Assessment Run B37 2024

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.	Tonsil	0%	Negative
2.	Uterine cervix	80-90%	Moderate to strong
3.	Breast carcinoma	95-100%**	Moderate to strong
4.	Breast carcinoma	10-70%**	Weak to moderate
5.	Breast carcinoma	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 95-100% of the neoplastic cells in the breast carcinoma no. 3.
- An at least weak to moderate distinct nuclear staining reaction in 10-70% of the neoplastic cells in the breast carcinoma no. 4.
- No nuclear staining reaction in the neoplastic cells in the breast carcinoma no. 5 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. 3 and 4 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. 3 and 4 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. 3 and 4 or false positive staining (≥ 1%) was seen in the breast carcinoma no. 5.

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.

KEY POINTS FOR PR IHC ASSAYS

- Tonsil and uterine cervix are highly recommendable to monitor analytical sensitivity and specificity.
- RTU systems provided a superior pass rate compared to assays based on concentrates.
- The widely used mAb clone **16** was found robust on all main IHC platforms.

Participation

Number of laboratories registered for PR, run B37	441
Number of laboratories returning slides	420 (95%)

At the date of assessment 95% 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

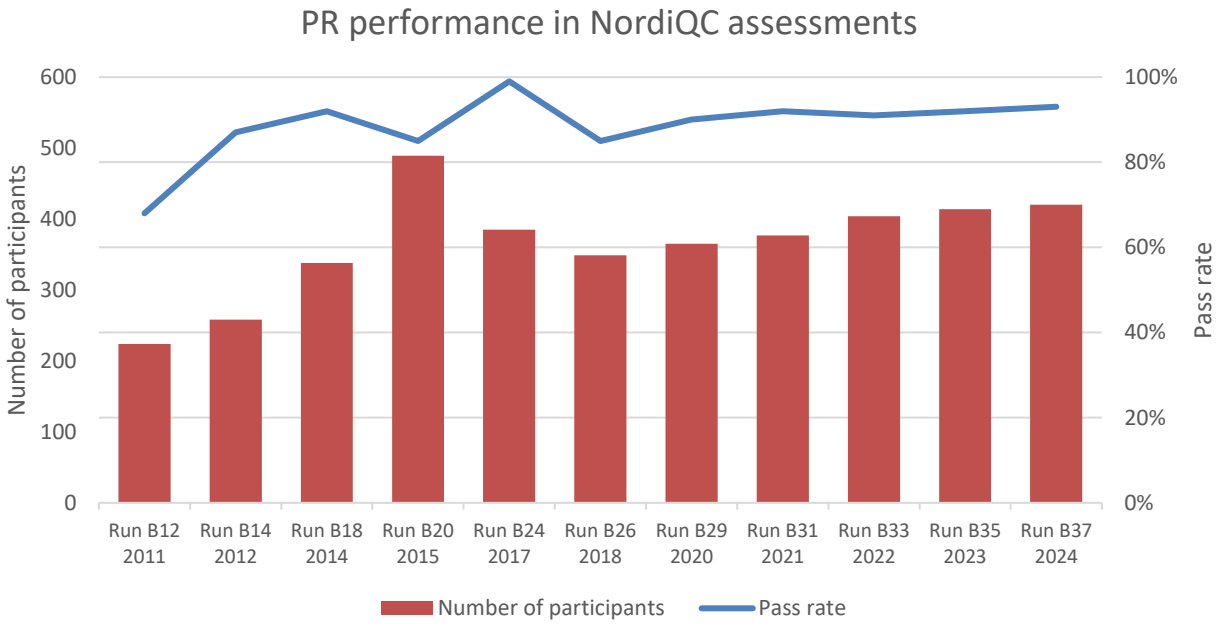
420 laboratories participated in this assessment. 93% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Use of detection systems with low analytical sensitivity
- Too short HIER time
- Unexplained technical issues

Performance history

This was the 16th 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 NordiQC assessments for PR**



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.

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.
88% 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". Especially the most commonly used RTU system from Ventana/Roche 790-2223/4296 based on rmAb clone 1E2 was very successful with a pass rate of 100% and 81% optimal results when applied accordingly to vendor instructions.
In this assessment, a too weak staining reaction or completely false negative result were the most predominant features of the insufficient results.

Table 1a. **Overall results for PR, run B37**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	51	32	11	7	1	84%	63%
Ready-To-Use antibodies	369	265	84	19	1	95%	72%
Total	420	297	95	26	2	-	
Proportion		71%	23%	6%	0%	94%	

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

Table 1b. **Concentrated antibodies and assessment marks for PR, run B37**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16**	30 1 1	Leica Biosystems Monosan DCS	20	7	4	1	84%	63%
mAb clone cocktail **16 + SAN27**	2	Leica Biosystems	1	0	1	0	-	-
mAb clone **1A6**	1	Leica Biosystems	0	1	0	0	-	-
mAb clone **PgR 636**	8	Dako/Agilent	4	2	2	0	75%	50%
mAb clone **PgR 1294**	6	Dako/Agilent	5	1	0	0	100%	83%
rmAb clone **BP6081**	1	Biolynx	1	0	0	0	-	-
rmAb clone **QR014**	1	Quartett	1	0	0	0	-	-
Total	51		32	11	7	1		
Proportion			63%	21%	14%	2%	84%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for PR, run 37**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **16 PA0312 (VRPS[3])**	16	Leica Biosystems	15	1	0	0	100%	94%
mAb clone **16 PA0312 (LMPS[4])**	18	Leica Biosystems	15	2	1	0	94%	83%
mAb clone **16 MAD-000670QD**	2	Master Diagnostica/Vitro	1	0	1	0	-	-
mAb **PgR 636 IR/IS068 (VRPS[3])**	12	Dako/Agilent	11	1	0	0	100%	92%
mAb **PgR 636 IR/IS068 (LMPS[4])**	12	Dako/Agilent	11	1	0	0	100%	92%
mAb **PgR 1294 GA090 (VRPS[3])**	43	Dako/Agilent	17	17	9	0	79%	40%
mAb **PgR 1294 GA090 (LMPS[4])**	29	Dako/Agilent	13	14	2	0	93%	45%
mAb clone **C4D10 CPM-0365**	1	Celnovte	1	0	0	0	-	-
mAb clone **MXR008 MAB-0854**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **1E2 790-2223/4296 (VRPS[3])**	90	Ventana/Roche	73	17	0	0	100%	81%
rmAb clone **1E2 790-2223/4296 (LMPS[4])**	138	Ventana/Roche	103	28	6	1	95%	75%
rmAb clone **278G8D6 PA246**	1	Abcarta	0	1	0	0	-	-
rmAb clone **SP2 GT205702**	1	GeneTech	1	0	0	0	-	-
rmAb clone **YR85 8360-C010**	2	Sakura Finetek	2	0	0	0	-	-
Ab clone **DA201 DRMD0249**	1	Dartmon	0	1	0	0	-	-
Ab clone **MSUA-570R MAD-000670QD**	1	Master Diagnostica/Vitro	0	1	0	0	-	-
Total	369		265	84	19	1		
Proportion			72%	23%	5%	0%	95%	

1) Proportion of sufficient stains (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) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
5) Product used on another platform than developed for

Detailed analysis of PR, run B37
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 (Dako/Agilent) (2/3)*, Cell Conditioning 1 (CC1, Ventana/Roche) (3/11), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (11/12) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (4/4) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:600, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 27/30 (90%) 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) (4/4) as retrieval buffer. The mAb was diluted in the range of 1:100-1:500, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 4/4 (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) as retrieval buffer. The mAb was diluted in the range of 1:50-1:100, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 5/5 (100%) 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[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	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**	-	-	2/3	-	3/11 (27%)	-	11/12 (92%)	4/4
mAb clone **PgR 636**	4/4	0/1	-	-	0/1	-	0/1	-
mAb clone **PgR 1294**	-	-	0/1	-	5/5 (100%)	-	-	-

* 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 Ultra, Ultra plus
3) Bond III, Prime

Ready-To-Use antibodies and corresponding systems
mAb clone **16**, product no. **PA0312**, Leica Biosystems, Bond MAX/Bond III/Bond Prime:
Protocols with optimal results were typically based on HIER using BERS1 or BERS2 (efficient heating time 10-30 min. at 95-100°C), 15-40 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.
Using these protocol settings, 32 of 33 (97%) 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, 24/24 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294** product no. **GA090**, Dako/Agilent, Omnis:
Protocols with optimal results were based on HIER using TRS High pH (efficient heating time 30 min.), 10-27 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (GV800/GV021) as detection system.
Using these protocol settings, 57/68 (84%) laboratories produced a sufficient staining result.

rmAb clone **1E2** product no. **790-2223/4296,** Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
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, 205/211 (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 mAb 16 **PA0312**	16/16 (100%)	15/16 (94%)	16/17 (94%)	15/17 (88%)
Dako Autotstainer mAb PgR 636 **IR068/IS068**	12/12 (100%)	11/12 (92%)	12/12 (100%)	11/12 (92%)
Dako Omnis mAb PgR 1294 **GA090**	34/43 (79%)	17/43 (40%)	27/29 (93%)	13/29 (45%)
Ventana BenchMark rmAb 1E2 **790-2223/790-4296**	90/90 (100%)	73/90 (81%)	131/138 (95%)	103/138 (75%)

* 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 B37 for PR, an overall pass rate of 94% was observed similar to the latest assessments. The prevalent 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 79% of the insufficient results (22/28). The majority of laboratories were able to demonstrate PR in the breast carcinoma, tissue core no. 3, with a high PR expression level expected in 95-100% of the neoplastic cells. However, the demonstration of PR in the basal squamous epithelial cells in the cervix and of central diagnostic importance the breast carcinoma, tissue core no. 4, in which at least a weak nuclear staining reaction of >10% of the neoplastic cells was seen in the reference staining, was more challenging and required a carefully calibrated protocol. The breast carcinoma, tissue core no. 4, showed a heterogenous expression pattern and a PR level in the range of 10-70% in the material circulated. 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 18% of the insufficient results (5/28), excessive background or an overall poor signal-to-noise ratio was observed compromising the read-out for PR expression in the neoplastic cells.
One insufficient result was characterized by a false positive nuclear staining reaction.

Ready-To-Use (RTU) Abs were used by 88% (369 of 420) of the participants. The proportion of participants using complete RTU systems including the pre-diluted primary Ab, specified ancillary reagents and IHC stainer platform has been steadily rising in the past years. This is both related to the classical and well-established vendors as Ventana/Roche, Dako/Agilent or Leica Biosystems, but also newly available systems from e.g. Sakura Finetek and Fuzhou Maixim (see Table 1c).

The Ventana/Roche RTU system, based on the rmAb **clone 1E2 (790-2223/4296)** to be performed on the BenchMark XT, Ultra and Ultra Plus platforms, was in this assessment the most widely used assay being used by 54% (228 of 420) of the participants and it gave an overall pass rate of 97%. 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. Ten participants used the RTU format on the Ventana BenchMark GX platform and obtained similar results as for the intended platforms with a pass rate of 100%, 90% optimal. In this assessment, the vendor recommended protocol settings, being used by 39% (90 of 228) of the laboratories, provided a pass rate of 100% being slightly superior compared to the level of 95% obtained by laboratory modified protocol settings – see Tables 1c and 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 17% of the participants (72 of 420) and gave an overall pass rate of 85%, that is comparable to level obtained in the previous two runs B33 and B35, however the proportion of optimal results was relatively low at a level of 42%. The pass rate of 93% for laboratory modified protocols was superior to the level of 79% obtained by vendor recommended protocol settings (see Table 3). The proportion of optimal results was also slightly higher for vendor recommended protocol settings at 45% compared to the modified protocols showing optimal rate of 40%. The insufficient results for the Dako Omnis **GA090** RTU system was primarily caused by too weak staining reaction and/or excessive background reaaction.
In this assessment, application of mouse linker to EnVision FLEX "transforming to FLEX+" was the most successful protocol modification, as all 22 protocols based on this modification provided a sufficient result. This was on par to the observations generated in previous assessments expect run 35, in which linker enhanced an excessive background reaction.

The Dako/Agilent RTU system **IR068/IS068** for Autostainer, based on the mAb **clone PgR 636**, provided an impressive pass rate of 100% (24/24). As shown in Table 3, identical pass rates and proportion of optimal results (92%) was obtained by vendor recommended and laboratory modified protocol settings.
The most commonly applied protocol modification were related to adjustment of HIER and antibody incubation times.

The Leica RTU system **PA0312** for Bond, based on the mAb **clone 16**, provided an overall pass rate of 97% (32/33) and 91% (30/33) optimal being similar to the levels obtained in recent runs. As shown in Table 3, all 16 protocols based on vendor recommended protocols provided a sufficient result and 15 of these assessed as optimal (94%). 52% (17/33) of the protocols based on the PA0312 system were applied by modified protocol settings giving a marginally reduced pass rate and proportion of optimal results of 94% and 88%, respectively. The most commonly applied protocol modifications were related to adjustment of antibody incubation times and HIER settings.

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 94% (152/161) and 72% (116/161) 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.
In addition as shown in table 1c, new RTU system providers as Sakura Finetek and Fuzhou Maixim are entering the diagnostic market and in general the majority deliver promising results.

12% (51 of 420) 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% was obtained, 63% optimal and thus inferior to the level obtained for corresponding RTU systems – see tables 1a – 1c.

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

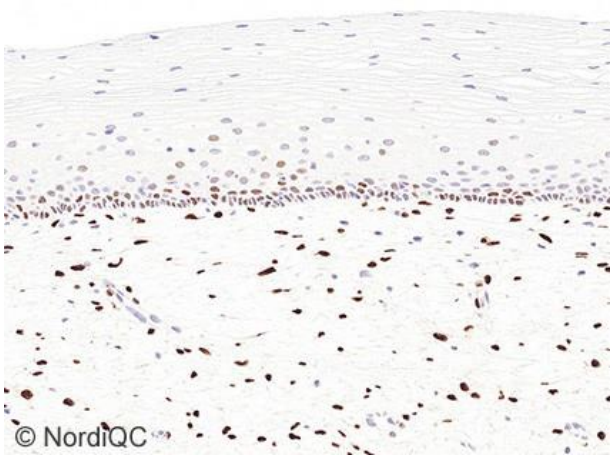


Fig. 1a
Optimal staining reaction for PR of the uterine cervix using the Leica Biosystems RTU system based on the mAb clone 16. The protocol was performed in compliance with the protocol settings recommend by the vendor using HIER in BERS2 for 20 min., 15 min. incubation in primary Ab and Bond™ Refine 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.

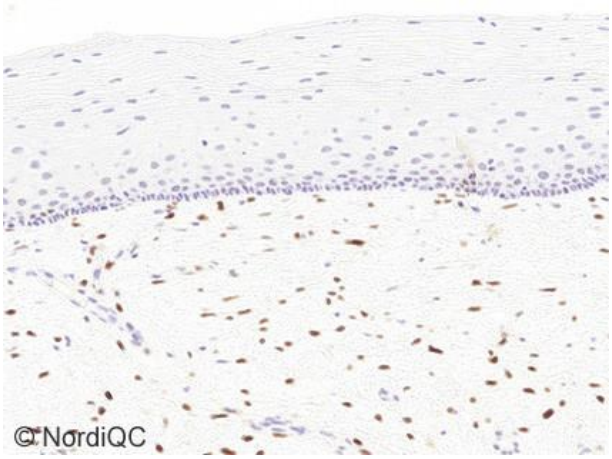


Fig. 1b
Insufficient staining reaction (assessed as borderline) for PR of the uterine cervix, using the Dako/Agilent RTU system for the Omnis staining platform based on the mAb clone PgR 1294. The protocol was applied accordingly to vendor recommendations but of unknown causes provided a too low analytical sensitivity.
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.

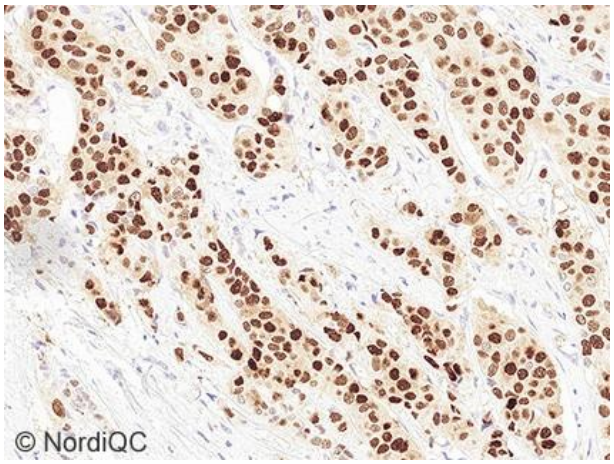


Fig. 2a
Optimal staining reaction for PR of the breast carcinoma no. 3 with 95-100% positive tumor cells using same protocol as in Fig. 1a. Virtually all neoplastic cells show a strong nuclear staining reaction.

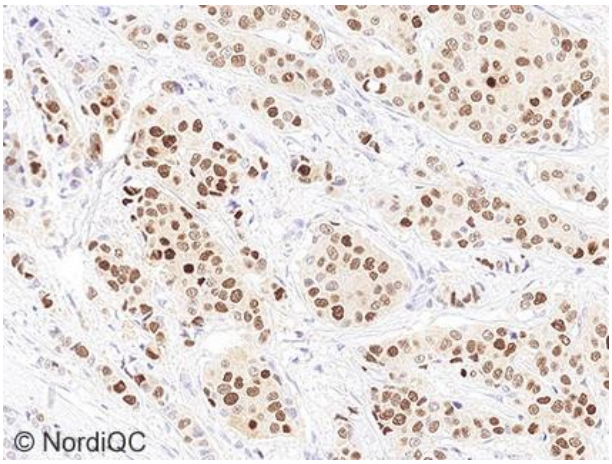


Fig. 2b
Staining reaction for PR of the breast carcinoma no. 3 with 95-100% positive tumor cells using same protocol as in Fig. 1b – same field as in Fig. 2a. The staining intensity is slightly reduced but the proportion of cells demonstrated is as expected.

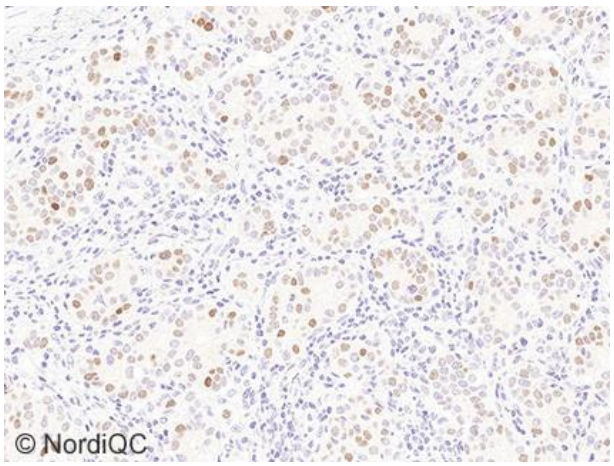


Fig. 3a
Optimal staining reaction for PR of the breast carcinoma no. 4, with at least 20-30% of the neoplastic cells showing a weak to moderate but distinct nuclear staining reaction - using same protocol as in Figs. 1a-2a.

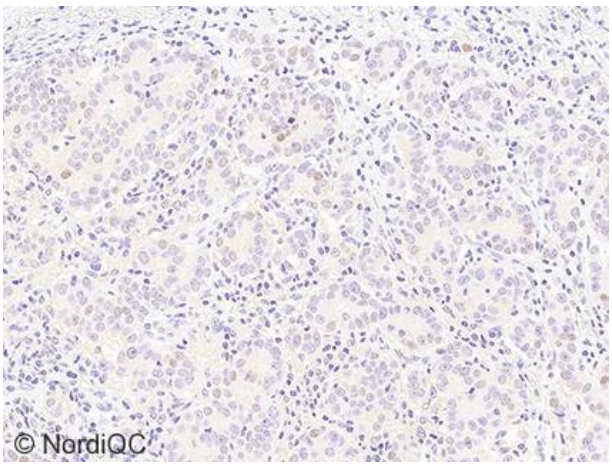


Fig. 3b
Insufficient staining reaction for PR of the breast carcinoma no. 4 expected to be positive in minimum 10% of the neoplastic cells – same field as in Fig. 3a. <10% and close to only 1-2% of the neoplastic cells are convincingly positive. Same protocol as used in Figs. 1b-2b.

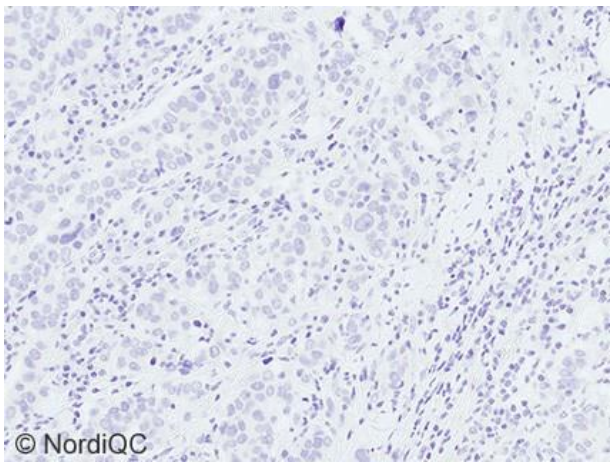


Fig. 4a
Optimal staining reaction for PR of the breast carcinoma no. 5 expected to be PR negative using same protocol as in Figs. 1a-3a. No nuclear staining reaction is seen.

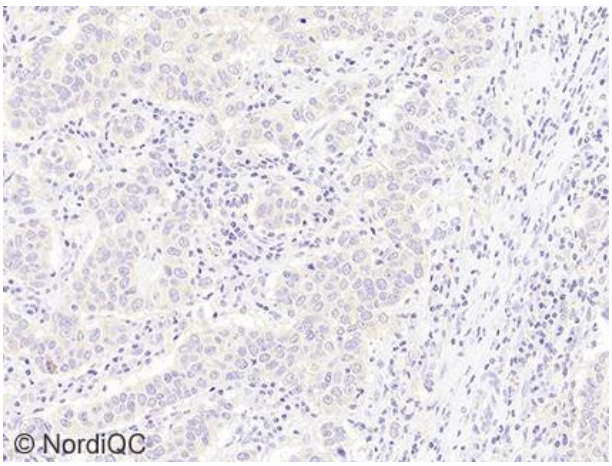


Fig. 4b
Staining reaction for PR of the breast carcinoma no. 5 expected to be PR negative using same protocol as in Figs. 1a-3a. No nuclear staining reaction is seen. However, a weak diffuse cytoplasmic staining reaction is observed in the neoplastic cells which in addition to the reduced analytical sensitivity shown in Figs 1b and 3b overall gave an inferior performance of the protocol.

SN/LE 19.04.2024