

### 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	0%	Negative
4.	Breast carcinoma	70-100%	Weak to strong
5.	Breast carcinoma	30-80%**	Weak to strong



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

\*\* PR expression heterogenous in the TMAs used for assessment (n=8)

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

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

- A moderate to strong, distinct nuclear staining reaction of virtually all columnar epithelial cells and most of the 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.
- An at least weak to moderate distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 4 and 5.
- No nuclear staining reaction in the neoplastic cells in the breast carcinoma no. 3
- 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  $\geq 10\%$  of the neoplastic cells in the breast carcinomas no. 4 and 5 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 columnar and basal squamous epithelial cells.

A PR IHC result was assessed as **borderline** if  $\geq 1\%$  and  $< 10\%$  of the neoplastic cells in one of the breast carcinomas no. 4 and 5 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 reaction ( $< 1\%$ ) was seen in one of the breast carcinomas no. 4 and 5 or a false positive result ( $\geq 1\%$ ) was seen in the breast carcinoma no. 3.

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 and LD assays performed equally providing high pass rates and proportion of optimal results
- In the latest 5 assessment of PR a high and satisfactory mean pass rate of 93% has been obtained.

### Participation

Number of laboratories registered for PR, run B39	467
Number of laboratories returning slides	427 (91%)

At the date of assessment 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

427 laboratories participated in this assessment. 98% achieved a sufficient mark (optimal or good). Table 1a, b and c summarize the antibodies (Abs) used and assessment marks (see page 3 and 4).

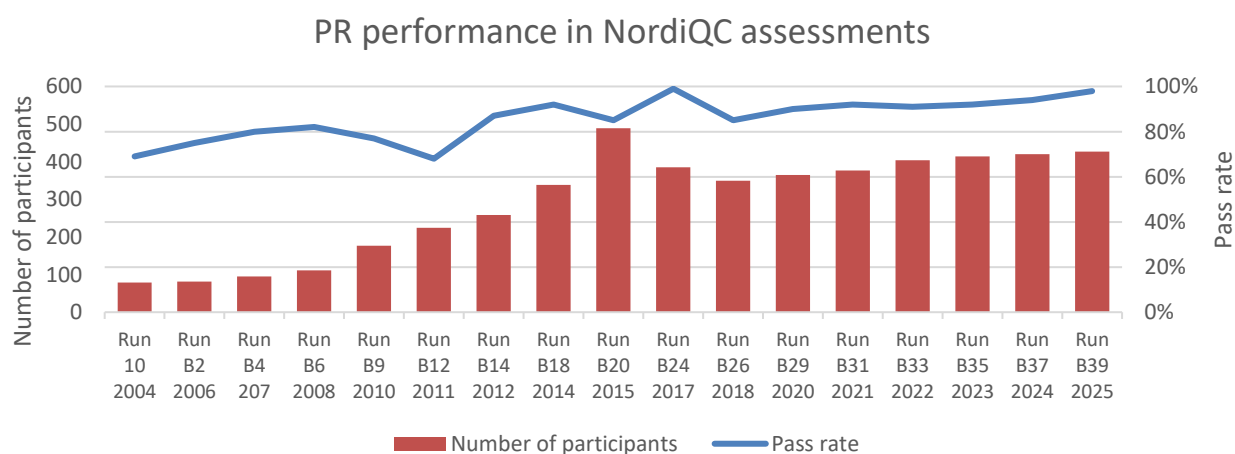
The most frequent causes of insufficient staining reactions were:

- Use of detection systems with low analytical sensitivity
- Too short HIER time

### Performance history

This was the 17<sup>th</sup> NordiQC assessment of PR. The pass rate was very satisfactory and in concordance with previous assessments as shown in Graph 1 with a mean level at 93,4% in the last 5 assessment runs:

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



### Control

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. 90% 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 99% when applying these assays as "plug-and-play". In this assessment, a too weak staining reaction or false positive result were the most predominant features of the insufficient results primarily caused by laboratory modifications.

Table 1a. **Overall results for PR, run B39**

	n	Optimal	Good	Borderline	Poor	Suff. <sup>1</sup>	OR <sup>2</sup>
Concentrated antibodies	41	31	9	-	1	98%	76%
Ready-To-Use antibodies	386	298	80	8	-	98%	77%
Total	427	329	89	8	1	-	
Proportion		77%	21%	1,8%	0,2%	98%	

1) Proportion of sufficient stains (optimal or good).

2) Proportion of Optimal Results.

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

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. <sup>1</sup>	OR <sup>2</sup>
mAb clone <b>16</b>	29	Leica Biosystems	23	6	-	-	100%	79%
mAb clone cocktail <b>16 + SAN27</b>	2	Leica Biosystems	2	-	-	-	-	-
mAb clone <b>PgR 636</b>	4	Dako/Agilent	2	1	-	1	-	-
mAb clone <b>PgR 1294</b>	3	Dako/Agilent	3	-	-	-	-	-
rmAb clone <b>BP6081</b>	1	Biolyntx	-	1	-	-	-	-
rmAb clone <b>QR014</b>	2	Quartett	1	1	-	-	-	-
Total	41		31	9	-	1	-	
Proportion			76%	22%	0%	2%	98%	

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

2) Proportion of Optimal Results.

Table 1c. **Ready-To-Use antibodies and assessment marks for PR, run B39**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. <sup>1</sup>	OR <sup>2</sup>
rmAb clone <b>1E2 790-2223/4296 (VRPS<sup>3</sup>)</b>	91	Ventana/Roche	73	17	1	-	99%	80%
rmAb clone <b>1E2 790-2223/4296 (LMPS<sup>4</sup>)</b>	153	Ventana/Roche	116	32	5	-	97%	76%
mAb <b>PgR 1294 GA090 (VRPS<sup>3</sup>)</b>	40	Dako/Agilent	32	8	-	-	100%	80%
mAb <b>PgR 1294 GA090 (LMPS<sup>4</sup>)</b>	36	Dako/Agilent	25	10	1	-	97%	69%
mAb <b>PgR 636 IR/IS068 (VRPS<sup>3</sup>)</b>	10	Dako/Agilent	10	-	-	-	100%	100%
mAb <b>PgR 636 IR/IS068 (LMPS<sup>4</sup>)</b>	7	Dako/Agilent	3	4	-	-	100%	43%
mAb clone <b>16 PA0312 (VRPS<sup>3</sup>)</b>	21	Leica Biosystems	19	2	-	-	100%	90%
mAb clone <b>16 PA0312 (LMPS<sup>4</sup>)</b>	14	Leica Biosystems	13	1	-	-	100%	93%
mAb clone <b>PgR 636 PM343</b>	1	Biocare Medical	-	1	-	-	-	-
rmAb clone <b>YR85 8360-C010</b>	2	Sakura Finetek	2	-	-	-	-	-
mAb clone <b>16 MAD-000670QD</b>	3	Master Diagnostica/Vitro	1	1	1	-	-	-
mAb clone <b>MXR008 MAB-0854</b>	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone <b>278G8D6 PA246</b>	1	Abcarta	1	-	-	-	-	-
rmAb clone <b>QR014 P006-30</b>	1	Quartett	-	1	-	-	-	-
rmAb clone <b>EP2 AN711-5M</b>	1	BioGenex	1	-	-	-	-	-
rmAb clone <b>SP2 GT205702</b>	1	Gene Tech	1	-	-	-	-	-
rmAb clone <b>SP2 RMPD002</b>	1	Diagnostic Biosystems	-	1	-	-	-	-
Ab clone <b>DY49836 4911422</b>	1	Dakewe	-	1	-	-	-	-
Ab clone <b>MSVA-570 MAD-001370QD</b>	1	Master Diagnostica	-	1	-	-	-	-
<b>Total</b>	<b>386</b>		<b>298</b>	<b>80</b>	<b>8</b>	<b>-</b>	<b>-</b>	
<b>Proportion</b>			<b>77%</b>	<b>21%</b>	<b>2%</b>	<b>0%</b>	<b>98%</b>	

1) Proportion of sufficient results (optimal or good). (≥5 assessed protocols).

2) Proportion of Optimal Results (OR).

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 applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 assessed protocols)

### Detailed analysis of PR, Run B39

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) (3/3)\*, Cell Conditioning 1 (CC1, Ventana/Roche) (7/10), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (9/10) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (4/5) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:400, depending on the total sensitivity of the protocol employed.

Using these protocol settings, 22/27 (82%) laboratories produced a sufficient staining result (optimal or good).

\* (number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for PR for the most commonly used antibody as concentrate on the four main IHC systems\***

Concentrated antibody	Dako/Agilent Autostainer <sup>1</sup>		Dako/Agilent Omnis		Ventana/Roche BenchMark <sup>2</sup>		Leica Biosystems Bond <sup>3</sup>	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone <b>16</b>	0/1**	-	3/3	-	7/10 (70%)	-	9/10 (90%)	4/5 (80%)

\* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

\*\* (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, Max

### Ready-To-Use antibodies and corresponding systems

mAb clone **1E2** product no. **790-2223/4296**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:

Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min.), 12-44 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, 218/223 (98%) 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.), 10-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (GV800/GV021) as detection system.

Using these protocol settings, 75/76 (99%) laboratories produced sufficient staining result.

1 laboratory used product no. GA090 for staining on Ventana Benchmark. 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 10-20 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, 16/16 (100%) laboratories produced sufficient staining result.

1 laboratory used product no. IR068 for staining on Dako Omnis. Data was not included in the description above

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 15-30 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, 33 of 33 (100%) laboratories produced a sufficient staining result (optimal or good).

2 laboratory used product no. PA0312 for staining on the Ventana Benchmark. Data was 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 strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for PR for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark rmAb 1E2 <b>790-2223/790-4296</b>	99% (90/91)	80% (73/91)	97% (148/153)	76% (116/153)
Dako Omnis mAb PgR 1294 <b>GA090</b>	100% (40/40)	80% (32/40)	97% (34/35)	71% (25/35)
Dako Autotstainer mAb PgR 636 <b>IR068/IS068</b>	100% (10/10)	100% (10/10)	100% (6/6)	33% (2/6)
Leica Bond mAb 16 <b>PA0312</b>	100% (21/21)	90% (19/21)	100% (12/12)	92% (11/12)

\* 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 B39 for PR, an overall pass rate of 98% was observed which was slightly higher than the latest five assessments (mean 93,4%, range 92-98%). The prevalent features of insufficient staining results were either characterized by generally too weak staining reactions or a weak false positive reaction in the B-cells of the tonsil.

A false negative or too weak staining reaction was the cause of 56% of the insufficient results (5/9). The majority of laboratories were able to demonstrate PR in the breast carcinoma, tissue core no. 4, with a high PR expression level expected in 70-100% 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. 5, in which at least a weak nuclear staining reaction of >10% of the neoplastic cells was seen in the reference staining, could be challenging and required a calibrated protocol. The breast carcinoma, tissue core no. 5, showed a heterogenous expression pattern and a PR level in the range of 30-80% 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 50<sup>th</sup> 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 44% of the insufficient results (4/9), false positive staining results especially in tonsil were observed compromising the read-out for PR expression in the neoplastic cells.

Ready-To-Use (RTU) Abs were used by 90% (386 of 427) 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 related both 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 platforms, was in this assessment the most widely used assay being used by 57% (244 of 427) of the participants and it gave an overall pass rate of 98%. 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 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. In this assessment, the vendor recommended protocol settings, being used by 37% (91 of 244) of the laboratories, provided a pass rate of 99% being slightly superior compared to the level of 97% 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 false positive staining reaction of the germinal center B-cells of the tonsil and occasionally also the squamous epithelial cells. This staining pattern was mainly observed with protocols with a prolonged Ab incubation time combined with short HIER (<64 min. as recommended). In previous assessments as runs B26 and B31 these modified protocol settings also have caused insufficient results due to false positive reaction in both breast carcinomas and tonsils.

The Dako/Agilent RTU system **GA090** for Omnis, using mAb **clone PgR 1294**, was utilized by 18% of the participants (76 out of 427), achieving an overall pass rate of 99%. This is comparable to previous runs B35 and B37. However, this assessment showed a significantly higher proportion of optimal results (75%), compared to 42% in run B37. The protocol settings recommended by the vendor displayed a high proportion of both sufficient and optimal results, whereas the laboratory modification required careful calibration. In this assessment, the application of mouse linker to EnVision FLEX "transforming to FLEX+" was the most effective protocol modification, used by 22 laboratories with 82% achieving optimal results. In comparison, 52 laboratories used the antibody as described by the vendor without the linker, with 72% achieving optimal results. However, application of linker also increases the risk of excessive background as noted in previous runs and overall protocol settings as primary antibody incubation and HIER time should be calibrated carefully.

The Dako/Agilent RTU system **IR068/IS068** for Autostainer, based on the mAb **clone PgR 636**, provided an impressive pass rate of 100% (17/17). As shown in Table 3, the vendor recommended protocol using the Flex+ system with mouse linker provided 100% optimal results, whereas the proportion of optimal results was only 33% applying laboratory modifications. Less successful modification was related to removal of the mouse linker resulting in a general weak staining reaction that compromised the scoring of the staining result.

The Leica RTU system **PA0312** for Bond, based on the mAb **clone 16**, provided an overall pass rate of 100% (35/35) and 91% (32/35) optimal results being similar to the levels obtained in recent runs. As shown in Table 3, all 21 protocols based on vendor recommended protocols provided a sufficient result and 19 of these assessed as optimal (90%). 40% (14/35) of the protocols based on the PA0312 system were applied by modified protocol settings giving a marginally increased proportion of optimal results of 92%. 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 99% (161/162) and 83% (134/162) 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.

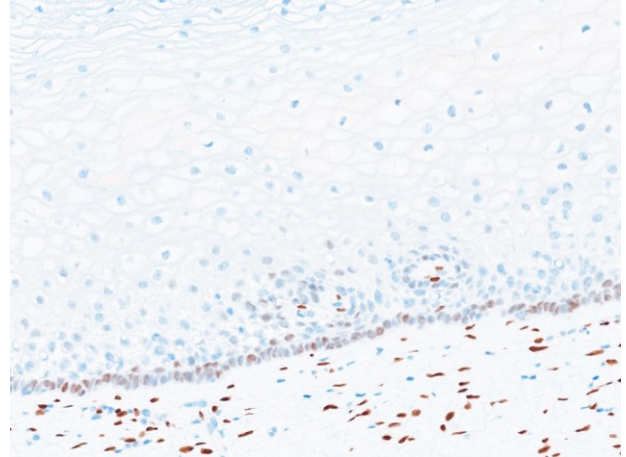
10% (41 of 427) of the participants used Abs as concentrated formats within laboratory developed (LD) assays. Similar to the data generated for the RTU systems, the Abs, mAb **clones 16, PgR 636 and PgR 1294** were the most used and could provide optimal results on the main IHC platforms (Ventana/Roche, 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 98% was obtained, 76% optimal and thus fully comparable 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

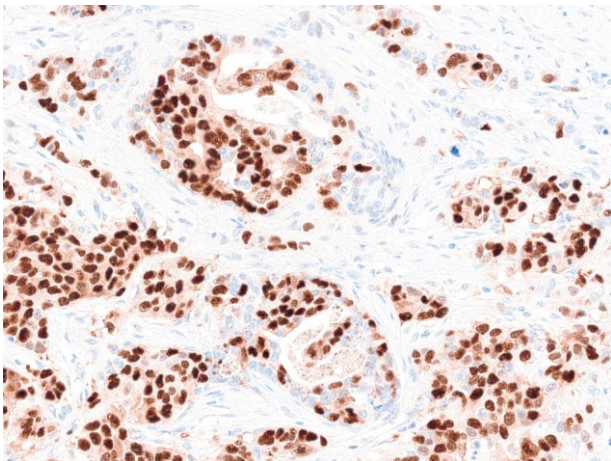




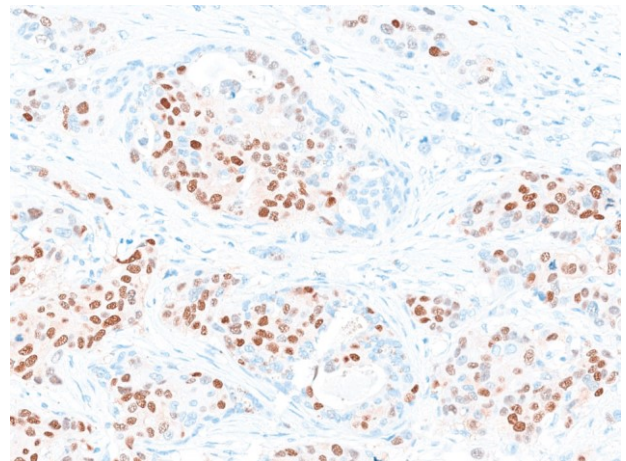
**Fig. 1a**  
 Optimal staining reaction for PR of the uterine cervix using the Ventana/Roche RTU system based on the rmAb clone 1E2 on Ventana Benchmark Ultra using OptiView as detection system. The vast majority of basal squamous epithelial cells show weak to moderate nuclear staining reaction, whereas 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 Ventana/Roche RTU system based on the rmAb clone 1E2. The protocol was applied accordingly to vendor recommendations using UltraView as detection system, 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 – 3b.

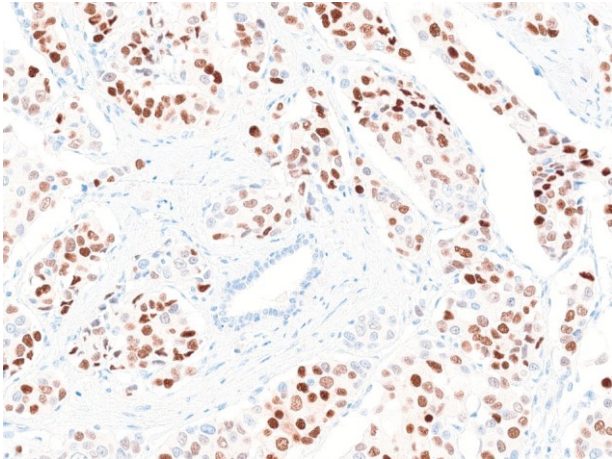


**Fig. 2a**  
 Optimal staining reaction for PR of the breast carcinoma, tissue core no. 4, with 70-100% positive tumor cells using same protocol as in Fig. 1a. Virtually all neoplastic cells show a strong nuclear staining reaction.

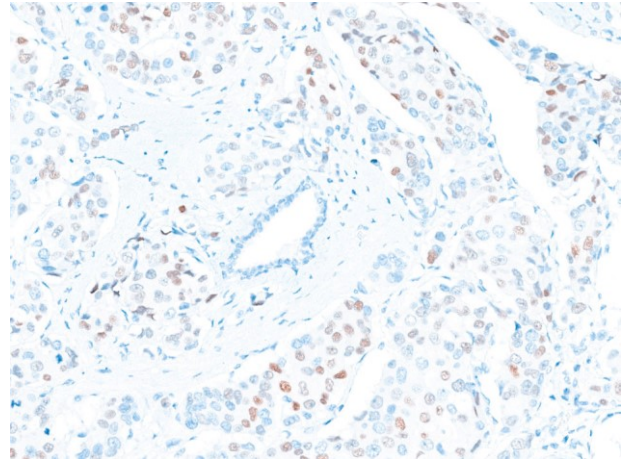


**Fig. 2b**  
 Staining reaction for PR of the breast carcinoma, tissue core no. 4, with 70-100% positive tumor cells using same protocol as in Fig. 1b – same field as in Fig. 2a. The staining intensity is slightly reduced but the proportion of cells demonstrated is as expected.

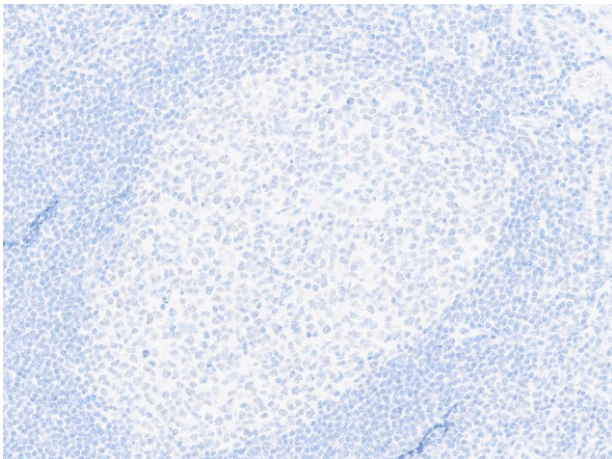




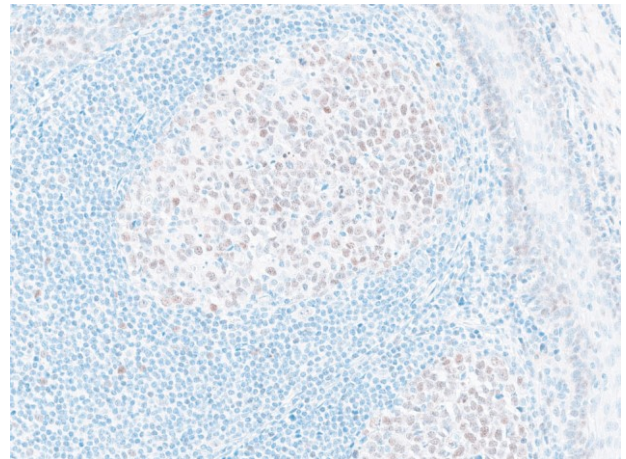
**Fig. 3a**  
Optimal staining reaction for PR of the breast carcinoma, tissue core no. 5, with at least 30-80% of the neoplastic cells showing a weak to moderate but distinct nuclear staining reaction - using same protocol as in Figs. 1a-2a.



**Fig. 3b**  
Insufficient staining reaction for PR of the breast carcinoma, tissue core no. 5, expected to be positive in minimum 30-80% of the neoplastic cells – same field as in Fig. 3a. A significantly reduced proportion of the neoplastic cells are convincingly positive. Same protocol as used in Figs. 1b-2b.



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



**Fig. 4b**  
Insufficient reaction for PR of the tonsil expected to be PR negative using the Ventana/Roche RTU system based on the rmAb clone 1E2. The protocol was performed with laboratory modifications using short HIER (24 min.), a prolonged Ab incubation (48 min.) and OptiView as detection system  
An unsuspected weak nuclear staining reaction is observed in the germinal center B-cells and in the squamous epithelia.

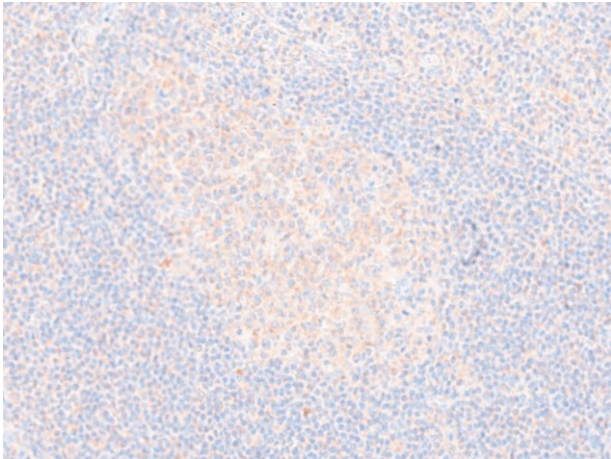


Fig. 5a (x200)  
 Staining reaction for PR of the tonsil using Dako/Agilent RTU system based on the mAb PgR 1294 clone. The protocol was performed with laboratory modifications by using the Flex+ system and prolonging the Ab incubation time causing a general excessive background created by too sensitive protocol settings. Same protocol used in Fig 5b.  
 The overall result assessed as good. No false positive nuclear staining reaction was observed.

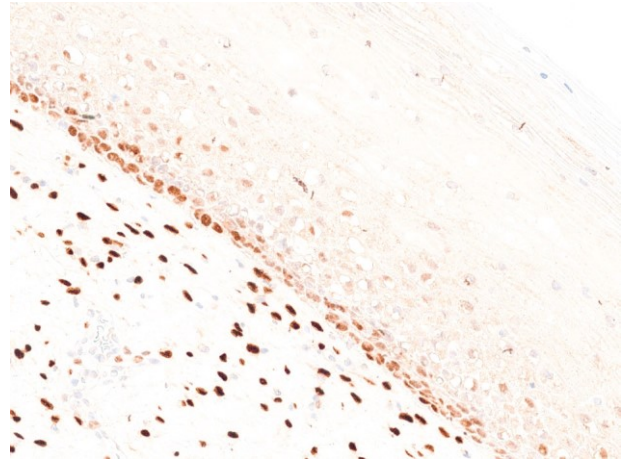


Fig. 5b (x200)  
 Sufficient staining reaction for PR of the cervix. The vast majority of basal squamous epithelial cells show moderate nuclear staining reaction, whereas the stromal cells show a strong nuclear staining reaction. A general excessive background staining reaction is displayed in the entire tissue.  
 Same protocol as in Figs. 5a  
 The overall result assessed as good. No false positive nuclear staining reaction was observed.

TJ/LE/SN 03.04.2025