Assessment Run B35 2023

Estrogen receptor (ER)

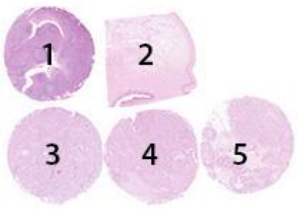
Purpose

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	90-100%	Moderate to strong
4.	Breast carcinoma	80-90%	Weak to moderate
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]

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

- A moderate to strong, distinct nuclear staining of virtually all squamous epithelial, columnar epithelial cells (if present) 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 moderate to strong distinct nuclear staining in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 3.
- An at least weak to moderate distinct nuclear staining in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 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 or poor morphology as described above could also result in a grade of **poor** where interpretation was severely hampered.

Participation

Number of laboratories registered for ER, B35	448
Number of laboratories returning slides	422 (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

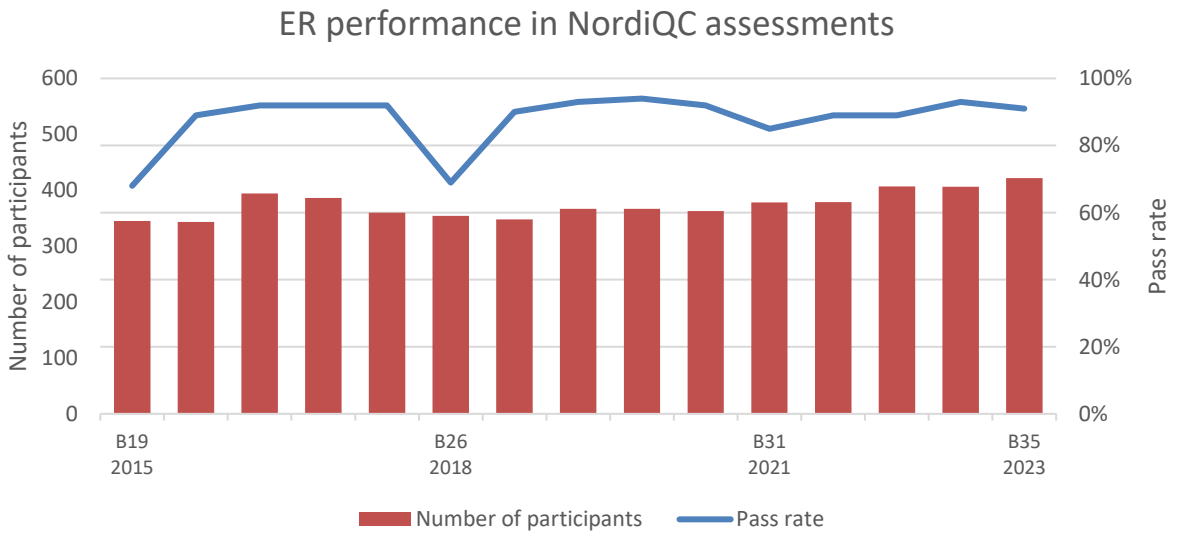
422 laboratories participated in this assessment. 385 of 422 (91%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 4).

The most common staining faults reported were weak staining and excessive counterstaining hindering interpretation, and the most frequent causes of insufficient staining reactions were:
- Insufficient Heat Induced Epitope Retrieval (HIER) time or HIER in acidic buffer (weak staining)
- HIER in an inappropriate buffer or for extended times (false positive staining)
- Use of detection systems with low sensitivity
- Unexplained / technical issues during staining process

Performance history

In this run the pass rate of 91% (proportion of sufficient results) was slightly lower than for run B34 (93%) although the pass rate has been stable at a high and satisfactory level in runs between 2015-2023, with the exception of runs B19 and B26 (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER from 2015 - 2023**



Fluctuations in pass rates (e.g. as seen in runs B19 and B26), is likely 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 incoming new participants, but in both this run and the previous run B34 similar pass rates were observed for both existing and new registered participants. In average 5% more participants were registered in these two runs.

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 (89%, 377 of 422) used Ready-To-Use (RTU) systems, with the majority of these (230 of 377, 61%) 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 92%, versus 84% for concentrated formats. "Plug and play" RTU assays (where a RTU clone was used on its intended fully automated platform) gave an overall pass rate of 96% across the two major manufacturers platforms (Ventana/Roche BenchMark: 95% and Dako/Agilent Omnis: 98%).

The commonest failing, accounting for 52% 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, leading to difficulties in scoring. In 6 cases, the counterstain was so excessive as to preclude interpretation entirely. In addition, as seen in previous runs, 4 instances of false positive staining of the negative tumour were seen, and in all cases the aberrant result was related to the mAb clone 6F11 being used with high pH HIER (specifically BERS2 pH 9 on Leica Bond).

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, whereas endothelial cells and lymphocytes must be negative. 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, B35**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **1D5**	1	Zytomed (BioCare)	0	0	1	0	-	-
mAb clone **6F11**	15	Leica Biosystems	6	8	1	0	93%	40%
rmAb clone **EP1**	8 3	Dako/Agilent Cell Marque	5	3	2	1	73%	45%
rmAb clone **SP1**	9 3 2 1 1	Thermo Sci./ePredia Cell Marque Zytomed AbCam DCS	13	2	1	0	94%	81%
rmAb clone **QR013**	1	Quartett	0	1	0	0	-	-
rmAb clone **ZR147**	1	Zeta Corporation	0	0	1	0	-	-
Ready-To-Use antibodies							Suff.[1]	OR[2]
mAb clone **6F11** **PA0009/PA0151 (VRPS[3])**	2	Leica Biosystems	0	2	0	0	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	19	Leica Biosystems	8	4	3	4	63%	42%
rmAb **EP1** **IR/IS084 (VRPS[3])**	3	Dako/Agilent	1	2	0	0	-	-
rmAb **EP1** **IR/IS084 (LMPS[4])**	29	Dako/Agilent	7	19	3	0	90%	24%
rmAb **EP1** **GA084 (VRPS[3])**	42	Dako/Agilent	29	12	0	1	98%	69%
rmAb **EP1** **GA084 (LMPS[4])**	27	Dako/Agilent	13	13	1	0	96%	48%
rmAb **EP1** **RMPD051**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb **EP1** **8361-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **SP1** **790-4324/4325 (VRPS[3])***	50	Ventana/Roche	30	20	0	0	100%	60%
rmAb clone **SP1** **790-4324/4325 (LMPS[4])***	184	Ventana/Roche	124	48	10	2	93%	67%
rmAb clone **SP1** **249R-17/18**	7	Cell Marque	3	3	1	0	86%	43%
rmAb clone **SP1** **M3011**	2	Spring Biosystems	0	0	2	0	-	-
rmAb clone **SP1** **MAD-000306QD/V** **MAD-000306QD-7/N**	2	Master Diagnostica Vitro SA	0	0	2	0	-	-
rmAb clone **SP1** **RMPD001**	2	Diagnostic BioSystems	2	0	0	0	-	-
rmAb clone **SP1** **KIT-0012**	1	Fuxhou Maixin	1	0	0	0		
rmAb clone **SP1** **BRB056**	1	Zytomed Systems	1	0	0	0	-	-
mAb clone **1D5** **AMC94-5M**	1	BioGenex	0	0	0	1	-	-
Ab clone **658G3A2** **PA212**	1	Abcarta	1	0	0	0	-	-
Ab clone **EAB-006** **01.09.70.01.18.01**	1	Zybio	0	1	0	0	-	-
Total	422		246	139	28	9		
Proportion			58%	33%	7%	2%	91%	

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 B35
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: Six of 15 laboratories obtained optimal results: five were based on high pH HIER, using Cell Conditioning 1 (CC1, Ventana/Roche) (1/2)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent)(1/1) or Bond Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica Biosystems) (3/10) as retrieval buffer. The mAb was diluted in the range of 1:25-1:200 and combined with a 2- or 3-layer detection system. Using these protocol settings, 13 of 13 (100%) of laboratories produced a sufficient staining result (optimal or good), and 5 of 13 (38%) of laboratories produced an optimal result. Low pH HIER (Leica Biosystems Bond Epitope Retrieval Solution 1, pH 6.0) was employed by two laboratories, with one optimal result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Five of 11 laboratories obtained optimal results, using protocols based on high pH HIER, using TRS pH 9 (3-in-1) (Dako/Agilent) (4/5) or BERS2 pH 9.0 (Leica Biosystems) (1/1) as retrieval buffer. The rmAb was diluted 1:50-1:100 and combined most often with a 2-layer detection system. Using these protocol settings, 5/5 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP1**: Thirteen of 16 laboratories obtained optimal results. Protocols with optimal results were typically based on high pH HIER, using CC1 (Ventana/Roche) (5/7), TRS pH 9 (Dako/Agilent) (3/3), BERS2 pH 9.0 (Leica Biosystems) (2/2) or Tris-EDTA/EGTA pH 9 (2/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:30-1:300 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 14 of 14 (100%) 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**	-	-	1/1	-	1/2**	-	3/10 (33%)	1/2
rmAb clone **EP1**	2/2	0/1	2/3	-	0/3	-	1/1	-
rmAb clone **SP1**	-	-	3/3	-	5/7 (71%)	-	2/2	-

* 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 20-40 min. (mode = 20 min.), 15-30 min. incubation of the primary Ab (mode = 15 min.) and Bond Polymer Refine Detection (DS9800) as detection system. Of the laboratories using these protocol settings, 11 of 16 (69%) produced a sufficient staining result (optimal or good).
Three laboratories were able to obtain a sufficient result using HIER with BERS1 (low pH), although none were optimal.

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 18 (83%) produced a sufficient staining result.
8 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.), 10-30 min. incubation of the primary Ab (mode = 10 min.) and EnVision FLEX (GV800) with or without rabbit linker (GV809) as detection system. Of the laboratories using these protocol settings, 59 of 60 (98%) produced a sufficient staining result.
4 laboratories used product no. GA084 on other platforms and are 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 8-92 min. (mode = 64 min.), 12-56 min. incubation of the primary Ab (mode = 16 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, 212 of 222 (96%) laboratories produced a sufficient staining result.
4 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**	3/3	1/3	18/21 (86%)	4/21 (19%)
Dako Omnis rmAb EP1 **GA084**	41/42 (98%)	29/42 (69%)	23/23 (100%)	12/23 (52%)
Leica Bond mAb 6F11 **PA009/PA0151**	2/2	0/2	12/19 (63%)	8/19 (42%)
VMS Ultra/XT/GX rmAb SP1 **790-4324/4325**	50/50 (100%)	30/50 (60%)	169/180 (94%)	121/180 (67%)

* 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 81% of the insufficient results (30 of 37). A weak or false negative staining reaction was further complicated by excessive counterstain in 16% (6 of 37) of the insufficient results. In one case, the counterstain was so excessive as to make resolution of the ER signal impossible. Clinically-relevant false positive staining reaction, where >1% of cells in the negative breast tumour (tissue core no. 5) stained unequivocally, was seen in 11% (4 of 37) of the insufficient results. Poor signal-to-noise ratio and excessive background were seen in 3% and 7% of insufficient staining results respectively.

Virtually all laboratories were able to demonstrate ER in the high-level ER-expressing breast carcinoma (tissue core no. 3), 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. 4), in which an at least weak nuclear staining reaction of 80-90% of the neoplastic cells was expected, was much more challenging.

In this run an increased number of sufficient results assessed as good was observed and characterized by either a poor-signal-to noise ratio, excessive background reactions and/or excessive "in-selective" nuclear counterstaining. The observation on both excessive background and "in-selective" nuclear counterstaining was primarily related to staining on the Omnis platform (Dako/Agilent). The primary cause for the background reaction problem is likely related to poor lots of the HRP Envision Flex reagents (Dako/Agilent), that has been on the marked since November-December 2022. Dako/Agilent is aware of this problem and it has not yet been solved by up-loading date of this report.

Ready-To-Use (RTU) Abs were used by 89% (377 of 422) of the participants. 92% (347 of 377) of these laboratories obtained a sufficient staining result, 59% optimal (222 of 377).

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 all the participants and gave an overall pass rate of 95%. Laboratory modified protocols (LMPS) were used by the majority (77%) 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 (LPMS) 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 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 a slightly reduced pass rate of 97% (36/37 users) versus 100% for the manufacturer's protocol, but with an optimal score rate of 78% (29 of 37) (see Table 3) compared to 60% using VRPS. 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 64% (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 97%.
The proportion of sufficient results of 98% and 100% obtained by the VRPS and LMPS, respectively was almost identical, whereas the VRPS provided 69% optimal results compared to 52% for laboratories applying LPMS. Five laboratories used a modified protocol including rabbit linker, with a pass rate of 100% and 17 laboratories increased the primary antibody incubation time to 15-30 minutes, with a pass rate of 100%.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb EP1 was used by 6% of the participants and provided an overall pass rate of 91%. As shown in Table 3, 88% (21 of 24) of the laboratories modified the protocol settings and obtained a slightly lower pass rate and reduced proportion of optimal results compared 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 15 laboratories: 87% of these obtained a sufficient mark (13/15) with 27% (4/15) optimal.

The Leica RTU system PA0009/PA0151 for BOND based on mAb 6F11, was used by 5% (21 of 422) of the participants and gave an overall pass rate of 67%. 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 both achieving sufficient results. Laboratories using a protocol modification increasing analytical sensitivity by using HIER in BERS2 (high pH) for 20 min. obtained a pass rate of 89% (8 of 9), 67% optimal (6/9). Extension of the primary antibody incubation time in conjunction with HIER in BERS1 (low pH) was performed by two laboratories, neither of which obtained satisfactory results. However, 4 instances of false positive staining of the negative tumour (breast carcinoma no. 5) were seen with high pH retrieval, as previously also noted in run B28 and sporadically in subsequent runs. This was linked to extended retrieval in BERS2 with or without extension of primary antibody incubation time.

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 11% (45 of 422) 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 Omnis (3 of 3, 100%) or Ventana platform (5 of 7, 71%). Overall, the rmAb clone SP1 was the most commonly used concentrated antibody, and the most reliable, with 81% 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 1 and 2. The overall pass rate for all laboratories using concentrated antibody formats in this run was 84% (38 of 45), with 53% (28 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 seen here and discussed in runs B28 and B15) were found to be the common core elements for an optimal performance.

In this run, 3-layer detection systems performed more successfully than 2-layer detection systems: the majority of the laboratories using concentrated antibody formats used a 3-layer detection system (53%, 24/45), with 47% (21 of 45) using a 2-layer system. Laboratories using a 3-layer system obtained a pass rate of 92%, with 45% optimal, compared to 2-layer detection system, with a pass rate of 76%, 62% 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

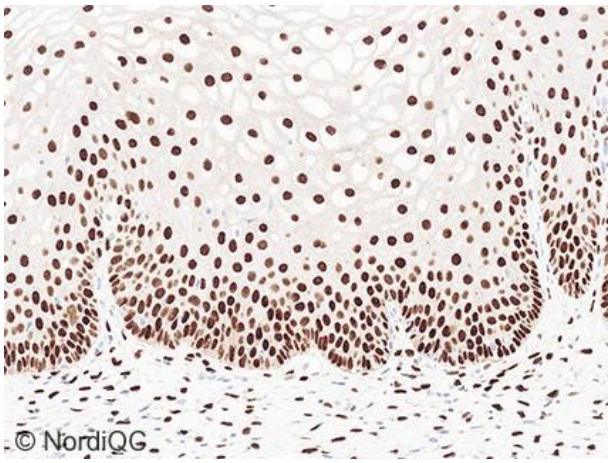


Fig. 1a
Optimal ER staining of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche by vendor recommended protocol settings using HIER in CC1 (pH 8.5), visualized by UltraView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a - 6a, same protocol.

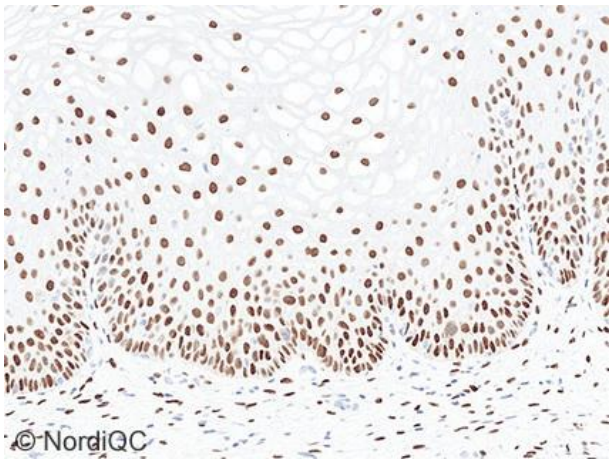


Fig. 1b
ER staining of the uterine cervix using the rmAb clone SP1 as concentrate, using HIER in an alkaline buffer and visualized by a 2-step detection system – same field as in Fig. 1a. The intensity of the ER staining reaction is reduced compared to the level seen in Fig. 1a, but still the expected proportion of cells are demonstrated. However, also compare with Figs. 2b - 5b, same protocol.

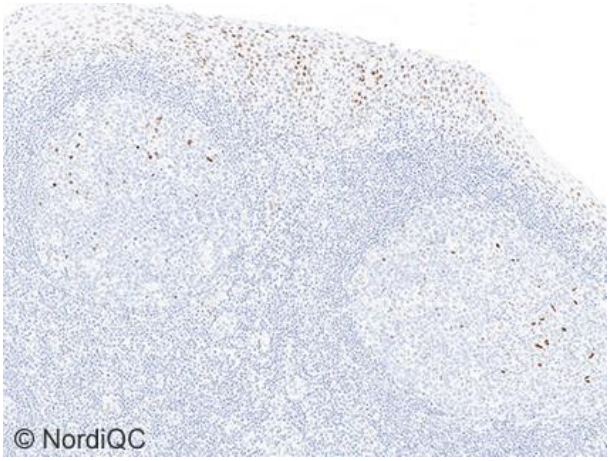


Fig. 2a
Optimal ER staining of the tonsil using the same protocol as in Fig. 1a.
A distinct nuclear staining reaction in both dispersed follicular dendritic cells in the germinal center and many squamous epithelial cells can be identified at even low magnification (5x).

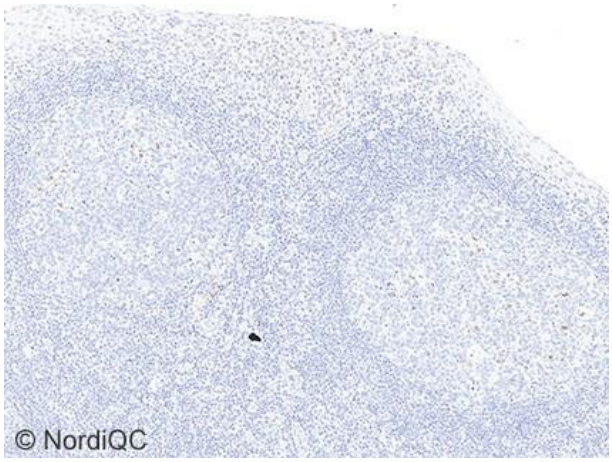


Fig. 2b.
Insufficient ER staining of the tonsil using the same protocol as in Fig. 1a.
At low magnification (5x), no definite nuclear staining reaction can be identified and only a weak hue is seen in few germinal center cells.
Also compare with Fig. 3b, same tonsil and area at 20x

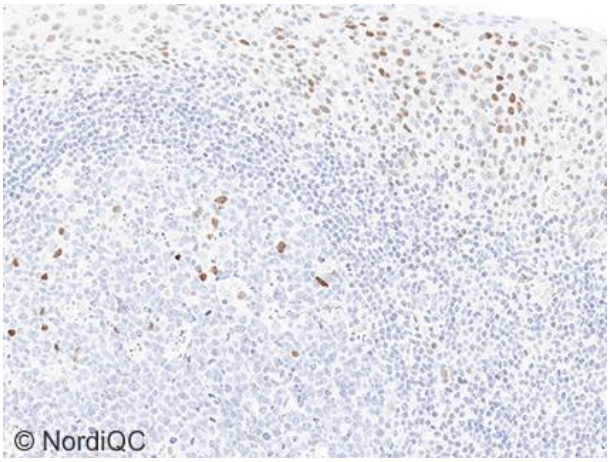


Fig. 3a
Optimal ER staining of the tonsil using the same protocol as in Figs. 1a and 2a.
A moderate, distinct nuclear staining reaction is seen in both dispersed follicular dendritic cells in the germinal center and many squamous epithelial cells.
No nuclear staining reaction is seen in the mantle zone B-cells and in general a high signal-to-noise ratio is seen.

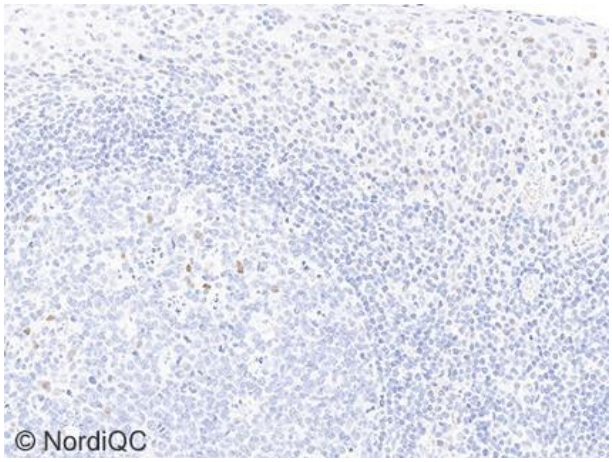


Fig. 3b
Insufficient ER staining of the tonsil using same protocol settings as in Figs. 1b and 2b.
Only a faint equivocal staining reaction is observed in few follicular dendritic cells in the germinal center and squamous epithelial cells.
Compare with Fig. 3a – same field.

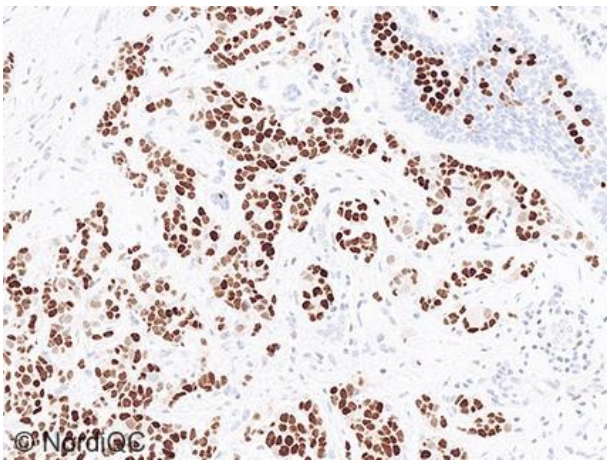


Fig. 4a
Optimal ER staining of the breast carcinoma, tissue core no. 3, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a – 3a.
The neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

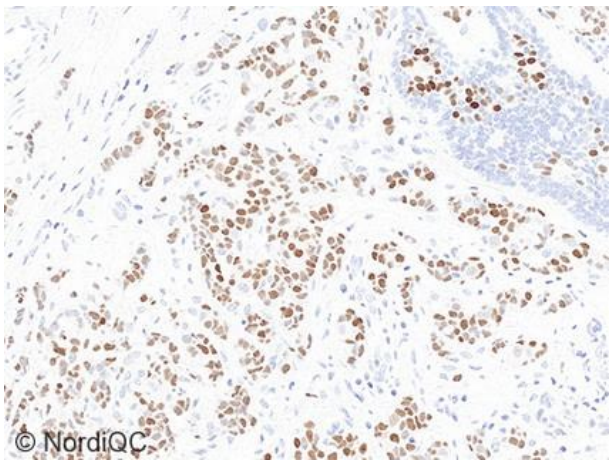


Fig. 4b
ER staining of the breast carcinoma, tissue core no. 3, with 90-100% cells being positive using the same protocol as in Fig. 1b - 3b.
The neoplastic cells display a clear positive staining reaction for ER. However also compare with Fig. 5b, same protocol.

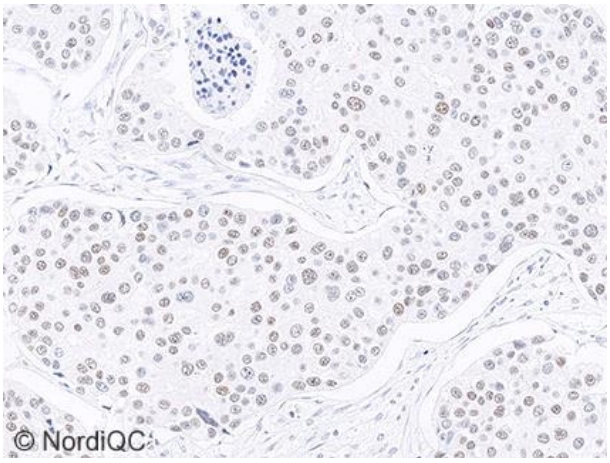


Fig. 5a
Optimal ER staining of the breast carcinoma, tissue core no. 4, with 80-90% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a – 4a.
The majority of the neoplastic cells display a weak but distinct nuclear staining reaction.

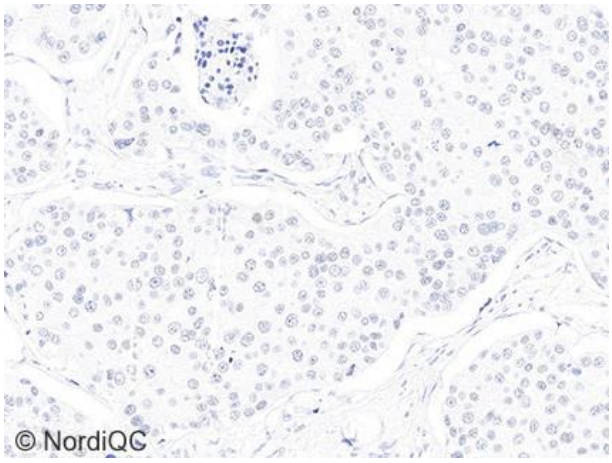


Fig. 5b
Insufficient and false negative ER staining of the breast carcinoma, tissue core no. 4, with 80-90% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1b - 4b.
No nuclear staining reaction for ER can be identified.

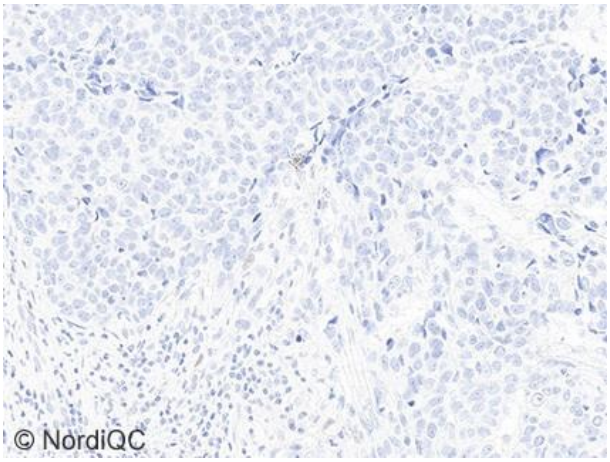


Fig. 6a
Optimal ER staining of the breast carcinoma no. 5 expected to be negative using same protocol as in Figs. 1a – 5a. No nuclear staining reaction is seen and a high signal-to-noise ratio is observed.

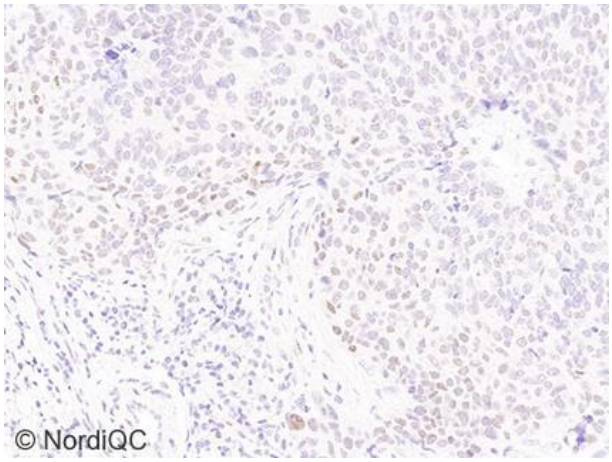


Fig. 6b
Insufficient ER staining of the breast carcinoma no 5 with no ER expression. A weak but distinct nuclear staining reaction is seen in >10% of the neoplastic cells.
The insufficient result was only seen for the mAb clone 6F11 and likely was caused by performing HIER in an alkaline buffer in combination with other protocol settings inducing a too high level of technical/analytical IHC sensitivity compromising the diagnostic specificity.

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