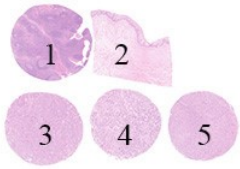
Assessment Run B38 2024

Estrogen receptor (ER)

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

Material
The slide to be stained for ER comprised:

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



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

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

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

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

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

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

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

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

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

KEY POINTS FOR ER IMMUNOASSAYS

- The rmAb clones **EP1** and **SP1** were most successful and highly recommendable.
- The mAb clone **6F11** was found less reproducible as also seen in several previous assessment runs giving both false negative and false positive results.
- RTU systems for ER gave a superior pass rate compared to assays based on concentrated Ab formats.

Participation

Number of laboratories registered for ER, B38	466
Number of laboratories returning slides	438 (94%)

At the date of assessment, 438 (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

438 laboratories were assessed in this assessment run. 380 (87%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 3-5).

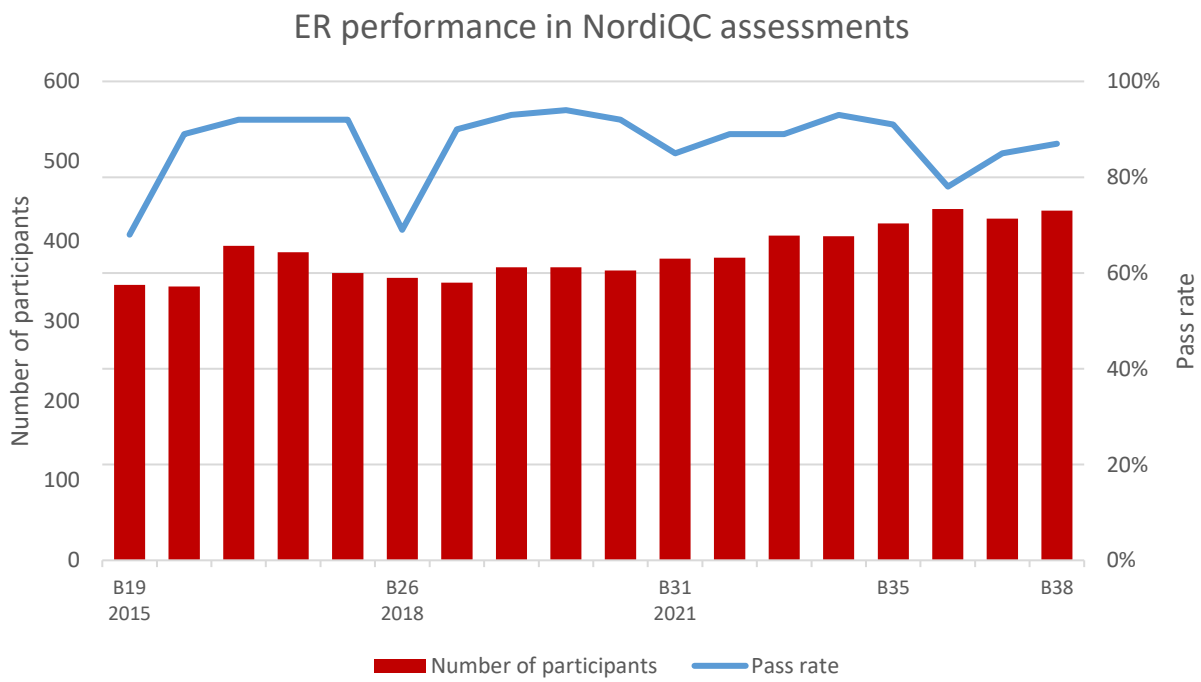
The most common staining faults reported were weak staining, poor signal to noise and excessive counterstaining hindering interpretation. False positive staining was seen in 1% of the submitted slides. The most frequent causes of insufficient staining reactions were:

- Use of detection systems with low sensitivity (weak staining)
- Insufficient Heat Induced Epitope Retrieval (HIER) time or HIER in acidic buffer (weak staining)
- Excessive HIER or primary Ab incubation time (scoring hindered by excessive background or poor signal/noise ratio)
- Use of mAb clone 6F11 with extended HIER in an alkaline buffer (false positive staining of the negative tumour)

Performance history

In this run the pass rate of 87% (proportion of sufficient results) was a small increase compared to run B37 (85%) although previously the pass rate had been stable at a high and satisfactory level in runs between 2015-2024, with the exception of runs B19 and B26 (see Graph 1). The pass rate for run B36 was slightly lowered (78%) and level of 87% for run B38 exactly on par to the average level for runs B19-B38.

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



Fluctuations in pass rates (e.g. as seen in runs B19 and B26), can be caused by the circulation of more challenging material. To ensure 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 may also be influenced by new participants and new participants have been registered for each of the last four runs. However, similar pass rates were observed for both existing and newly registered participants. In the previous two runs, a reduced pass rate was in particular largely attributed to an inferior performance using the most common combination of primary antibody and staining platform, RTU format of rmAb clone SP1 (790-4324/790-4325) on the Ventana BenchMark platform group. Over half of the participants used this combination over the last four runs (B35: 61%; B36: 58%; B37: 55%; B38: 56%). The pass rate for this group fell from 95% in run B35 to 78% in Run B36, rising back to 91% in this run. The insufficient results across all four runs were mainly characterized by reduced analytical sensitivity.

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 (92%, 403 of 438) used Ready-To-Use (RTU) systems, with the majority of these (261 of 403, 65%) 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 88% (356/403), versus 69% (24/35) for concentrated formats. "Plug and play" RTU assays (where a RTU format was used on its intended fully automated platform) gave an overall pass rate of 91% across the two major manufacturers platforms, with both Ventana/Roche BenchMark and Dako/Agilent Omnis delivering a pass rate of 91%. In this run, the most robust performance was seen equally using clone EP1 for Omnis or clone SP1 on Benchmark Ultra/Ultra PLUS/GX used as a true "plug and play" (vendor-recommended) assay, each with a pass rate of 95% and an optimal rate of 50% and 58% respectively.

The commonest failing, accounting for 51% (39/58) of insufficient results in this assessment, was low analytical sensitivity giving a weak or false negative staining reaction. Low analytical sensitivity and weak demonstration of ER was often further complicated by excessive or "inselective" counterstaining (where nuclei were difficult to distinguish from cytoplasm), or poor signal-to-noise ratios, leading to difficulties in scoring. Four submissions showed clinically-relevant false positive staining of the tumour expected to be negative for ER.

Uterine cervix and tonsil continue to be 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 1a. **Overall results for ER, run B38**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	35	12	12	8	3	69%	34%
Ready-To-Use antibodies	403	224	132	39	8	88%	55%
Total	438	236	144	47	11		
Proportion		54%	33%	11%	3%	87%	

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

Table 1b. **Concentrated antibodies and assessment marks for ER, run B38**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11**	16	Leica Biosystems	3	7	4	2	63%	19%
rmAb clone **EP1**	4 1	Dako/Agilent CellMarque	3	2	0	0	100%	60%
rmAb clone **SP1**	7 3 1 1 1	Thermo Sci./ePredia Cell Marque Diagnostic BioSystems AbCam BioCare	6	3	4	0	69%	46%
rmAb clone **QR013**	1	Quartett	0	0	0	1	-	-
Total	35		12	12	8	3		
Proportion			34%	34%	23%	9%	69%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for ER, run B38**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11** **PA0009/PA0151 (VRPS[3])**	3	Leica Biosystems	0	0	2	1	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	16	Leica Biosystems	1	5	5	5	38%	6%
rmAb **EP1** **IR084/IS084 (VRPS[3])**	5	Dako/Agilent	2	1	2	0	60%	40%
rmAb **EP1** **IR084/IS084 (LMPS[4])**	23	Dako/Agilent	12	10	1	0	96%	52%
rmAb **EP1** **GA084 (VRPS[3])**	40	Dako/Agilent	20	18	2	0	95%	50%
rmAb **EP1** **GA084 (LMPS[4])**	34	Dako/Agilent	18	12	4	0	88%	53%
rmAb **EP1** **RMPD051**	1	Diagnostic BioSystems	1	0	0	0	-	-
rmAb **EP1** **8361-C010**	2	Sakura Finetek	1	1	0	0	-	-
rmAb clone **SP1** **790-4324/4325 (VRPS[3])***	74	Ventana/Roche	43	27	3	1	95%	58%
rmAb clone **SP1** **790-4324/4325 (LMPS[4])***	188	Ventana/Roche	118	52	17	1	90%	63%
rmAb clone **SP1** **249R-17/18**	4	Cell Marque	3	0	1	0	-	-
rmAb clone **SP1** **MAD-000306QD/V** **MAD-000306QD-7/N**	2	Master Diagnostica Vitro SA	0	1	1	0	-	-
rmAb clone **SP1** **RMPD001**	1	Diagnostic BioSystems	1	0	0	0	-	-
rmAb clone **SP1** **GT205602**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **SP1** **BRB053**	4	Zytomed Systems	0	3	1	0	-	-
rmAb clone **SP1** **ALR 301 G7**	1	BioCare Medical	1	0	0	0	-	-
rmAb clone **SP1** **Kit-0012**	1	Fuzhou Maixin	1	0	0	0	-	-
rmAb clones **SP1+6F11** **PM308**	1	BioCare Medical	0	1	0	0	-	-
Ab clone **658G3A2** **PA212**	1	Abcarta	0	1	0	0	-	-
Ab clone **MXR030** **RMA-1065**	1	Fuzhou Maixin	1	0	0	0	-	-
Total	403		224	132	39	8		
Proportion			56%	33%	10%	2%	88%	

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 B38

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **6F11**: Three of 16 laboratories obtained optimal results: all were based on high pH HIER, using Cell Conditioning 1 (CC1, Ventana/Roche) (2/3)* or Bond Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica Biosystems) (1/13) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 and combined with a 2- or 3-layer detection system. Using these protocol settings, 7 of 12 (58%) laboratories produced a sufficient staining result (optimal or good).

As seen in previous runs, HIER at high pH could lead to false positive staining of tumour, tissue core number 3 (expected negative), and this was seen in 2 of 6 of the insufficient results. Low pH HIER was not employed by any laboratory.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: Three of 5 laboratories obtained an optimal result, using a protocol based on high pH HIER, using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (2/2) or BERS2 pH 9.0 (Leica Biosystems) (1/1) as retrieval buffer. The rmAb was diluted 1:50 and combined with a 2-layer or 3-layer detection system. An optimal result was obtained by all 3 labs using these parameters.

rmAb clone **SP1**: Six of 13 laboratories obtained optimal results. Protocols with optimal results were all based on high pH HIER, using CC1 (Ventana/Roche) (3/6), TRS pH 9 (Dako/Agilent) (2/3) or BERS2 pH 9.0 (Leica Biosystems) (1/3) as retrieval buffer. The rmAb was typically diluted in the range of 1:30-1:200 and combined with either a 2- or 3-layer detection system. Using these protocol settings, 9 of 12 (75%) laboratories produced a sufficient staining result. One laboratory used low pH retrieval (Bond Epitope Retrieval Solution 1(BERS1) pH 6.0, Leica Biosystems), but did not obtain 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**		**Leica Bond III/Prime**	
	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**	-	-	-	-	2/3**	-	1/13 (8%)	-
rmAb clone **EP1**	0/1	-	2/2	-	0/1	-	1/1	-
rmAb clone **SP1**	-	-	2/3	-	3/6 (50%)	-	1/3	-

* 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/Bond PRIME:
Only one optimal result was obtained, using the following protocol: HIER in BERS2 (high pH) for 20 min., 60 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Of the 19 laboratories using this antibody, 6/14 (43%) were able to achieve sufficient results using a protocol based on HIER using BERS2 (15-30 min.), 15-60 min. incubation of primary Ab and Bond Polymer Refine or Bond PRIME Polymer DAB Detection. Four laboratories used a protocol using HIER with BERS1 (low pH), but none achieved a sufficient result.

rmAb clone **EP1**, product no. **IR084/IS084**, Dako/Agilent, Dako Autostainer+/Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 97-98°C; mode = 20 min.), 20-40 min. incubation of the primary Ab (mode = 20 min.) and EnVision FLEX (K8000/SM802, K8010/DM822) or EnVision FLEX+ (K8002/SM802) as detection system, with or without Rabbit Linker (K8009, K8019). Of the laboratories using these protocol settings, 15 of 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 20-40 min. at 97°C, mode = 30 min.), 10-30 min. incubation of the primary Ab (mode = 10 min.) and EnVision FLEX (GV800/GV823) with or without rabbit linker (GV809) as detection system. Of the laboratories using these protocol settings, 65 of 71 (91%) produced a sufficient staining result.
3 laboratories used product no. GA084 on another platform and are not included in the description above.

rmAb clone **SP1**, product no. **790-4324/4325**, Ventana/Roche, BenchMark XT, ULTRA, ULTRA Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 4-92 min. (mode = 64 min.), 4-64 min. incubation of the primary Ab (modes = 16 and 32 min.) and UltraView (760-500) with or without UltraView Amplification kit (760-080), or OptiView (760-700) without amplification as detection system. Using these protocol settings, 227 of 249 (91%) laboratories produced a sufficient staining result, 152 (61%) optimal.
10 laboratories used product no 790-4324/4325 on other platforms. These were not included in the description above.

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

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

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 rmAb EP1 **IR084/IS084**	3/5 (60%)	2/5 (40%)	14/15 (93%)	7/15 (47%)
Dako Omnis rmAb EP1 **GA084**	38/40 (95%)	20/40 (50%)	27/31 (87%)	17/31 (55%)
Leica Bond III/Max/Prime mAb 6F11 **PA0009/PA0151**	0/3	0/3	6/16 (38%)	1/16 (6%)
VMS Ultra/XT/Ultra Plus rmAb SP1 **790-4324/4325**	70/74 (95%)	43/74 (58%)	160/178 (90%)	110/178 (62%)

* 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 in 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 79% of the insufficient results (46/58). A weak or false negative staining reaction was further complicated by excessive or "inselective" counterstain in 10% (6/58) of the insufficient results. Clinically relevant false positive staining reaction, where >1% of cells in the negative breast tumour (tissue core no. 3) stained unequivocally, was seen in four of the insufficient results. Poor signal-to-noise ratio was noted in 12% 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. 5), in which an at least weak nuclear staining reaction of 80-100% of the neoplastic cells was expected, was more challenging.

In the previous two runs, an increased number of sufficient results assessed as good (B36: 56% and B37: 50% of all results) were observed in comparison to Run B35 and previous runs, largely characterized by reduced analytical sensitivity manifesting as significantly fewer cells staining positive for ER than expected when using the Roche SP1 RTU on the Ventana Benchmark platform group. In this run, the optimal rate returned to B35 levels, with over half of all participants (54%) achieving an optimal staining result and 33% good. Other features of insufficient results in this run included a poor-signal-to noise ratio, associated largely with the Leica Bond platform and excessive or "inselective" nuclear counterstaining (associated with the Dako Omnis and Ventana Benchmark platforms respectively). False positive staining observed in this run was related to use of clone 6F11 with a HIER in a high pH buffer on the Leica Bond platform, most commonly on the Bond PRIME instrument.
Ready-To-Use (RTU) Abs were used by 92% (403/438) of the participants. 88% (356/403) of these laboratories obtained a sufficient staining result, 56% optimal (224/403).

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 57% (252/438) of all the participants and gave an overall pass rate of 91% (230/252), 61% optimal. Laboratory modified protocols (LMPS) were used by the majority (71%, 178/252) of participants using this system. Optimal results could be obtained both by the vendor recommended protocol settings (VRPS) (16 min. incubation of the primary Ab, HIER in CC1 for 64 min. and UltraView or iView as detection kit) and by laboratory modified protocol settings (LMPS) adjusting incubation time of the primary Ab, HIER time, detection systems and use of amplification

as shown in Table 3. In this assessment, VRPS were used by only 29% (74/252) of the laboratories and provided a slightly improved pass rate compared to LMPS as shown in Tables 1 and 3, although the optimal rate was somewhat higher with LMPS. Increasing the incubation time in primary antibody to 32 min. was the most commonly used successful single modification to the VRPS, with 95% (21/23) laboratories using this protocol achieving adequate results, 65% optimal. Use of OptiView as a substitute for UltraView detection as the sole modification to the VRPS gave a pass rate of 100% (9/10), 90% optimal. Protocol modifications using OptiView detection (with or without alteration of primary incubation and HIER time) were largely successful, resulting in a pass rate of 96% (43/45 users) versus 95% for the manufacturer's protocol, but with an improved optimal score rate of 80% (36/45 users) compared to 58% using VRPS (see Table 3). Use of UltraView amplification in addition to the base detection system gave a pass rate of 100% (18/18) and optimal rate of 78% (14/18).

The Dako/Agilent RTU system GA084 for Omnis, based on rmAb clone EP1 was used by 16% of the participants and gave an overall pass rate of 91%, 52% optimal. The proportion of sufficient results was superior when using VRPS (95%) versus LMPS (87%), whereas the VRPS provided 50% optimal results compared to 55% for laboratories applying LPMS. Seven laboratories used the VRPS with the addition of rabbit Linker, resulting in a pass rate of 71%, 43% optimal. Overall, modified protocols including rabbit linker obtained a pass rate of 87% (13/15), 67% optimal. Seven laboratories increased the primary antibody incubation time to 20-30 minutes as the sole protocol modification, obtaining a pass rate of 100%, 71% optimal.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb EP1 was used by 5% (20/438) of the participants and provided an overall pass rate of 85%, 45% optimal. As shown in Table 3, 75% (15/20) of the laboratories modified the protocol settings and obtained a superior pass rate (93%) to laboratories using the RTU system according to the Dako/Agilent recommendations (60% optimal). However, the optimal rates were similar (VRPS: 40%; LMPS: 47%). The commonest and most successful modification included use of a rabbit linker and was used by 10 laboratories: 100% of these passed, 60% (6/10) optimal.

The Leica RTU system PA0009/PA0151 for BOND based on mAb 6F11, was used by 4% (19/438) of the participants and gave an overall pass rate of 32%, 5% optimal. In this assessment, VRPS based on HIER in BERS1 (low pH) for 20 min., 15 min. incubation of the primary Ab and Bond Refine as detection system was used by three participants, with none achieving sufficient results. Extension of the HIER time in BERS1 to 50 minutes was used as a protocol modification by one laboratory, but this did not successfully increase analytical sensitivity and produced an overall insufficient (weak) staining reaction. Laboratories using a protocol modification increasing analytical sensitivity by using HIER in BERS2 (high pH) for 20 min. without extending the incubation time in primary antibody obtained a pass rate of 60% (3/5), although no optimal results were obtained. Use of BERS2 for 30 minutes and increasing the primary Ab incubation time to 60 min. gave one optimal result (1/1). However, 40 min. HIER in BERS2 without extension of the primary antibody incubation time resulted in poor signal to noise compromising interpretation (1/1). In this run, as it has been observed sporadically in previous runs, false positive staining of the negative tumour (breast carcinoma no. 3) was produced by two participants, using HIER in BERS2 for 30-40 minutes and Bond-PRIME polymer DAB detection.

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 8% (35 of 438) of the participants, continuing the downward trend from 11% in run B35. The three most commonly applied Abs mAb clone 6F11, rmAb clones EP1 and SP1 used in a LD assay could all provide an optimal result on the tissues supplied in this run. Overall, the rmAb clone 6F11 was the most commonly used concentrated antibody, however this did not perform well, with just 19% (3/16) of laboratories attaining an optimal result across all platforms. Notably, false positive staining of the negative tumour was seen in 12% (2/16) of laboratories using this clone, as seen in previous runs. Once again this was associated with HIER in an alkaline buffer. However, 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 most robust antibody clone in this run was EP1, with an overall pass rate of 100%, 60% optimal. The overall pass rate for all laboratories using concentrated antibody formats in this run was 69% (24/35), with 34% (12/35) 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 here, and in runs B35, B28 and B15) were found to be the common core elements for an optimal performance.

In this run, 2-layer detection systems performed slightly more successfully than 3-layer detection systems: the majority of the laboratories using concentrated antibody formats used a 3-layer detection system (69%, 24/35) and 31% (11/35) used a 2-layer system. The opposite trend was seen with laboratories using RTU antibody formats, where the majority (322/403, 80%) used 2-layer detection systems. (Overall, laboratories using a 3-layer system obtained a pass rate of 66%, with 25% optimal, compared to 2-layer detection system, with a pass rate of 73%, 55% 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, tissue cores no. 3 and 4. In addition, tonsil can be used as supplementary negative tissue control, as B-cells in mantle zones and within germinal centers must be negative.

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

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

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

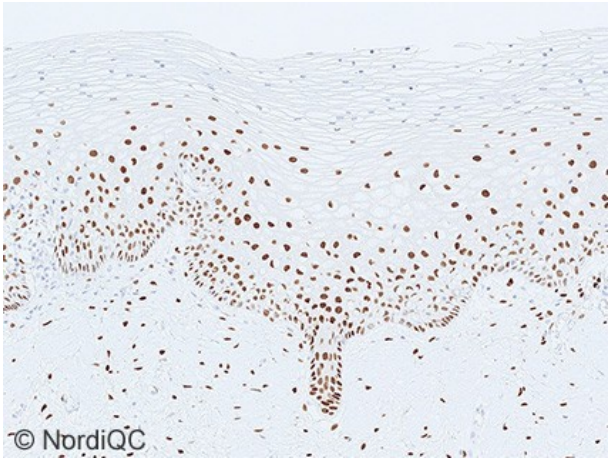


Fig. 1a
Optimal ER staining reaction of the uterine cervix using the rmAb clone SP1 as RTU format (790-4324) from Ventana/Roche, using HIER in CC1 (pH 8.5) for 40 min., incubation time 16 min. in primary Ab and visualized by OptiView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a – 5a, same protocol

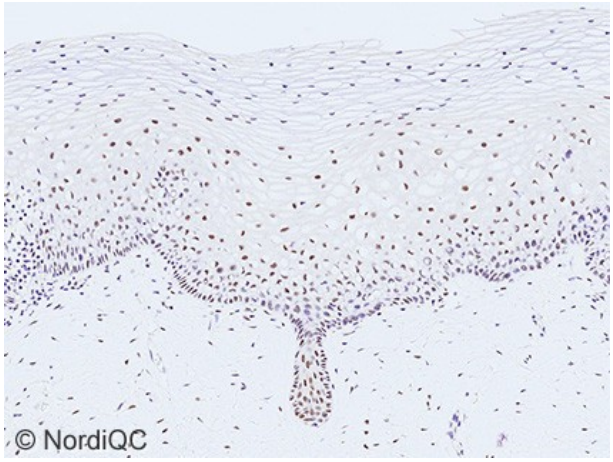


Fig 1b
Insufficient ER staining reaction of the uterine cervix using the rmAb clone 6F11 as RTU format (PA0009/PA0151) from Leica Biosystems by HIER in alkaline buffer (BERS2) for 30 min., primary Ab incubation for 15 min. and Bond Refine as detection system.1a.
A reduced proportion and too weak nuclear staining reaction is seen in the squamous epithelial cells.
Also compare with Figs. 2b – 4b, same protocol,

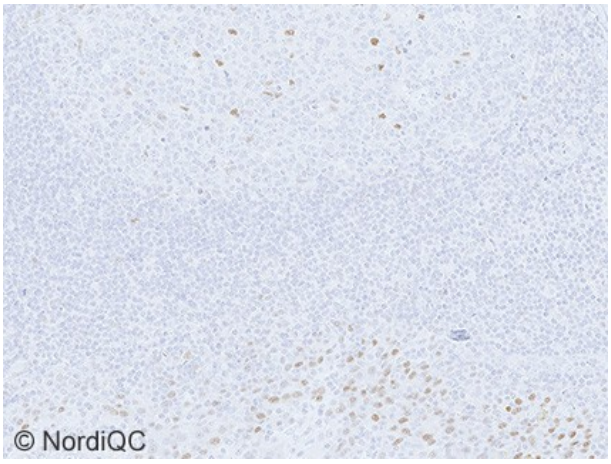


Fig. 2a
Optimal ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
A distinct nuclear staining reaction in both dispersed follicular dendritic cells/T-cells in the germinal center and many squamous epithelial cells can be identified at even low magnification (10x).

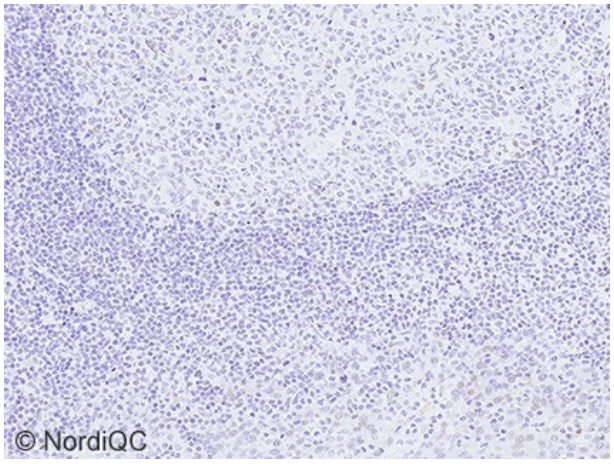


Fig. 2b.
Insufficient ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
Only a faint equivocal staining reaction in few follicular dendritic cells/T-cells in the germinal center and squamous epithelial cells is observed.

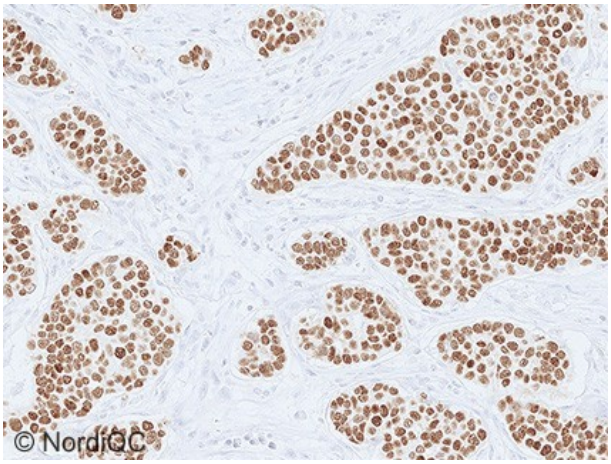


Fig. 3a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 4, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a – 2a.
The neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

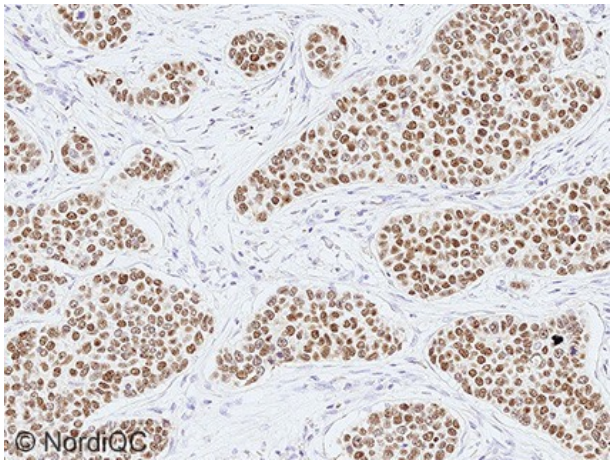


Fig. 3b.
ER staining reaction of the breast carcinoma, tissue core no. 4, with 90-100% cells being positive using the same protocol as in Figs. 1b – 2b.
The neoplastic cells display a clear positive staining reaction for ER.
However also compare with Fig. 4b, same protocol.

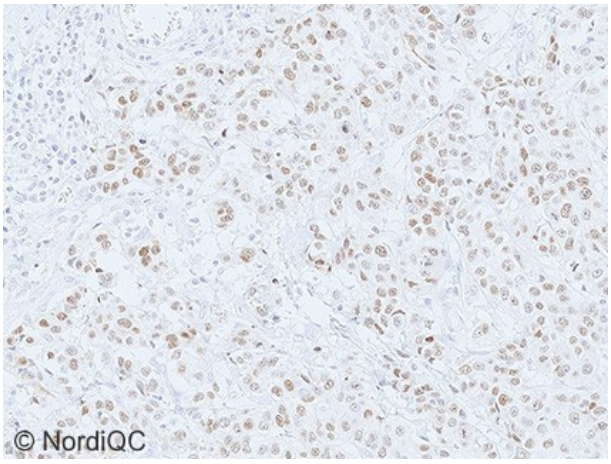


Fig. 4a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 5, with 80-100% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a – 3a.
The majority of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

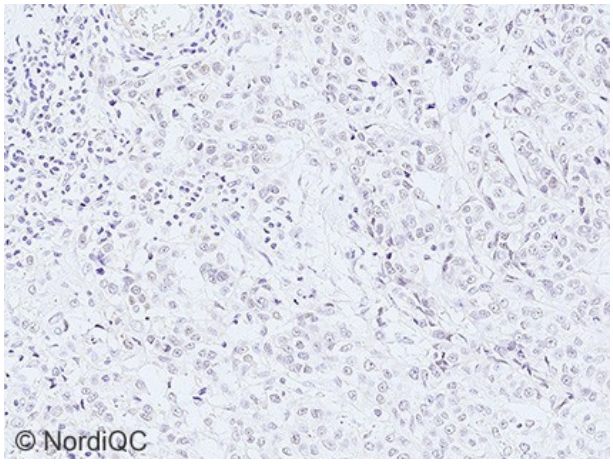


Fig. 4b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 5, with 80-100% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1b – 4b.
Only scattered (<1% overall) neoplastic cells show a weak and equivocal nuclear staining reaction for ER.

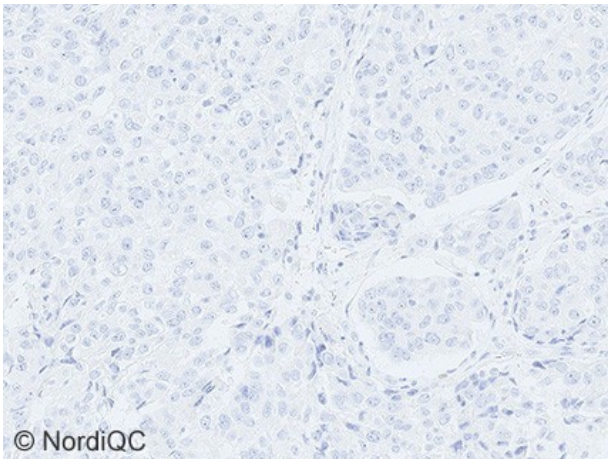


Fig. 5a
Optimal ER staining of the breast carcinoma expected to be ER negative, tissue core no. 3, using the same protocol as in Figs. 1a - 4a.
No staining reaction is seen.
The negative reaction was characterized by the NordiQC reference laboratory using rmAb clones SP1 and EP1 and same result was obtained by 99% of all participants.

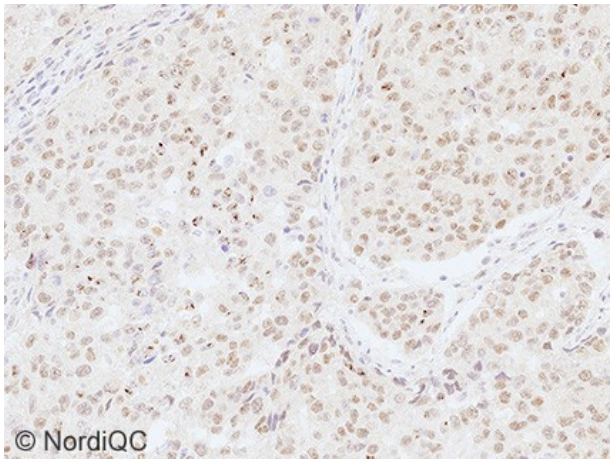


Fig. 5b
Insufficient ER staining of the breast carcinoma expected to be ER negative, tissue core no. 3. The majority of neoplastic cells show a weak to moderate positive nuclear staining reaction.
The protocol was based on the mAb 6F11 as concentrate using HIER in an alkaline buffer and performed on Bond Prime, Leica Biosystems. The mAb clone 6F11 gave an aberrant false positive result in this sample in four protocols.

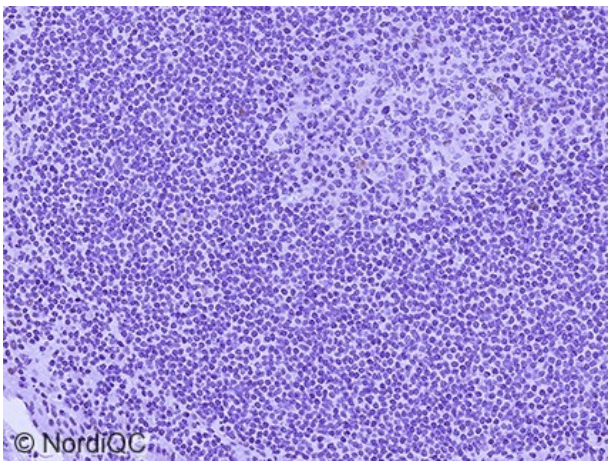


Fig. 6a
Insufficient ER staining reaction of the tonsil using the rmAb SP1 by a protocol providing a combination of reduced analytical sensitivity and excessive counterstaining compromising the evaluation of the ER IHC assay quality. The intense counterstaining makes it virtually impossible to evaluate if the immunohistochemical critical assay performance controls (squamous epithelial cells and follicular dendritic cells/T-cells in germinal centres) are positive or negative. Also compare with Fig. 6b, same protocol.

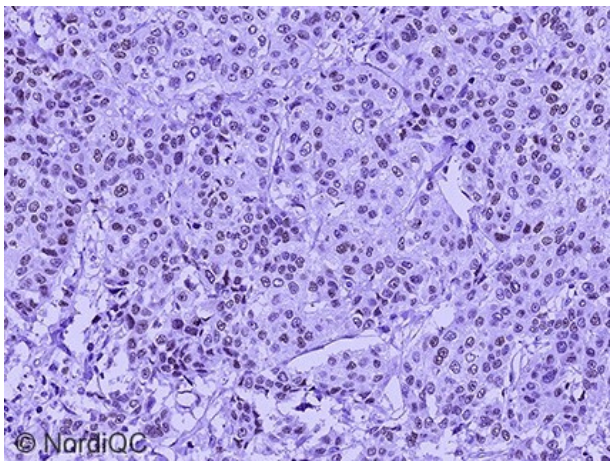


Fig. 6b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 5, with 80-100% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Fig. 5a. The excessive counterstaining obscures the evaluation of ER level in the neoplastic cells.

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