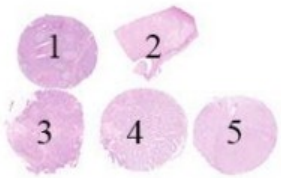
Assessment Run B39 2025

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	20-60%	Weak to moderate
4.	Breast carcinoma	0%	Negative
5.	Breast carcinoma	90-100%	Moderate to strong



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

KEY POINTS FOR ER IHC ASSAYS

- Tonsil and uterine cervix are highly recommendable tissue controls to monitor analytical sensitivity and specificity.
- RTU assays was used by 93% of all participants.
- The rmAb clone EP1 was most successful with high pass rates both applied as RTU or LD assay.
- The Ventana/Roche RTU system based on rmAb clone SP1 showed inferior performance.

Participation

Number of laboratories registered for ER, B38	474
Number of laboratories returning slides	430 (91%)

At the date of assessment, 430 (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.

Results

430 laboratories participated in this assessment run. 329 (77%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks given (see page 3 and 4).

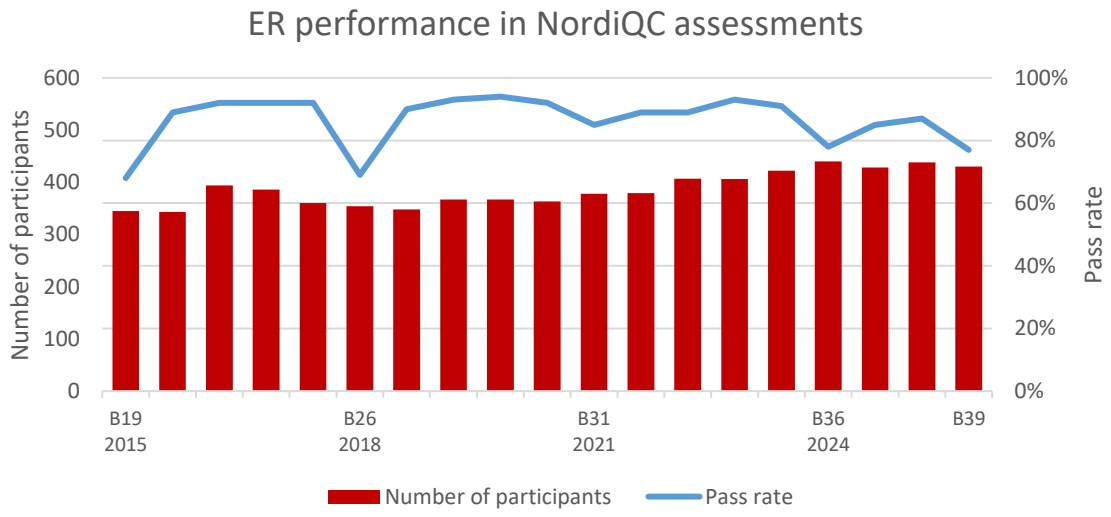
The commonest failing, accounting for 95% of the insufficient results in this assessment, was low analytical sensitivity giving a too weak (73%) or false negative staining reaction (12%). This generally manifested as a severely reduced number of cells showing positive staining compared to the reference slide. Low analytical sensitivity and weak demonstration of ER was occasionally 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. Three submissions showed clinically relevant false positive result of the tumour expected to be negative for ER.

The most frequent causes of insufficient staining reactions were:
- Use of detection systems with low analytical sensitivity– in particular, use of UltraView detection kit with RTU formats of clone SP1 was associated with a weaker than expected staining reaction.
- Insufficient Heat Induced Epitope Retrieval (HIER) time or HIER in acidic buffer (weak staining)
- Use of mAb clone 6F11 with extended HIER in an alkaline buffer (false positive staining result)

Performance history

In this run the pass rate of 77% (proportion of sufficient results) showed a sharp decline compared to run B38 (87%), returning to the level of the previous anomalous reduced pass rate of 78% seen in B36. Prior to B36, the pass rate had been stable at a high and satisfactory level of 85-94% in runs between 2015-2024, with the exception of runs B19 and B26 (see Graph 1).

Graph 1. **Participant numbers and pass rates for ER from 2015 - 2025**



Fluctuations in pass rates in between assessment runs can be caused by many factors both related to the protocols applied by the participants, circulation of more challenging material, changed assessment criteria

or similar factors. To ensure the consistency of the material circulated, NordiQC evaluates the selected tissue materials with two reference standard methods and in addition monitor the ER expression levels throughout all TMAs used in the assessment. NordiQC also strives to include testing material that show comparable and diagnostic relevant levels of antigen expression in between each run. Fluctuation in pass rates may also be influenced by new participants and new participants continue to be registered. However, similar pass rates continue to be observed for both existing and newly registered participants. As for run B36 (and to a lesser extent in B37), a reduced pass rate in this run was largely attributed to an inferior performance of the Ventana/Roche RTU system based on rmAb clone SP1 (790-4324/790-4325) on the BenchMark platform group. Over the last five runs, this RTU system was employed by an average of 57% of participants (range 55% to 58%). The overall pass rate for the system fell from 95% in run B35 to 77% in the current run. The insufficient results across this period were overwhelmingly 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 (93%, 400 of 430) used Ready-To-Use (RTU) systems, with the majority of these (250 of 400, 63%) using the Ventana/Roche SP1 RTU on the BenchMark platform. Both RTU and concentrated primary antibody formats could be used successfully: however, the pass rate for participants using RTU antibodies was 77% (309 of 400) and slightly superior to 67% (20 of 30) for concentrated formats (Table 1a). "Plug and play" RTU assays (where a RTU format was used on its intended fully automated platform) gave an overall pass rate of 80% across the two major manufacturers platforms (Ventana/Roche and Dako/Agilent), with Ventana/Roche BenchMark delivering a pass rate of 77% and Dako/Agilent Omnis a pass rate of 91%. In this run, the most robust performance was seen using clone EP1 for Omnis used as a true "plug and play" (vendor-recommended) assay, with a pass rate of 93% and an optimal rate of 43% (Tables 1a, 1c and 3).

Uterine cervix and tonsil continue to be recommended as positive tissue controls for ER. In uterine cervix, virtually all squamous 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 B39**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	30	5	15	4	6	67%	17%
Ready-To-Use antibodies	400	104	205	72	19	77%	26%
Total	430	109	220	76	25		
Proportion		25%	51%	18%	6%	77%	

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

Table 1b. **Concentrated antibodies and assessment marks for ER, run B39**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **6F11**	14	Leica Biosystems	3	7	2	2	71%	21%
rmAb clone **EP1**	4 2	Dako/Agilent CellMarque	1	4	0	1	83%	17%
rmAb clone **SP1**	4 3 1	Thermo Sci./ePredia Cell Marque AbCam	1	2	2	3	38%	13%
Ab clone **BP6139**	1	Biolynx	0	1	0	0	-	-
rmAb clone **QR013**	1	Quartett	0	1	0		-	-
Total	30		5	15	4	6		
Proportion			17%	50%	13%	20%	67%	

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 B39**

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	0	3	-	-
mAb clone **6F11** **PA0009/PA0151 (LMPS[4])**	16	Leica Biosystems	2	7	3	4	56%	13%
rmAb **EP1** **IR084/IS084 (VRPS[3])**	2	Dako/Agilent	0	2	0	0	-	-
rmAb **EP1** **IR084/IS084 (LMPS[4])**	21	Dako/Agilent	7	10	4	0	81%	33%
rmAb **EP1** **GA084 (VRPS[3])**	42	Dako/Agilent	18	21	3	0	93%	43%
rmAb **EP1** **GA084 (LMPS[4])**	29	Dako/Agilent	10	15	4	0	86%	34%
rmAb **EP1** **AN710**	1	BioGenex	0	0	1	0	-	-
rmAb **EP1** **8361-C010**	2	Sakura Finetek	1	1	0	0	-	-
rmAb clone **SP1** **790-4324/4325 (VRPS[3])***	75	Ventana/Roche	8	49	12	6	76%	11%
rmAb clone **SP1** **790-4324/4325 (LMPS[4])***	185	Ventana/Roche	55	88	38	4	77%	30%
rmAb clone **SP1** **249R-17/18**	8	Cell Marque	1	7	0	0	100%	13%
rmAb clone **SP1** **MAD-000306QD-3/V** **MAD-0003060-DS-2** **MAD-000306QD**	3	Master Diagnostica Vitro SA	0	0	2	1	-	-
rmAb clone **SP1** **RMPD001**	2	Diagnostic BioSystems	0	2	0	0	-	-
rmAb clone **SP1** **GT205602**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **SP1** **BRB053**	3	Zytomed Systems	0	2	1	0	-	-
rmAb clone **SP1** **ALR 301 G7**	1	BioCare Medical	0	0	3	0	-	-
rmAb clone **SP1** **M3011**	1	Spring Biosystems	0	0	1	0	-	-
rmAb clones **SP1+6F11** **PM308**	1	BioCare Medical	0	1	0	0	-	-
Ab clone **DY49837** **4911432**	1	Dakewe/BioSci	0	0	1	0	-	-
rmAb clone **QR013** **P-E001-30**	1	Quartett	0	0	0	1	-	-
Ab clone **658G3A2** **PA212**	1	Abcarta	1	0	0	0	-	-
Ab clone **MXR030** **RMA-1065**	1	Fuzhou Maixin	0	0	1	0	-	-
Total	400		104	205	72	19		
Proportion			26%	51%	18%	5%	77%	

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 B39
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **6F11**: Three of 14 laboratories obtained optimal results: all were based on HIER using Cell Conditioning 1 (CC1, Ventana/Roche) (2/3)* or Bond Epitope Retrieval Solution 2 (BERS2) pH 9.0 (Leica Biosystems) (1/10) as retrieval buffer. The mAb was diluted in the range of 1:25-1:100 and combined with a 2- or 3-layer detection system. Using these protocol settings, 9 of 11 (82%) laboratories produced a sufficient staining result (optimal or good).
As seen in previous runs, HIER at high pH could lead to false positive staining in non-ER expressing tumours (tissue core number 4 expected negative).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP1**: One of 6 laboratories obtained an optimal result, using a protocol based on HIER using BERS2 pH 9.0 (Leica Biosystems) (1/2) as retrieval buffer. The rmAb was diluted 1:50 and combined with a 3-layer detection system. A sufficient result was obtained by 2/2 labs using these parameters.

rmAb clone **SP1**: One of 7 laboratories obtained optimal results. The protocol with optimal result was based on HIER using Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (1/2) as retrieval buffer. The rmAb was diluted in 1:100 and combined with a 2-layer detection system.

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

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

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS High pH 9.0	TRS Low pH 6.1	TRS High pH 9.0	TRS Low pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **6F11**	-	-	-	-	2/3**	-	1/10 (10%)	0/1
rmAb clone **EP1**	-	-	0/2	-	0/2	-	1/2	-
rmAb clone **SP1**	-	-	1/2	-	0/4	-	0/1	-

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

Ready-To-Use antibodies and corresponding systems
mAb clone **6F11,** product. no. **PA0009/PA0151**, Leica Biosystems Bond III/Bond Max/Bond PRIME:
Two optimal results were obtained, using the following protocol: HIER in BERS2 (high pH) for 30 min., 15 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) or Bond-PRIME Polymer DAB Detection (DS9284) as detection system. Of the 19 laboratories using this RTU format, 9 of 14 (64%) were able to achieve sufficient results using a protocol based on HIER using BERS2 (15-40 min.), 15-30 min. incubation of primary Ab and Bond Polymer Refine or Bond PRIME Polymer DAB Detection. Five laboratories used a protocol using HIER with Bond Epitope Retrieval Solution 1 (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 10-25 min. at 97-98°C; mode = 20 min.), 20-40 min. incubation of the primary Ab and EnVision FLEX (K8000/SM802, K8024/SM802) or EnVision+ Rabbit (K4003) as detection system, with Rabbit Linker (K8009, K8019). Of the laboratories using these protocol settings, 7 of 8 (88%) produced a sufficient staining result.
5 laboratories used product no IR084/IS084 on other platforms. These were not included in the description above.

rmAb clone **EP1**, product no. **GA084**, Dako/Agilent, Dako Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 20-30 min. at 97°C, mode = 30 min.), 10-27 min. incubation of the primary Ab (mode = 10 min.) and EnVision FLEX (GV800/GV823) with or without rabbit linker (GV809) as detection system. Of the laboratories using these protocol settings, 61 of 66 (93%) produced a sufficient staining result, 28 of 66 (42%) optimal.
2 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 12-64 min. (mode = 64 min.), 12-44 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, 182 of 231 (79%) laboratories produced a sufficient staining result, 57 of 231 (25%) 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**	2/2	0/2	13/16 (81%)	5/16 (31%)
Dako Omnis rmAb EP1 **GA084**	39/42 (93%)	18/42 (43%)	24/27 (89%)	10/27 (37%)
Leica Bond III/Prime mAb 6F11 **PA0009/PA0151**	0/3	0/3	9/16 (56%)	2/16 (13%)
VMS Ultra/XT/Ultra Plus rmAb SP1 **790-4324/4325**	57/75 (76%)	8/75 (11%)	135/175 (77%)	49/175 (28%)

* 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 95% of the insufficient results (96 of 101), occasionally further complicated by excessive or "inselective" counterstain. Clinically relevant false positive staining reaction, where >1% of cells in the negative breast tumour (tissue core no. 4) stained unequivocally, was seen in three of the insufficient results (3%).

Virtually all laboratories were able to demonstrate ER in the high-level ER-expressing breast carcinoma (tissue core no. 5), in which 90-100% of the neoplastic cells were expected to be demonstrated and by the NordiQC reference standard methods, the cells showed a moderate to strong intensity. Demonstration of ER in the heterogeneous mid-level ER-expressing breast carcinoma (tissue core no. 3), in which an at least weak nuclear staining reaction of 20-60% of the neoplastic cells was expected (depending on TMA), was more challenging.

In this run the proportion of sufficient results assessed as good (220 of 430, 51%) was significantly increased, as also noted in two previous outlying runs showing a reduced pass rate (B36: 56% and B37: 50% of all results assessed as good). The results assessed as good were again overwhelmingly characterized by reduced analytical sensitivity manifesting as significantly fewer cells staining positive for ER than expected.

Ready-To-Use (RTU) Abs were used by 93% (400 of 430) of the participants. 77% (309 of 400) of these laboratories obtained a sufficient staining result, 26% optimal (104 of 400).

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 58% (250 of 430) of all the participants and gave an overall pass rate of 77% (192 of 250), 23% optimal. Laboratory modified protocols (LMPS) were used by the majority (70%, 175 of 250) 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 30% (75/250) of the laboratories and provided a near identical pass rate compared to LMPS as shown in Tables 1c and 3, although the optimal rate was higher with LMPS. Increasing the incubation time in primary antibody to 32 min. was the most commonly used single modification to the VRPS. This modification gave an identical pass rate of 77% to the VRPS for laboratories using this protocol (20/26), and a slightly increased optimal rate of 15% versus 11% for VRPS. Use of OptiView as a substitute for UltraView detection as the sole modification to the VRPS was the most successful modification and gave a pass rate of 100% (10/10), 80% optimal. Protocol modifications using OptiView detection (with or without alteration of primary incubation and HIER time) were in general highly successful, resulting in a pass rate of 98% (42/43 users), and an improved optimal score rate of 60% (26/43 users) compared to the 11% using VRPS (see Table 3). Use of UltraView amplification in addition to the base detection system gave a pass rate of 82% (14/17) and optimal rate of 53% (9/17). Together these observations suggest a reduced analytical sensitivity is seen when using the recommended UltraView detection system.

The Ventana/Roche RTU system for ER has in the 5 latest assessment runs B35-B39 given a mean pass rate of 85% (range 77%-95%) applying all protocol settings. This is a relatively significant decline from the mean rate of 94% (range 90%-97%) obtained in the 5 previous runs B30-B34. At present no single factor as batch numbers of primary abs, detection system or similar to explain the reduced performance has been identified.
To exclude that the inferior performance of the Ventana/Roche RTU system for ER is not directly related to the NordiQC material circulated similar long-term analysis of pass rate for the Dako/Agilent ER system for Omnis as been effectuated. The Dako Omnis gave as such a mean pass rate of 93% (range 91-97% in runs B35-B39 and 96% (range 93%-98%) in runs B30-B34 indicating a more stable performance.

The Dako/Agilent RTU system GA084 for Omnis, based on rmAb clone EP1 was used by 16% of the participants (69 of 430) and gave an overall pass rate of 91%, 41% optimal. The proportion of sufficient results was superior when using VRPS (93%) versus LMPS (89%), and in addition the VRPS provided 43% optimal results compared to 37% for laboratories applying LPMS. Five laboratories used the VRPS with the addition of rabbit Linker, resulting in a pass rate of 100%, 20% optimal. Overall, modified protocols including rabbit linker obtained a pass rate of 100% (11/11), 55% optimal. Fourteen laboratories increased the primary antibody incubation time to 15-30 minutes as the sole modification from VRPS, obtaining a pass rate of 93%, 50% optimal.

The Dako/Agilent RTU system IR084/IS084 for Autostainer, also based on the rmAb EP1 was used by 4% (18 of 430) of the participants and provided an overall pass rate of 83%, 28% optimal. As shown in Table 3, 89% (16 of 18) of the laboratories modified the protocol settings and obtained a pass rate of 81% (31% optimal). The number of laboratories using VRPS was too limited to be reliably analyzed. The commonest and most successful modification included use of a rabbit linker and was used by 9 laboratories: 89% of these passed, with 56% optimal.

The Leica RTU system PA0009/PA0151 for BOND based on mAb 6F11, was used by 4% (19 of 430) of the participants and gave an overall pass rate of 47%, 11% 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 or Bond-PRIME Polymer DAB as detection system was used by three participants, with none achieving sufficient results. One laboratory extended incubation of the primary Ab to 20 minutes but did not achieve a sufficient result. 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 50% (2/4), with no optimal results. In this run, extending use of BERS2 to 30-40 minutes without increasing the primary Ab incubation time was a successful modification, with 100% sufficient (4/4) and 50% optimal 2/4). In this run, false positive staining of the negative tumour (breast carcinoma no. 4) was produced by one participant, using an extended primary incubation time of 30 minutes, HIER in BERS2 for 30 minutes and Bond-PRIME polymer DAB detection. These observations are in line with previous assessment runs indicating the mAb clone 6F11 is challenging to optimize securing both the diagnostic sensitivity and specificity at the same 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 7% (30 of 430) 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, and sufficient results were obtained with all three Abs on the main IHC platforms (Dako/Agilent, Leica Biosystems and Ventana/Roche), see Tables 1b and 2. The overall pass rate for all laboratories using concentrated antibody formats in this run was 67% (20/30), with 17% (5/30) obtaining optimal results.
The rmAb clone 6F11 was the most commonly used concentrated antibody, however as seen in previous runs, this clone could give both false negative and false positive results.
Notably, as seen in previous runs, false positive staining was associated with HIER in an alkaline buffer and too high concentration of the antibody. The most robust antibody clone as concentrate in this run was EP1, with an overall pass rate of 83%, 17% optimal.

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 and 3-layer detection systems performed very similarly. Overall, laboratories using a 3-layer system obtained a pass rate of 77%, with 36% optimal, compared to 2-layer detection system, with a pass rate of 76%, 22% optimal. The majority of the laboratories using concentrated antibody formats used a 3-layer detection system (70%, 21/30) and 30% (9/30) used a 2-layer system, with pass rates of 62% for 3-layer and 78% for 2-layer systems. The opposite trend was seen with laboratories using RTU antibody formats, where the majority (322/400, 80%) used 2-layer detection systems. For laboratories using RTU formats, 2-layer systems gave a pass rate of 76% (22% optimal) with 3-layer systems giving a pass rate of 81% (44% 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 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

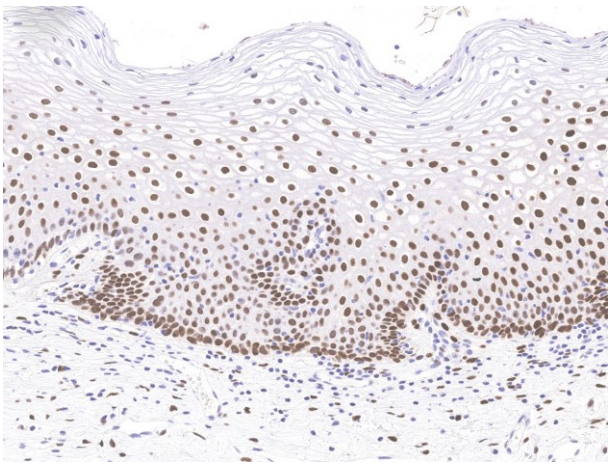


Fig. 1a
Optimal ER staining reaction of the uterine cervix using the rmAb clone EP1 as RTU format (790-4324) from Ventana/Roche, using HIER in CC1 (pH 8.5) for 48 min., incubation time 24 min. in primary Ab and visualized by OptiView and performed on BenchMark Ultra.
Virtually all squamous epithelial and stromal cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 2a – 4a, same protocol.

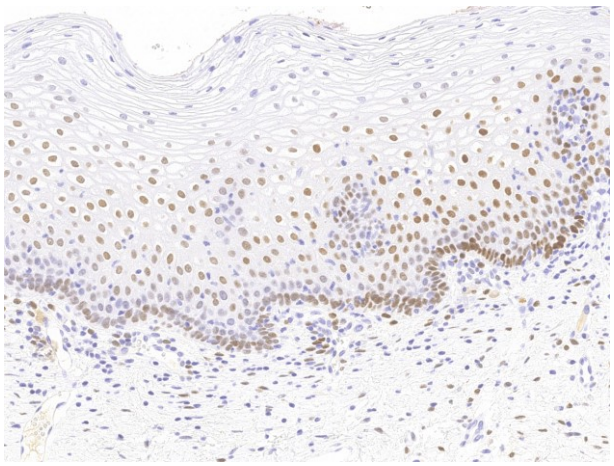


Fig 1b
ER staining reaction of the uterine cervix using the rmAb clone SP1 as a concentrated format within a protocol providing a general too low level of analytical sensitivity.
A reduced intensity and proportion of squamous epithelial cells demonstrated is seen.
Also compare with Figs. 2b – 4b, same protocol.

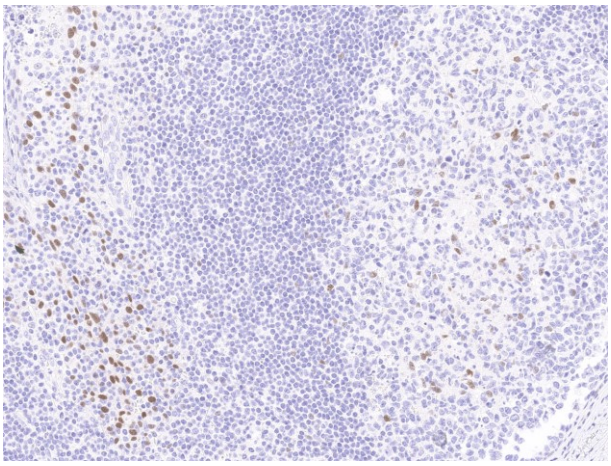


Fig. 2a
Optimal ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
A distinct nuclear staining reaction in both dispersed follicular dendritic cells/T-cells in the germinal center and many squamous epithelial cells can be identified at even low magnification (10x).

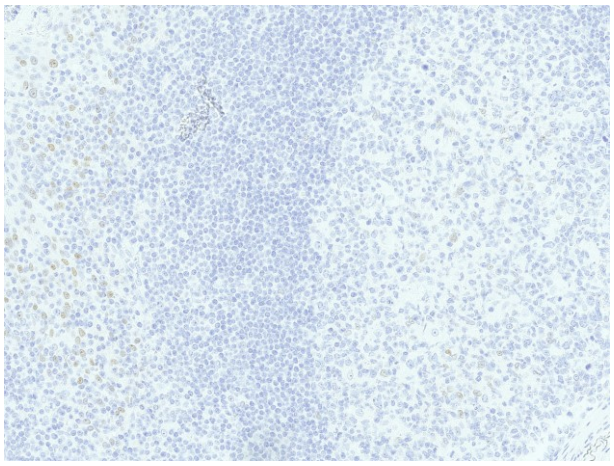


Fig. 2b.
Insufficient ER staining reaction of the tonsil using the same protocol as in Fig. 1a.
Only a faint equivocal staining reaction in few follicular dendritic cells/T-cells in the germinal center and squamous epithelial cells is observed.

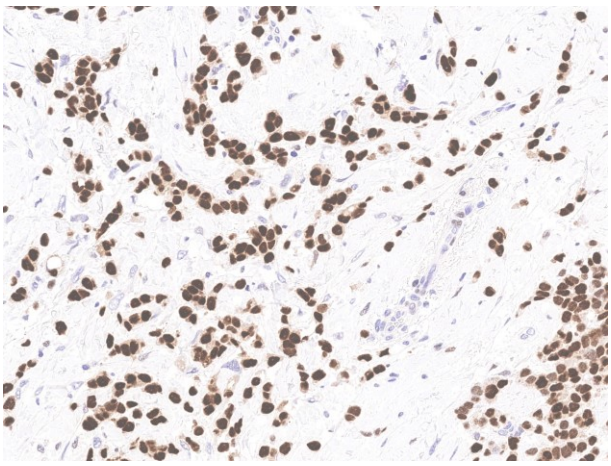


Fig. 3a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 5, with 90-100% cells being positive (moderate to strong) using same protocol as in Figs. 1a – 2a.
The neoplastic cells display a moderate to strong and distinct nuclear staining reaction.

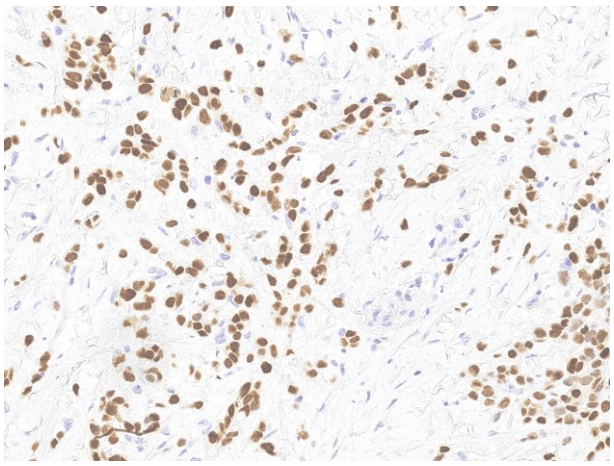


Fig. 3b.
ER staining reaction of the breast carcinoma, tissue core no. 5, with 90-100% cells being positive using the same protocol as in Figs. 1b – 2b.
The neoplastic cells display a clear positive staining reaction for ER.
However also compare with Fig. 4b, same protocol.

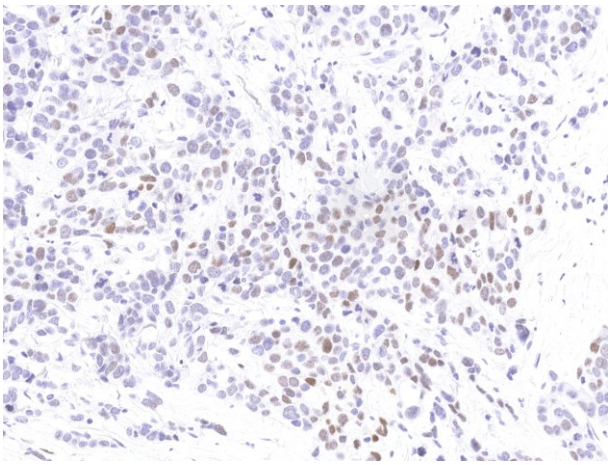


Fig. 4a
Optimal ER staining reaction of the breast carcinoma, tissue core no. 3, with 20-60% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1a – 3a.
About 40-50% of the neoplastic cells display a weak to moderate but distinct nuclear staining reaction.

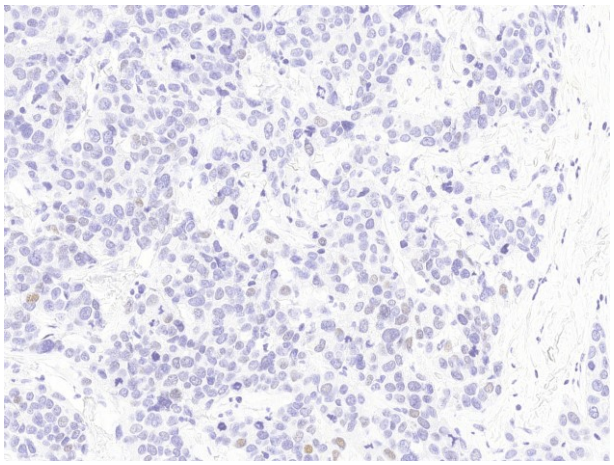


Fig. 4b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 3, with 20-60% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Figs. 1b – 3b.
Only scattered (<1% overall) neoplastic cells show a weak and equivocal nuclear staining reaction for ER.

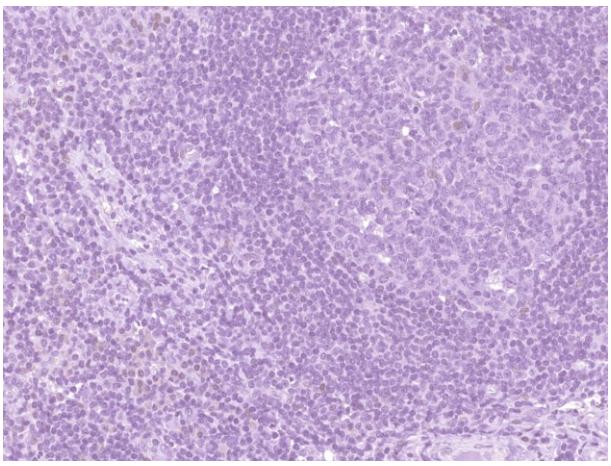


Fig. 5a
Insufficient ER staining reaction of the tonsil using the rmAb SP1 by a protocol providing a combination of reduced analytical sensitivity and excessive counterstaining compromising the evaluation of the ER IHC assay quality. The intense counterstaining makes it virtually impossible to evaluate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

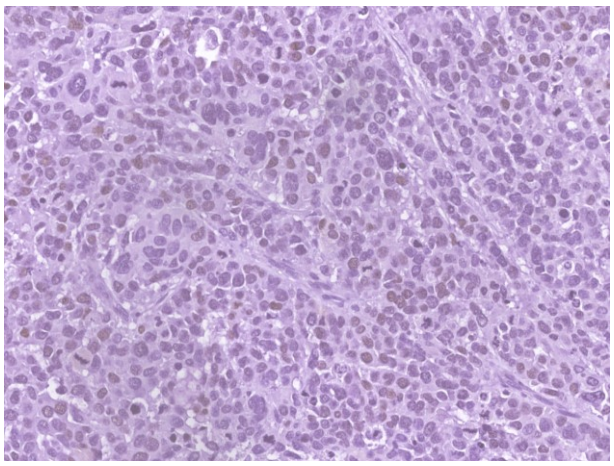


Fig. 5b
Insufficient ER staining reaction of the breast carcinoma, tissue core no. 3, with 20-60% of the neoplastic cells expected to be positive (weak to moderate) using same protocol as in Fig. 5a. The excessive counterstaining obscures the evaluation of ER level in the neoplastic cells.

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