. Use of OptiView detection was a successful protocol modification, resulting in an optimal staining result for 94% (29/31) of users, compared to 76% 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 100% (16 of 16) and an optimal rate of 81% (13 of 16).

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 95%. The proportion of sufficient and optimal results obtained by the vendor recommended protocol settings was slightly decreased compared to laboratory modified protocols as shown in Table 3. The modified protocols either increased incubation time of the primary Ab and/or added a rabbit linker for the detection system. A modification including rabbit linker was used by 10 laboratories: 100% obtained a sufficient mark and 50% being optimal staining results.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb clone EP1 was used by 10% of the participants and provided an overall pass rate of 75%. As shown in Table 3, 89% (32 of 36) 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 17 laboratories: 82% obtained a sufficient mark (14/17) and 24% (4/17) being optimal staining results.

The Leica RTU system PA009/PA0151 for BOND based on mAb clone 6F11, was used by 4% of the laboratories and gave an overall pass rate of 60%. In this assessment, vendor recommended protocol settings 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, all with insufficient 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), 29% optimal (2 of 7). These two optimal results were obtained using BERS2 for 30 min., and 100% (3 of 3) laboratories using this protocol obtained a sufficient result. 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 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 12% (45 of 379) of the participants. All three mAb clones (6F11, EP1 and SP1) used in a LD assay could provide an optimal result on the tissues supplied in this run, with the highest rate of optimal results seen with rmAb SP1 on the Ventana platform (6 of 8, 75%). Sufficient results were obtained with all three mAbs 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 73% (33 of 45), with 38% (17 of 45) 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, one poor, false positive result (positive staining in the negative breast tumour) was seen using mAb clone 6F11 and high pH retrieval (BERS2 on Leica Bond).

In this run, both 2- and 3-layer detection systems performed evenly: 51% (23 of 45) of the laboratories using concentrated antibody formats used a 3-layer detection system, with 49% (22 of 45) using a 2-layer system. Laboratories using a 3-layer system obtained a pass rate of 70%, with 43% optimal, compared to 2-layer detection system, with a pass rate of 77%, 32% 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 the level of 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. 2 and 5. 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

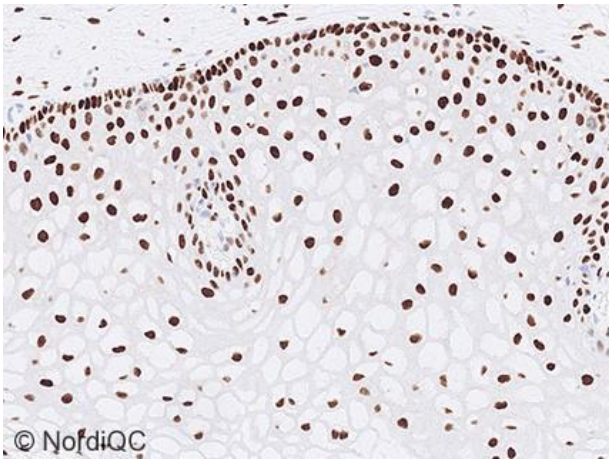


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, using HIER in CC1 (pH 8.5), visualized by a 3-step detection system and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a-5a, same protocol.

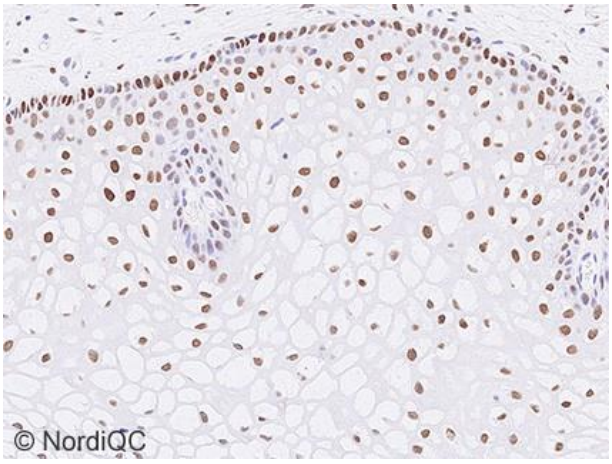


Fig. 1b
ER staining of the uterine cervix using the mAb clone 6F11 based RTU format (PA0151) from Leica Biosystems, using HIER in BERS1 (pH 6), visualized by a 3-step detection system and performed on BOND – same field as in Fig. 1a.
The intensity of the ER staining reaction is reduced compared to the level seen in Fig. 1a, but still the expected proportion of cells demonstrated as expected.
However, also compare with Figs. 2b- 4b, same protocol.

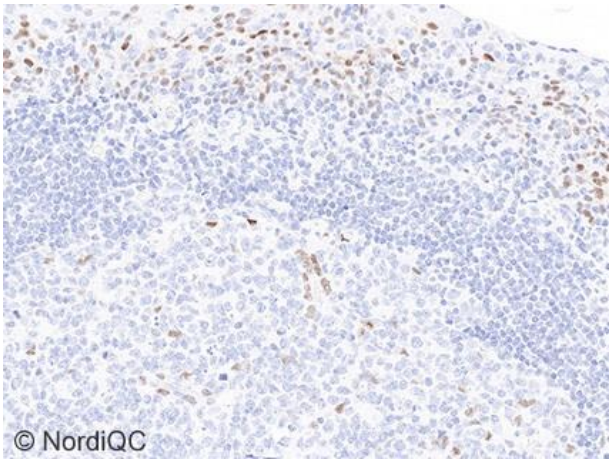


Fig. 2a
Optimal ER staining of the tonsil using same protocol settings as in Fig. 1a.
A moderate, distinct nuclear staining reaction is seen in dispersed follicular dendritic cells in the germinal center and squamous epithelial cells.
No nuclear staining reaction is seen in the mantle zone B-cells and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

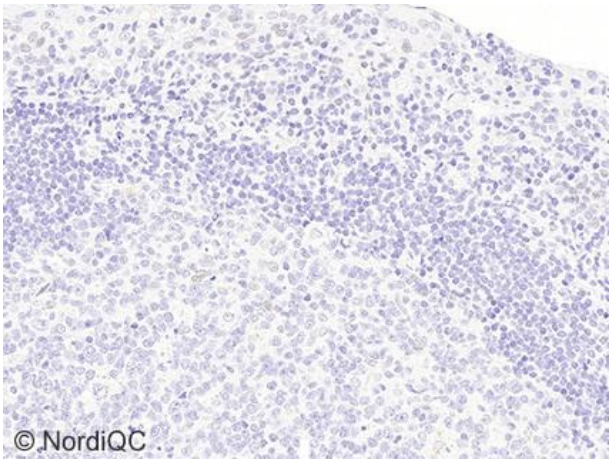


Fig. 2b
Insufficient ER staining of the tonsil using same protocol settings as in Fig. 1b.
Only a faint equivocal staining reaction is observed in few follicular dendritic cells in the germinal center and squamous epithelial cells.
Compare with Fig. 2a – same field.
Tonsil was found to be superior as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

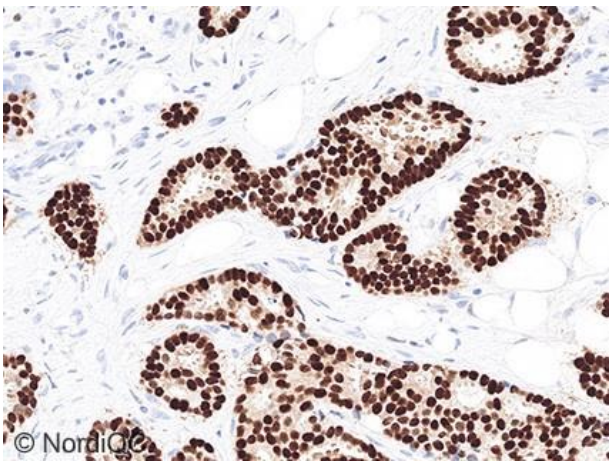


Fig. 3a
Optimal ER staining of the breast carcinoma no. 2 with 90-100% cells being positive using same protocol as in Figs. 1a-2a.
The neoplastic cells display a strong and distinct nuclear staining reaction.

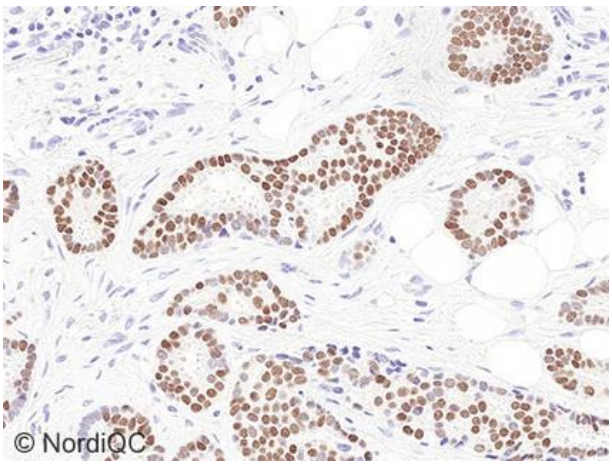


Fig. 3b
ER staining of the breast carcinoma no. 5 with expected 90-100% cells being positive using same protocol as in Figs. 1b-2b – same field as in Fig. 3a.
The staining intensity of positive cells is reduced compared to the optimal result in Fig. 3a. However, the protocol still provides the expected proportion of positive cells, as the ER expression level is high in the neoplastic cells

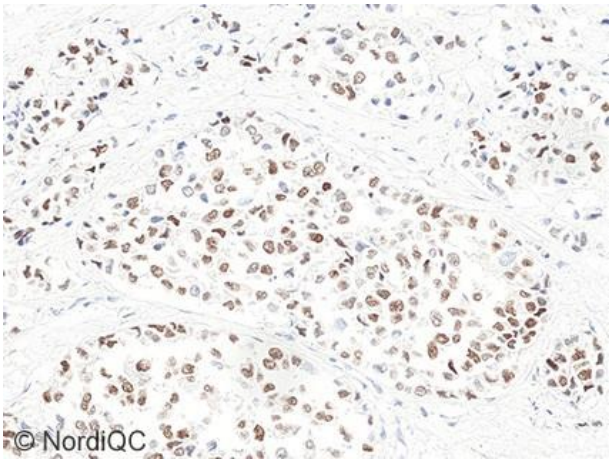


Fig. 4a
Optimal ER staining of the breast carcinoma no. 5 with 50-90% of the neoplastic cells expected to be positive using same protocol as in Figs. 1a-3a.
The vast majority of the neoplastic cells display a weak and distinct nuclear staining reaction.

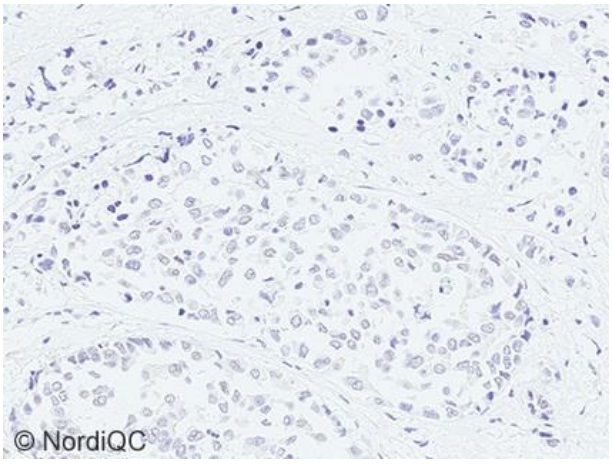


Fig. 4b
Insufficient and false negative ER staining of the breast carcinoma no. 5 with expected 50-90% cells being positive using same protocol as in Figs. 1b-3b.
<1% of the neoplastic cells are positive.

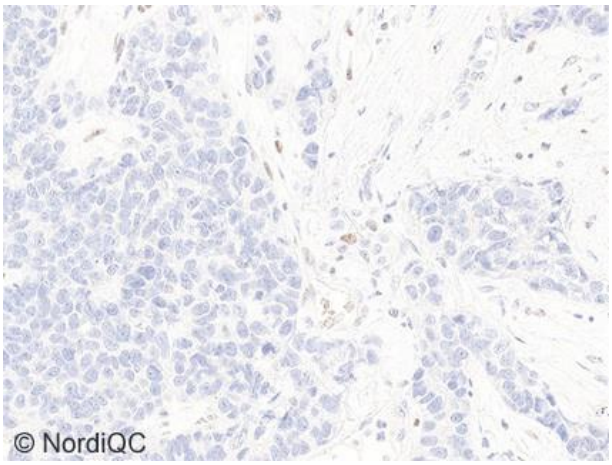


Fig. 5a
Optimal ER staining of the breast carcinoma no. 4 expected to be negative using same protocol as in Figs. 1a-4a.
No nuclear staining reaction is seen in the neoplastic cells and a high signal-to-noise ratio is observed facilitating the read-out.
Note, dispersed stromal cells show a weak positive 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.

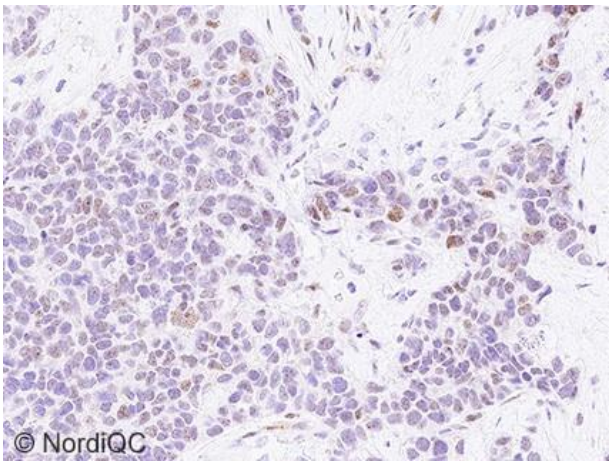


Fig. 5b
Insufficient and false positive ER staining of the breast carcinoma no. 4 expected to be negative. The protocol based on the mAb clone 6F11 in a concentrated format, using HIER in an alkaline buffer and a 3-step polymer-based detection system.
An aberrant false positive nuclear staining reaction is seen in >1% of the neoplastic cells.
Similar aberrant nuclear staining reaction in breast carcinomas expected to be negative has been seen in previous NordiQC assessment, when mAb clone 6F11 is used by IHC protocols with high technical sensitivity.

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