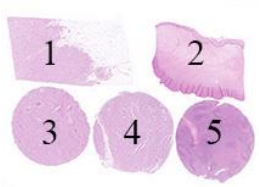
Assessment Run B35 2023

Progesterone receptor (PR)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests performed by the NordiQC participants for demonstration of Progesterone receptor (PR) expression in breast carcinomas. IHC, based on the mAb clones 16 and PgR 1294, 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 PR. 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. regular measurement of PR results) is needed.

Material
The slide to be stained for PR comprised the following tissues:

No.	Tissue	PR-positivity*	PR-intensity*
1.	Breast carcinoma	80-100%	Moderate to strong
2.	Uterine cervix	80-90%	Moderate to strong
3.	Breast carcinoma	20-50%**	Weak to moderate
4.	Breast carcinoma	0%	Negative
5.	Tonsil	0%	Negative



* PR-status and staining pattern as characterized by NordiQC reference laboratories using the mAb clones 16 and PgR 1294.
** PR expression heterogenous.

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

Criteria for assessing a PR IHC result as **optimal** included:

- A moderate to strong, distinct nuclear staining reaction in most stromal cells (with the exception of endothelial cells and lymphoid cells), and at least a weak nuclear staining reaction in most basal squamous epithelial cells in the uterine cervix. If present, a moderate to strong nuclear staining reaction in virtually all columnar epithelial cells.
- An at least moderate to strong distinct nuclear staining reaction in 80-100% of the neoplastic cells in the breast carcinoma no. 1.
- An at least weak to moderate distinct nuclear staining reaction in 20-50% of the neoplastic cells in the breast carcinoma no. 3.
- No nuclear staining reaction in the neoplastic cells in the breast carcinoma no. 4 and no more than a weak cytoplasmic reaction in cells with a strong nuclear staining.
- No staining reaction in the tonsil.

A PR IHC result was classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas no. 1 and 3 showed an at least weak nuclear staining reaction but significantly reduced proportion compared to the reference range. An at least weak to moderate nuclear staining reaction seen in the majority of the stromal, columnar and basal squamous epithelial cells in the uterine cervix.

A PR IHC result was assessed as **borderline** if ≥ 1% and < 10% of the neoplastic cells in one of the breast carcinomas no. 1 and 3 showed a nuclear staining reaction. A significantly reduced number of neoplastic cells demonstrated in combination with a negative staining reaction in cervix can also be marked as **borderline**.

A PR IHC result can 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 hampering the interpretation.

A PR IHC result was assessed as **poor** if a false negative staining (< 1%) was seen in one of the breast carcinomas no. 1 and 3. or false positive staining (≥ 1%) was seen in the breast carcinoma no. 4.

A PR IHC result can also be assessed as **poor** in case of extreme poor signal-to-noise ratio, impaired morphology etc. hampering the interpretation.

Participation

Number of laboratories registered for PR, run B35	446
Number of laboratories returning slides	417 (93%)

At the date of assessment 93% 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.

Three laboratories were excluded from the assessment due to the use of an inappropriate antibody, having excess water under coverslip or extensive section damage compromising interpretation.

Results

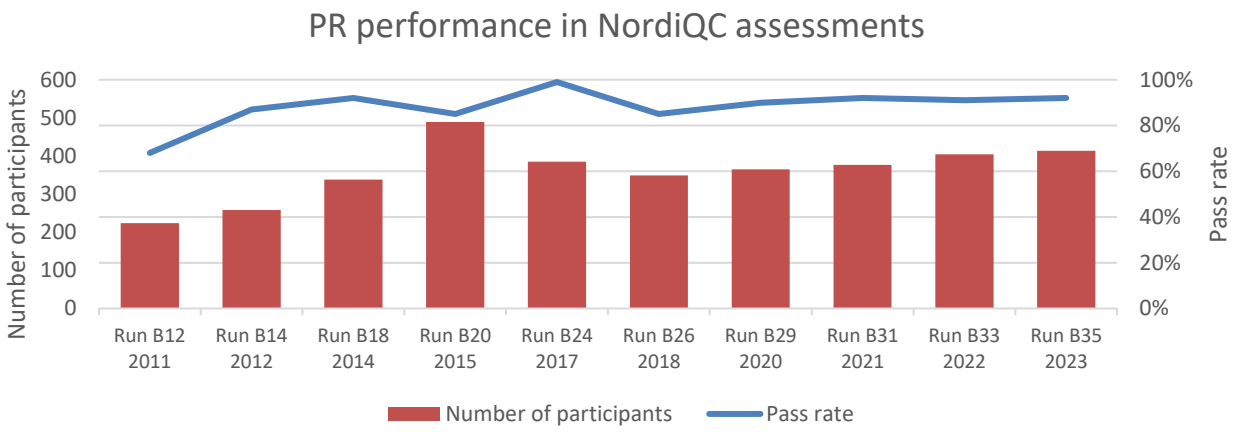
414 laboratories participated in this assessment. 92% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Use of detection systems with low analytical sensitivity
- Use of detection systems with low analytical specificity - problematic lots of EnVision FLEX (Dako/Agilent)
- Inefficient HIER
- Less successful primary Abs
- Unexplained technical issues

Performance history

This was the 15th NordiQC assessment of PR. The pass rate was in concordance with previous assessments as shown in Graph 1:

Graph 1. **Pass rate in the last ten NordiQC assessments for PR**



Conclusion

The widely used mouse monoclonal antibodies (mAb) clones **16**, **PgR 636**, **PgR 1294** and the rabbit monoclonal Ab (rmAb) clone **1E2** could all be used to provide an optimal result for PR.
84% of the participants used Ready-To-Use (RTU) systems from Ventana/Roche, Dako/Agilent and Leica Biosystems and in total obtained a pass rate of 95% when applying these assays as "plug-and-play".
In this assessment, a weak staining reaction was the predominant feature of the insufficient results, but excessive background was also observed.
Uterine cervix and tonsil in combination can be recommended as positive and negative tissue controls for PR. In uterine cervix, virtually all stromal cells and columnar epithelial cells must show a moderate to strong nuclear staining reaction. Most critically, the majority of basal squamous epithelial cells must show an at least weak but distinct nuclear staining reaction. Note, PR expression level can be reduced in some samples of uterine cervix e.g. due to post-menopausal status or phase of menstrual cycle. Tonsil is an appropriate negative tissue control – no nuclear staining reaction should be seen.

Table 1. **Antibodies and assessment marks for PR, run B35**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16**	34 1 1	Leica Biosystems Monosan DCS	22	11	2	1	92%	61%
mAb clone cocktail **16 + SAN27**	3	Leica Biosystems	0	2	1	0	-	-
mAb clone **PgR 636**	10	Dako/Agilent	6	3	1	0	90%	60%
mAb clone **PgR 1294**	11	Dako/Agilent	7	2	2	0	82%	64%
rmAb clone **BP6081**	1	Biolynx	0	0	1	0	-	-
rmAb clone **EP2**	1	Epitomics	0	0	1	0	-	-
rmAb clone **SP2**	1	Thermo Fisher Scientific	1	0	0	0	-	-
rmAb clone **SP42**	1	Zytomed	0	1	0	0	-	-
rmAb clone **QR014**	1	Quartett	0	1	0	0	-	-
rmAb clone **ZR290**	2	Zeta Corporation	2	0	0	0	-	-
rmAb clone **ZR4**	1	Zeta Corporation	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **16 PA0312 (VRPS[3])**	13	Leica Biosystems	13	0	0	0	100%	100%
mAb clone **16 PA0312 (LMPS[4])**	16	Leica Biosystems	14	2	0	0	100%	88%
mAb clone **16 MAD-000670QD**	3	Master Diagnostica	0	0	3	0	-	-
mAb **PgR 636 IR/IS068 (VRPS[3])**	8	Dako/Agilent	7	1	0	0	100%	88%
mAb **PgR 636 IR/IS068 (LMPS[4])**	16	Dako/Agilent	11	3	2	0	88%	69%
mAb **PgR 1294 GA090 (VRPS[3])**	43	Dako/Agilent	15	23	5	0	88%	35%
mAb **PgR 1294 GA090 (LMPS[4])**	25	Dako/Agilent	2	20	3	0	88%	8%
rmAb clone **1E2 790-2223/4296 (VRPS[3])**	64	Ventana/Roche	40	22	2	0	97%	63%
rmAb clone **1E2 790-2223/4296 (LMPS[4])**	153	Ventana/Roche	108	39	6	0	96%	71%
rmAb clone **278G8D6 PA246**	1	Abcarta	1	0	0	0	-	-
rmAb clone **MXR008 RMA-0895**	1	Fuzhou Maixin	1	0	0	0	-	-
rmAb clone **EP2 AN711-5M**	1	BioGenex	0	1	0	0	-	-
rmAb clone **SP2 RMPD002**	1	Diagnostic BioSystems	0	0	0	1	-	-
Ab clone **EAB-008 01.09.70.01.20.01**	1	Zybio	0	1	0	0	-	-
Total	414		250	132	30	2		
Proportion			60%	32%	7%	0%	92%	

1) Proportion of sufficient results (optimal or good) (≥5 asessed protocols).
2) Proportion of optimal results (≥5 asessed 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 PR, run B35
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **16**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) High pH (3-in-1) (Dako/Agilent) (1/1)*, TRS pH 9 (Dako/Agilent) (2/3), Cell Conditioning 1 (CC1, Ventana/Roche) (3/14), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (11/13) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (5/5) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:800, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 33/36 (92%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **PgR 636**: Protocols with optimal results were based on HIER using TRS High pH (3-in-1) (Dako/Agilent) (4/5), BERS2 (Leica Biosystems) (1/1) or Tris-EDTA buffer (1/1). The mAb was typically diluted in the range of 1:100-1:500, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 7/7 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294**: Protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (4/5), TRS High pH (Dako/Agilent) (2/5) or TRS High pH (3-in-1) (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50-1:200, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 8/10 (80%) laboratories produced a sufficient staining result.

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

Concentrated antibodies	**Dako/Agilent Autostainer Link48**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra / GX**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0 (3-in-1)	TRS pH 6.1 (3-in-1)	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **16**	1/1**	-	2/3	-	3/14 (21%)	-	11/13 (85%)	5/5 (100%)
mAb clone **PgR 636**	4/5 (80%)	-	-	-	0/2	-	1/1	-
mAb clone **PgR 1294**	1/1	-	2/4	-	4/5 (80%)	-	-	-

* 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 **16**, product no. **PA0312**, Leica Biosystems, Bond Max/Bond III:
Protocols with optimal results were typically based on HIER using BERS1 or BERS2 (efficient heating time 10-30 min. at 95-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.
Using these protocol settings, 25 of 25 (100%) laboratories produced a sufficient staining result (optimal or good).
1 laboratory used product no. PA0312 for manual staining. Data was not included in the description above.

mAb clone **PgR 636**, product no. **IR068/IS068**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-25 min. at 95-98°C), 12-20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems.
Using these protocol settings, 18/18 (100%) laboratories produced a sufficient staining result.
2 laboratories used product no. ISO068/IR068 outside of the Autostainer+/Link (Dako/Agilent) platform. Data was not included in the description above.

mAb clone **PgR 1294** product no. **GA090**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min.), 10-15 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (GV800/GV021) as detection system.
Using these protocol settings, 45/51 (88%) laboratories produced a sufficient staining result.

rmAb clone **1E2** product no. **790-2223/4296,** Ventana/Roche, BenchMark GX/XT/Ultra:
Protocols with optimal result were typically based on HIER using CC1 (efficient heating time 24-64 min.), 12-64 min. incubation of the primary Ab and UltraView (760-500) with or without amplification (760-080) or OptiView (760-700) as detection system.
Using these protocol settings, 193/198 (97%) laboratories produced a sufficient staining result.

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 protocol settings (LPMS) changing basal protocol settings. Only protocols performed on the intended 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
Leica BOND MAX/ BOND III mAb 16 **PA0312**	13/13 (100%)	13/13 (100%)	15/15 (100%)	14/15 (93%)
Dako Autotstainer+/ Autostainer Link mAb PgR 636 **IR068/IS068**	8/8 (100%)	7/8 (88%)	13/14 (93%)	10/14 (71%)
Dako Omnis mAb PgR 1294 **GA090**	38/45 (88%)	15/43 (35%)	22/25 (88%)	2/25 (8%)
Ventana BenchMark XT/Ultra rmAb 1E2 **790-2223/790-4296**	62/64 (97%)	40/64 (63%)	140/146 (96%)	103/146 (71%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer are included.

Comments
In this NordiQC assessment B35 for PR, an overall pass rate of 92% was observed similar to most of the previous assessments except run B24 where the pass rate was exceptionally high. The features of insufficient staining results were either characterized by false negative/too weak staining reactions, excessive background or a general poor signal-to-noise ratio.
A false negative or too weak staining reaction was the cause of 75% of the insufficient results (24/32). The majority of laboratories were able to demonstrate PR in the breast carcinoma no. 1 with a high PR expression level expected in 80-100% of the neoplastic cells. However, the demonstration of PR in the basal squamous epithelial cells in the cervix and the breast carcinoma no. 3, in which at least a weak nuclear staining reaction of >20% of the neoplastic cells was seen in the reference staining, was more challenging and required a carefully calibrated protocol. In order to account for heterogeneity and monitor the target analyte (PR) expression levels in the individual tumour cores included in NordiQC TMA blocks, reference slides are always made throughout the blocks. Every 50th slide throughout the blocks were thus stained for PR by the two reference standard methods and used during the assessment meeting as reference points.
In 22% of the insufficient results (7/32), excessive background or an overall poor signal-to-noise ratio was observed and mainly caused by issues with certain lots of the EnVision FLEX detection system (Dako/Agilent).
No insufficient results characterized by false positive nuclear staining reaction was observed in this assessment run.

Ready-To-Use (RTU) Abs were used by 84% (346 of 414) of the participants. 95% (330 of 346) of the laboratories used a complete RTU system including the pre-diluted primary Ab, specified ancillary reagents and IHC stainer platform from either Ventana/Roche, Dako/Agilent or Leica Biosystems (see Table 3). The proportion of participants using RTU systems has been steadily rising in the past years.

The Ventana/Roche RTU system, based on the rmAb clone 1E2 (790-2223/4296) to be performed on the BenchMark XT and Ultra platforms, was in this assessment the most widely used assay being used by 52% (217 of 414) of the participants and it gave an overall pass rate of 96%. 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 protocols adjusting incubation time of the primary Ab, HIER time and detection system as shown in Table 3. 7 participants used the rmAb clone 1E2 on other Ventana BenchMark staining platforms such as GX and LT. In this assessment, the vendor recommended protocol settings, being used by 29% (64 of 217) of the

laboratories, provided a similar overall pass rate of 97% compared to laboratory modified protocol settings giving a pass rate of 96%, however the proportion of optimal results was higher with LMPS as seen in Table 3. The insufficient results for the Ventana RTU system based on rmAb clone 1E2 were mainly characterized by a too weak staining reaction.

The Dako/Agilent RTU system GA090 for Omnis, based on mAb clone PgR 1294 was used by 16% of the participants (68 of 414) and gave an overall pass rate of 88%, that is comparable to 86% in the previous run B33, however the proportion of optimal results decreased from 55% (34/64) to 25% (17/68) in this assessment. The pass rate for the vendor recommended protocol settings and laboratory modified protocols was exactly the same – 88% (see Table 3). The proportion of optimal results was higher for VRPS at 35% (15/43) compared to the modified protocols showing optimal rate of 8% (2/25). The most successful protocol modification previously has been the use of a more sensitive detection system EnVision FLEX+ instead of EnVision FLEX, providing an optimal rate of 77% and 76% in the last two PR assessment runs. However, in this run it was evident that the use of a mouse linker enhanced the unspecific background staining most likely caused by problematic lots of the EnVision FLEX detection system and sent out to customers from November/December 2022 (see Figs. 5a-5b). In this assessment run, this led to a decreased proportion of optimal results of 10% (2/20) for laboratories using a 3-layer detection system on the Dako Omnis and it was observed that 17 of the 20 (85%) protocols gave an excessive background staining compromising the read-out.

The Dako/Agilent RTU system IR068/IS068 for Autostainer, based on the mAb clone PgR 636, provided an overall pass rate of 92% (22/24). As shown in Table 3, amongst participants using the RTU system on the intended Dako Autostainer staining platform 58% (14/22) modified the protocol settings obtaining a pass rate of 88% (14/16), 69% (11/16) optimal and 2 participants did not use the mAb on the intended platform. Laboratories using VRPS achieved a pass rate of 100% (8/8), 88% (7/8) optimal. The one non-optimal result (good) was downgraded due to an uneven staining reaction most likely caused by technical issues on the Autostainer. The most common staining protocol adjustments were changes in HIER and antibody incubation times.

The Leica RTU system PA0312 for Bond, based on the mAb clone 16, provided an overall pass rate of 100% (29/29) and 93% (27/29) optimal. As shown in Table 3, all participants (13/13) implementing VRPS received an optimal result. 54% (15/28) of the laboratories modified the protocol settings obtaining a pass rate of 100% and 93% (14/15) optimal, one participant used the mAb for manual staining. 6 participants used the BERS1 instead of the alkaline BERS2 for HIER providing a pass rate of 100%, 83% (5/6) optimal.

Overall, the RTU systems from the above mentioned three main vendors being applied in full compliance with the recommended protocol settings gave a pass rate of 95% (121/128) and 59% (75/128) optimal. In general, it must be emphasized that modifications of vendor recommended protocol settings for RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process by the end-users. As seen in this assessment, modifications can be successful but potentially also generate aberrant results and therefore must be carefully monitored.

16% (68 of 414) of the participants used Abs as concentrated formats within laboratory developed (LD) assays. Similar to the data generated for the RTU systems, the Abs, mAb clones 16, PgR 636 and PgR 1294 were widely used and could all provide sufficient and optimal results on the main IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems), see Tables 1 and 2. Irrespective of the clone applied, a careful calibration of the primary Ab in combination with efficient HIER, preferable in an alkaline buffer, and use of a sensitive 3-layer detection system were found to be the core elements for an optimal performance. For LD assays based on concentrated formats an overall pass rate of 85% (58/68) was obtained, 38% (38/68) optimal.

Controls

As observed in previous NordiQC assessments of PR, uterine cervix is an appropriate positive tissue control to monitor the level of analytical sensitivity for the PR assay: With an optimal protocol, virtually all columnar epithelial cells and stromal cells should show a moderate to strong nuclear staining reaction with only a minimal cytoplasmic reaction, whereas the majority of basal squamous epithelial cells must show an at least weak and distinct nuclear staining reaction. No staining must be seen in endothelial cells and lymphocytes. However, it must be taken into consideration that the PR expression level can be reduced in the uterine cervix of e.g. post-menopausal women and thus especially demonstration of PR in basal squamous epithelial cells hereby can be compromised. From in-house NordiQC data, the usage of uterine cervix as positive tissue control will require a screening of the samples with a validated PR IHC protocol for appropriate selection of a sample with the described expression pattern.
Tonsil is recommendable as negative tissue control, in which no nuclear staining should be seen.

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

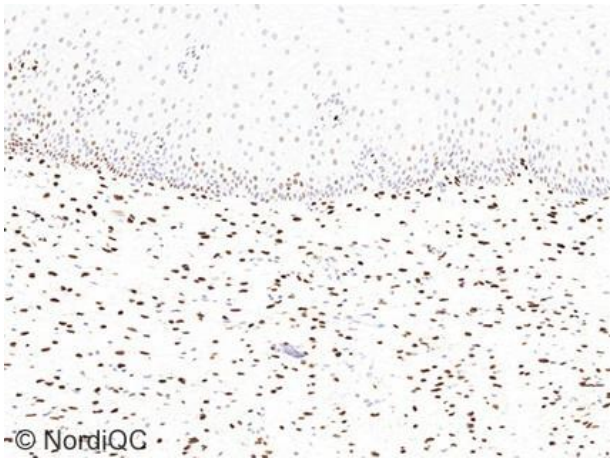


Fig. 1a
Optimal staining result for PR of the uterine cervix using the Leica Biosystems RTU system based on the mAb clone 16. The protocol was performed in compliance with the protocol settings recommend by the vendor using HIER in BERS2 for 20 min., 15 min. incubation in primary Ab and Bond™ Refine as detection system and applied on Bond III. The vast majority of basal squamous epithelial cells show weak to moderate nuclear staining reaction, whereas the stromal cells show a moderate to strong nuclear staining reaction. Same protocol as in Figs. 2a – 4a.

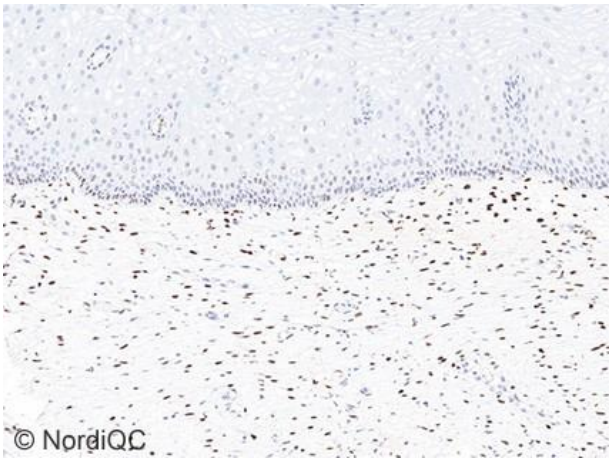


Fig. 1b
Insufficient staining result for PR of the uterine cervix, using the Ventana/Roche RTU system for the BenchMark staining platform based on the rmAb clone 1E2. The protocol provided a too low analytical sensitivity primarily caused by a decreased HIER time to 8 min. The majority of stromal cells are demonstrated whereas only scattered basal squamous epithelial cells show a faint nuclear staining reaction – same field as in Fig. 1a. Same protocol as in Figs. 2b – 4b.

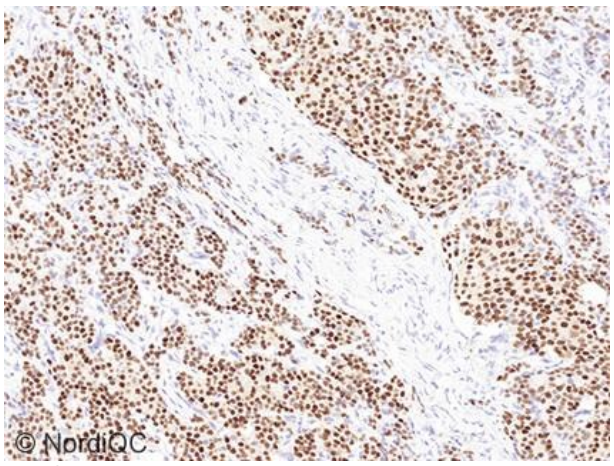


Fig. 2a
Optimal staining for PR of the breast carcinoma no. 1 with 80-100% positive tumor cells using same protocol as in Fig. 1a. Virtually all neoplastic cells show a strong nuclear staining reaction.

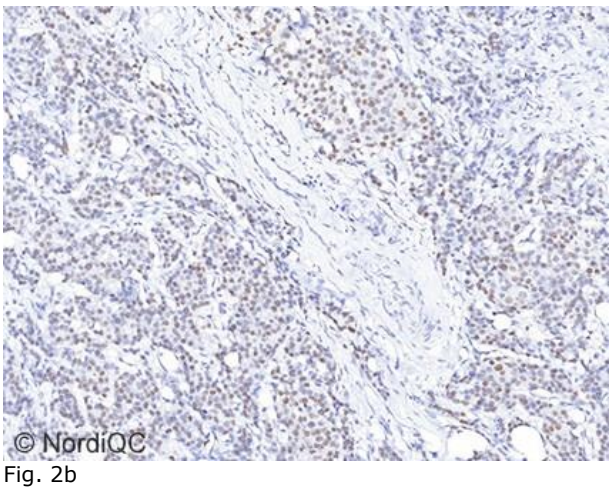


Fig. 2b
Insufficient staining for PR of the breast carcinoma no. 1 with 80-100% positive tumor cells using same protocol as in Fig. 1b – same field as in Fig. 2a. A significant reduction in both the staining intensity and proportion of positive tumor cells is seen.

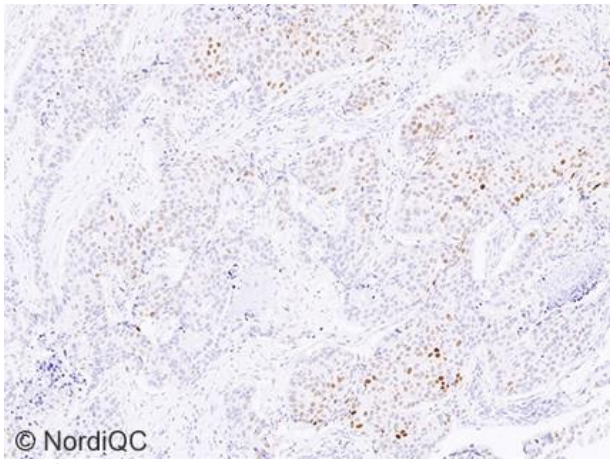


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 3, with at least 20% of the neoplastic cells showing a weak to moderate but distinct nuclear staining reaction - using same protocol as in Figs. 1a-2a.

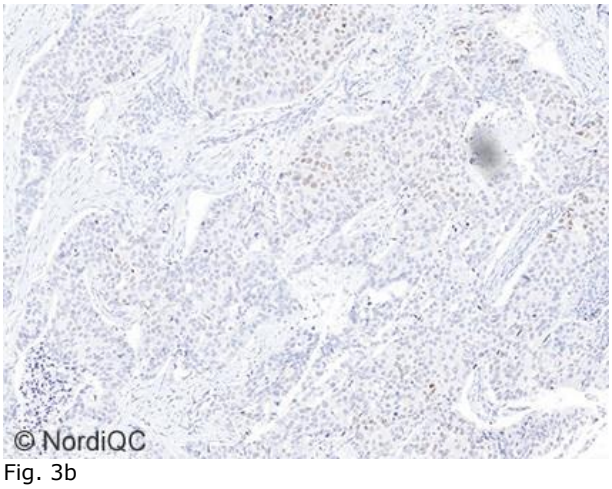


Fig. 3b
Insufficient staining for PR of the breast carcinoma no. 3 expected to be positive in minimum 20% of the neoplastic cells – same field as in Fig. 3a. <10% of the neoplastic cells are convincingly positive. Same protocol as used in Figs. 1b-2b.

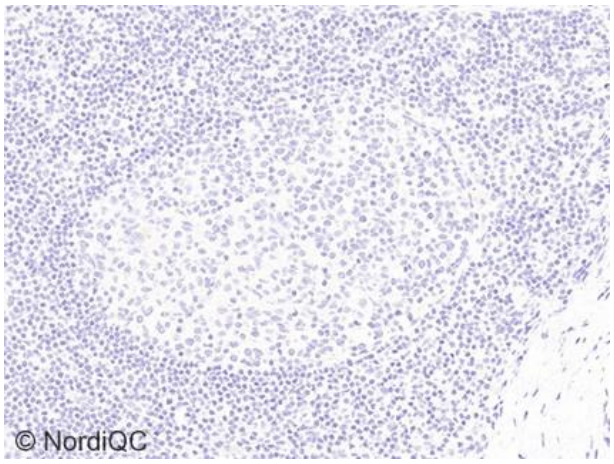


Fig. 4a
Optimal staining result for PR of the tonsil using same protocol as in Figs. 1a-3a. No nuclear staining reaction is seen.

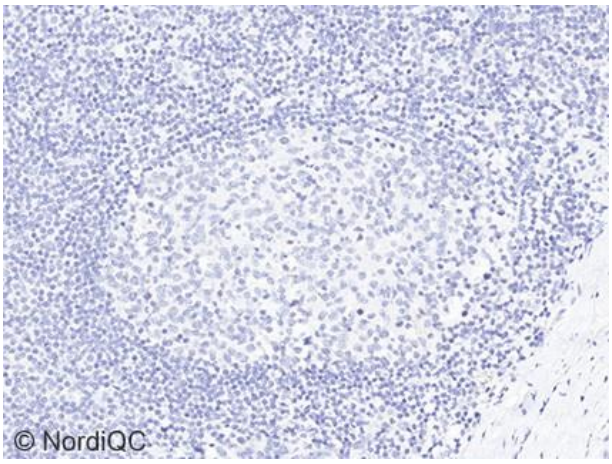


Fig. 4b
Staining result for PR of the tonsil using same protocol as in Figs. 1b-3b. No nuclear staining reaction is seen.

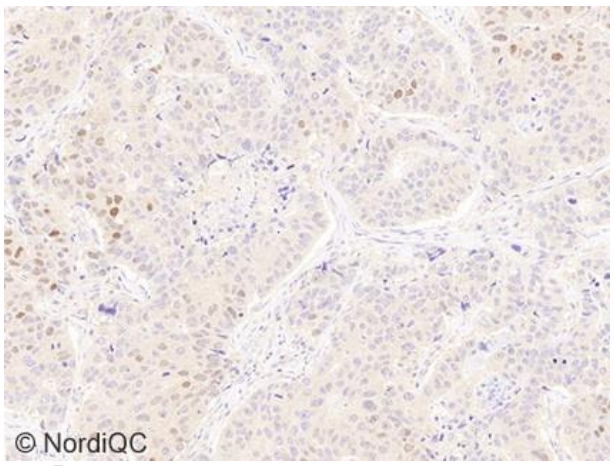


Fig. 5a
Insufficient staining for PR using the Dako/Agilent RTU system based on the mAb clone PgR 1294 on the Dako Omnis. An excessive background staining interfering the interpretation is seen in all tumor cells, caused by some lots of HRP EnVision FLEX detection system. It was noted that the background staining was more intense when Mouse Linker was added to the protocol as in this case. Compare with Fig. 3a.

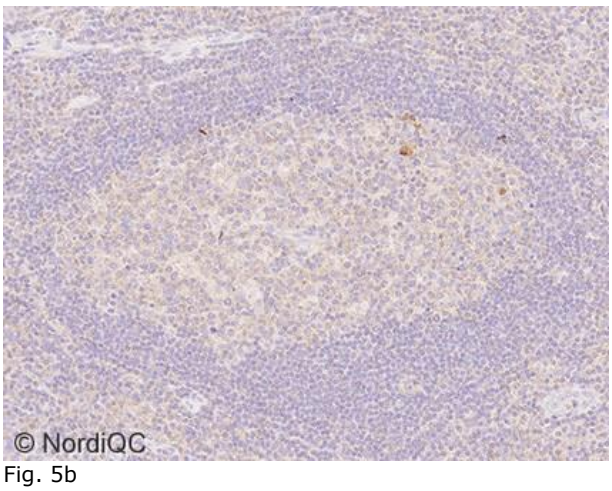


Fig. 5b
Insufficient staining result for PR of the tonsil using same protocol as in Fig. 5a. Although no specific nuclear staining reaction is seen, an overall strong background staining is interfering interpretation. Compare with Figs. 4a-4b.

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