Assessment Run B26 2018

Progesteron receptor (PR)

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

No.	Tissue	PR-positivity*	PR-intensity*
1.	Uterine cervix	80-90%	Moderate to strong
2.	Tonsil	0%	Negative
3.	Breast carcinoma	0%	Negative
4.	Breast carcinoma	90 - 100%	Moderate to strong
5.	Breast carcinoma	70 - 90%	Weak to moderate
6.	Breast carcinoma	40 - 60%	Weak to moderate

*PR-positivity and intensity as characterized by NordiQC reference laboratories using the mAb clone 16

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

Criteria for assessing PR staining as **optimal** were:

- A moderate to strong, distinct nuclear staining reaction of both columnar and basal squamous epithelial cells and most of the stromal cells (with the exception of endothelial and lymphoid cells) in the uterine cervix.
- An at least weak to moderate distinct nuclear staining reaction in the appropriate proportion (see table above) of neoplastic cells in the breast ductal carcinomas no. 4, 5 and 6.
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 3.
- Not more than a weak cytoplasmic staining reaction in cells with strong nuclear staining reaction - for the mouse monoclonal antibody (mAb) clone PgR636, moderate to strong cytoplasmic staining reaction in columnar epithelial cells of the uterine cervix was accepted.
- No nuclear staining reaction of cells in the tonsil.

The staining reactions were classified as **good** if ≥ 10% of the neoplastic cells in the breast carcinomas no. 4, 5 and 6 showed an at least weak nuclear staining reaction but less than the range of the reference laboratories.

The staining reactions were classified as **borderline** if 1) ≥ 1 % and < 10% of the neoplastic cells showed a nuclear staining reaction in one or more of the breast carcinomas no. 4, 5 and 6. 2) If a distinct nuclear staining reaction was seen in ≥ 10% of germinal centre cells in tonsil.

The staining reactions were classified as **poor** if a false negative or false positive staining reaction was seen in one of the breast carcinomas.

Participation

Number of laboratories registered for PR, run B26	366
Number of laboratories returning slides	349 (95%)

One laboratory returned an ER slide with a PR Ab. This laboratory was not included in the results below.

Results
348 laboratories participated in this assessment. 85% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary Ab
- Protocols based on rabbit mnolclonal Ab (rmAb) clone 1E2 gave a false positive result (no single reason was identified)

Conclusion
The most widely used Ab clones **16**, **PgR 636, PgR 1294** and **1E2** could all be used to obtain an optimal result.

Irrespective of the clone applied, efficient HIER and careful calibration of the primary antibody were mandatory for optimal performance. In this assessment, Ready-To-Use (RTU) systems and laboratory developed assays showed a similar performance.
Uterine cervix is an appropriate positive tissue control – almost all columnar epithelial cells, basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining reaction with only a minimal cytoplasmic staining.
Tonsil is an appropriate negative tissue control – no nuclear staining reaction should be seen.
In concordance with the observations seen in previous PR assessments, it is highly advisable to include both PR negative and PR positive breast tumours and well characterized PR negative tissues as tonsil in the validation/verification process of the PR assay and meticulously monitor the PR expression results and metrics produced in the laboratory.

Table 1. **Antibodies and assessment marks for PR, run B26**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	33 1 1	Leica/Novocastra Biocare Vector	22	6	4	3	82%	82%
mAb clone cocktail **16 + SAN27**	4	Leica/Novocastra	-	4	-	-	-	-
mAb clone **1A6**	1	Leica/Novocastra	-	1	-	-	-	-
mAb clone **PgR 636**	19	Dako Agilent	14	4	1	-	95%	100%
mAb clone **PgR 1294**	10	Dako Agilent	7	2	1	-	90%	89%
rmAb clone **SP2**	3 1	Thermo Scientific Diagnostic BioSystems	2	1	-	1	-	-
rmAb clone **SP42**	2 1 1	Zytomed Spring Biosystems Cell Marque	2	1	1	-	-	-
rmAb clone **Y85**	1	Cell Marque	-	-	1	-	-	-
rmAb clone **P21-S**	1	DB Biotech	-	-	-	1	-	-
Ready-To-Use antibodies								
rmAb clone **Y85**	1	Sakura Finetek	1	-	-	-	-	-
mAb clone **16 PA0312**	11	Leica/Novocastra	9	2	-	-	100%	100%
mAb clone **16 MAD-000670QD**	1	Master Diagnostica	-	-	1	-	-	-
mAb **PgR 636 IR/IS068**	35	Dako Agilent	29	2	1	3	89%	97%
mAb **PgR 1294 GA090**	38	Dako Agilent	23	11	3	1	89%	89%
mAb clone **PgR 1294 K4071/SK310**	1	Dako Agilent	1	-	-	-	-	-
rmAb clone **1E2 790-2223/4296**	180	Ventana	118	31	27	4	83%	83%
rmAb clone **SP2 Kit-0013**	2	Maixin	1	1	-	-	-	-
Total	348		229	66	40	13	-	
Proportion			66%	19%	11%	4%	85%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of PR, run B24
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) pH 9 (3-in-1) (Dako) (3/3)*, TRS High pH (Dako) (2/3), Cell Conditioning 1 (CC1, Ventana) (3/13), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (5/6), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (5/6), Epitope Retrieval Solution pH 6 (Novocastra) (1/1), Tris-EDTA pH 9 (TE)

(1/1) or Citrate pH 6 (2/2) 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, 28/34 (82%) 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 pH 9 (3-in-1) (Dako) (6/7), TRS High pH (Dako) (1/1), BERS2 (Leica) (4/5), BERS1 (Leica) (1/1), TE (1/2), or TRS pH 6.1 (3-in-1) (Dako) (1/2) 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, 18/18 (100%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294**: Protocols with optimal results were based on HIER using TRS High pH (Dako) (4/4) or CC1 (Ventana) (3/5) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:220, depending on the total sensitivity of the protocol employed.
Using these protocol settings, 8/9 (89%) 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**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica 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**	3/3	-	2/3	-	3/13 (23%)	-	5/6 (83%)	5/6 (83%)
rmAb clone **PgR 636**	6/7 (86%)	1/2	1/1	-	-	-	4/5 (80%)	1/1
mAb clone **PgR1294**	-	-	4/4	-	3/5 (60%)	-	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)

Ready-To-Use antibodies and corresponding systems
mAb clone **16**, product no. **PA0312**, Leica, Bond Max, Bond III: Protocols with an optimal result were typically based on HIER using BERS1 or BERS2 (Bond, Leica) (efficient heating time 10-30 min. at 100°C), 15-60 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.
Using these protocol settings, 11 of 11 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **PgR 636**, product no. **IS068/IR068**, Dako, 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 95-99°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems.
Using these protocol settings, 29/30 (97%) laboratories produced a sufficient staining result.
4 laboratories used product no. ISO068/IR068 on other platforms than Autostainer+/Link (Dako). Data was not included in the description above.

mAb clone **PgR 1294** product no. **GA090**, Dako, Omnis: Protocols with optimal results were based on HIER using TRS High pH (Dako) (efficient heating time 20-30 min.), 10-25 min. incubation of the primary Ab and EnVision Flex/Flex+ (GV800/GV021) as detection system.
Using these protocol settings, 31/35 (89%) laboratories produced a sufficient staining result.
2 laboratories used product no. GA090 on other platforms than Omnis (Dako). Data was not included in the description above.

rmAb clone **1E2** product no. **790-2223/4296,** Ventana, BenchMark GX, XT/Ultra:
Protocols with optimal result were typically based on HIER using CC1 (efficient heating time 8-64 min.), 8-64 min. incubation of the primary Ab and iView (760-091), UltraView (760-500) or OptiView (760-700) as detection system.
Using these protocol settings, 190/193 (98%) laboratories produced a sufficient staining result.

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**	4/4	4/4	7/7 (100%)	5/7 (71%)
Dako Autotstainer+/ Autostainer Link mAb PgR 636 **IS068/IR068**	13/15 (87%)	13/15 (87%)	16/16 (100%)	14/16 (88%)
Dako Omnis mAb PgR 1294 **GA090**	20/23 (87%)	14/23 (61%)	12/13 (92%)	7/13 (54%)
Ventana BenchMark GX/XT/Ultra rmAb 1E2 **790-2223/790-4296**	42/47 (89%)	35/47 (74%)	107/133 (80%)	83/133 (62%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit
Only protocols performed on the specified vendor IHC stainer are included.

Comments

In this NordiQC assessment B26 for PR, an overall pass rate of 85% was observed similar to most of the previous assessments, except the latest run B24. The features of insufficient staining results (n=53) were a weak/false negative staining reaction, 55% (29 of 53), or a false positive staining reaction in germinal centre cells in tonsil, 36% (19 of 53). The remaining 5 insufficient results were caused by false positive staining in the breast carcinoma core no. 3, expected to be PR negative, or poor signal-to-noise ratio.

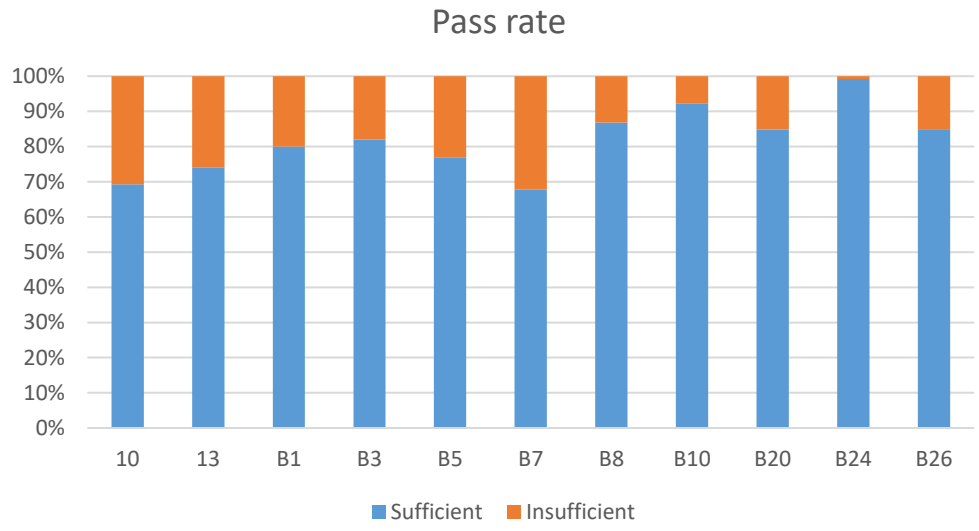
Using a laboratory developed (LD) assay, the three most widely used antibodies, mAb clones 16, PgR 636 and PgR 1294 were all successful and could provide sufficient and optimal results on the main IHC systems (Dako, Leica and Ventana). Both HIER in alkaline and non-alkaline buffers could be used to obtain a sufficient and optimal result (see Table 2). The main prerequisite for optimal performance seemed to be a careful calibration of the primary Ab and thus adjustment of the titre to the overall level of sensitivity of the IHC system.
The corresponding RTU system for mAb clones16 (Leica) provided a pass rate of 100% compared to 82% for the concentrated format. The RTU systems for mAb clones PgR 636 (Dako) and PgR 1294 (Dako) both provided pass rates similar to the LD assays based on the same clones – see Table 1.
Optimal results could be obtained both by the officially recommended protocols provided by the companies but also by laboratory defined modifications of the protocol e.g. adjustment of incubation time of the primary Ab and/or reduced HIER time (see Table 3).

An aberrant and false positive staining reaction was seen in 36% (19 of 54) of the insufficient results and was characterized by a weak distinct nuclear staining reaction of germinal centre B-cells in the tonsil. The false positive staining reaction was only seen for the RTU format of rmAb clone 1E2 (Ventana). No single reason for this reaction was identified.
Optimal results could be obtained both by the vendor recommended protocol settings (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. However, off-label use must be meticulously validated by the end-user. In total, only 47 laboratories used the Ventana RTU system based on the rmAb clone 1E2 according to the official recommendations. 89% (n=42) were evaluated as sufficient and 11% insufficient compared to the modified protocol settings with a pass rate of 80% (107 of 133).

Performance history

This was the eleventh NordiQC assessment of PR. The pass rate was in concordance with previous assessments, except the latest run B24 which was exceptionally high, as shown in Graph 1:

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



Controls

As observed in the previous NordiQC assessments of PR, uterine cervix is an appropriate positive tissue control for evaluation of the sensitivity of PR staining: With an optimal protocol almost all columnar epithelial cells, the majority of basal squamous epithelial cells and most of the stromal cells must show a strong and distinct nuclear staining with only a minimal cytoplasmic reaction. No staining must be seen in endothelial cells and lymphocytes. However, it must be taken into consideration that the PR expression level is reduced in the uterine cervix of post-menopausal women and thus especially demonstration of PR in squamous epithelial cells can be compromised.

Tonsil is recommendable as negative tissue control, in which no nuclear staining should be seen.

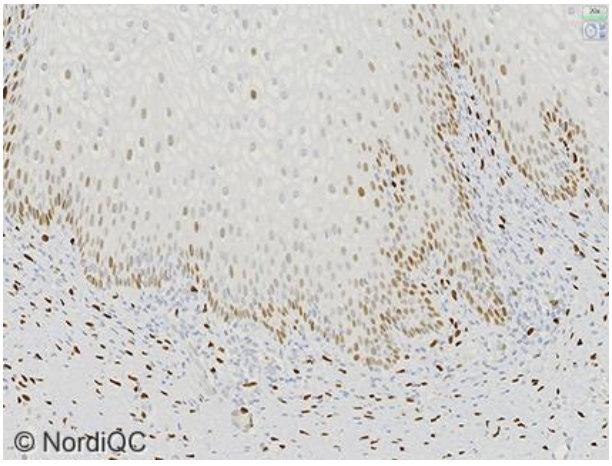


Fig. 1a

Optimal staining for PR of the uterine cervix using the mAb clone 16 optimally calibrated at a titre of 1:200, efficient HIER in an alkaline buffer using a 3-step multimer based detection system.

The vast majority of basal squamous epithelial cells show a weak to moderate nuclear staining reaction, whereas the majority of columnar epithelial cells and stromal cells show a moderate to strong nuclear staining reaction.

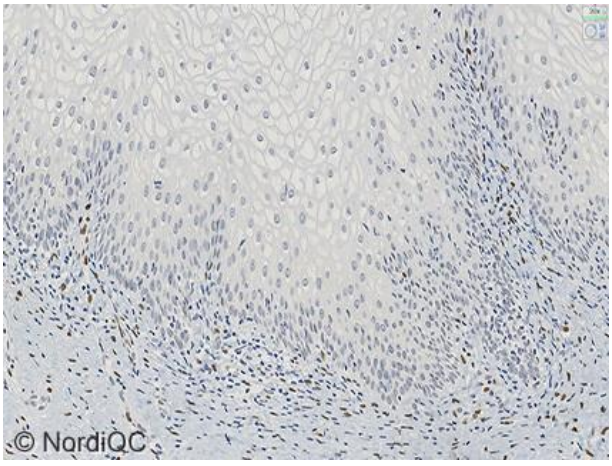


Fig. 1b

Insufficient staining for PR of the uterine cervix, using the rmAb clone 1E2 with laboratory modified protocol settings with inefficient HIER giving a too low sensitivity - same field as in Fig. 1a.

The stromal cells are demonstrated, but the squamous epithelial cells are virtually negative. Also compare with Figs. 2b-3b – same protocol.

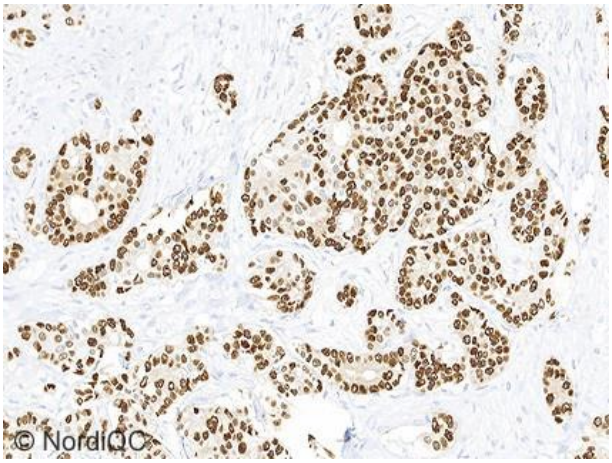


Fig. 2a
Optimal staining for PR of the breast carcinoma no. 4 with 90 - 100% cells positive using same protocol as in Fig. 1a.
A strong nuclear staining reaction is seen. A weak cytoplasmic staining reaction in the positive neoplastic cells is seen and accepted. No unspecific background staining was seen.

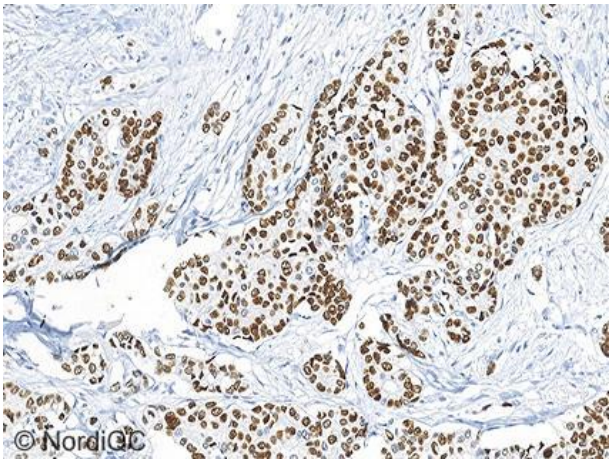


Fig. 2b
Staining for PR of the breast carcinoma no. 4 with 90-100% cells positive using same protocol as in Fig. 1b. - same field as in Fig. 2a.
A moderate to strong distinct nuclear staining reaction in virtually all neoplastic cells is seen.
However also compare with Fig. 3b – same protocol.

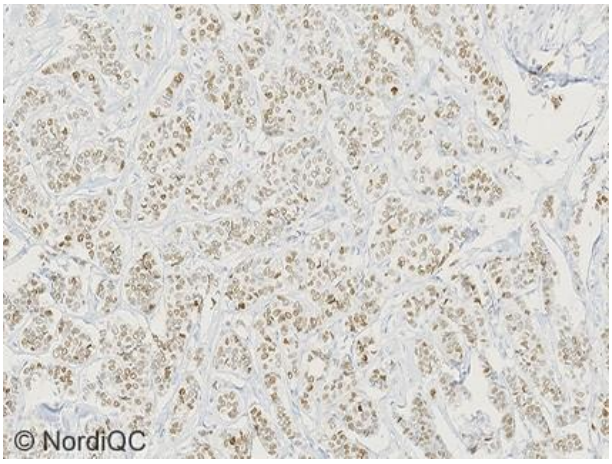


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 5 with 70-90% cells positive using same protocol as in Figs. 1a-2a.
The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

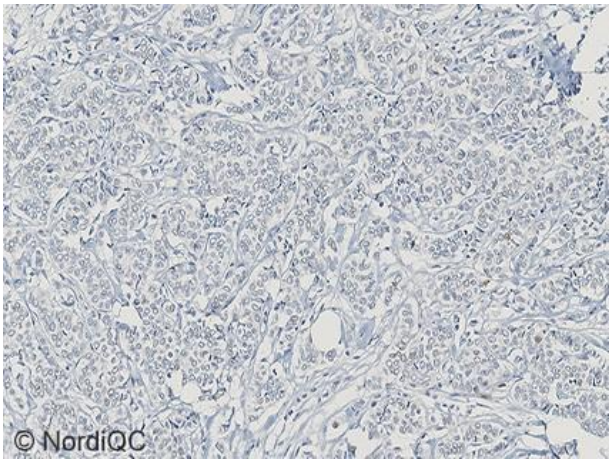


Fig. 3b
Insufficient staining for PR of the breast carcinoma no. 5 with 70-90% cells positive using same protocol as in Figs. 1b-2b – same field as in Fig. 3a.
Only dispersed cells are demonstrated and the tumuor is virtually negative.

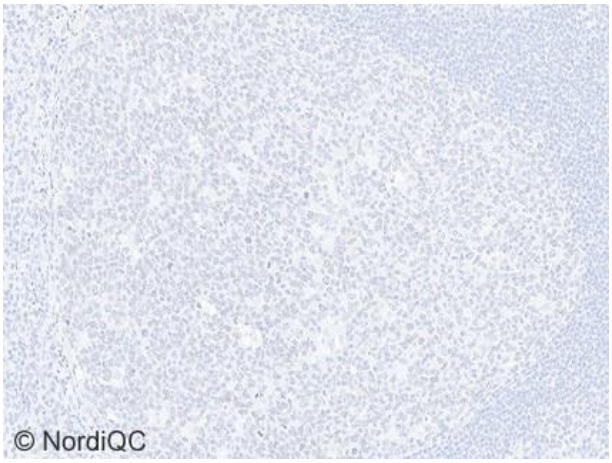


Fig. 4a
Optimal staining for PR of tonsil using same protocol as in Figs. 1a-3a.
No nuclear staining reaction is seen.

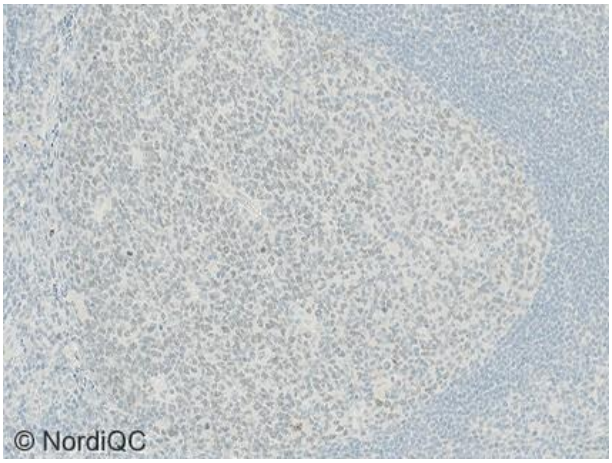


Fig. 4b
Insufficient staining for PR of tonsil – same field as in Fig. 4a.
The majority of germinal cells show a weak and aberrant false positive nuclear staining reaction.
This aberrant staining reaction was only seen for rmAb clones 1E2 (RTU, Ventana). No single reason for this staining pattern was identified.

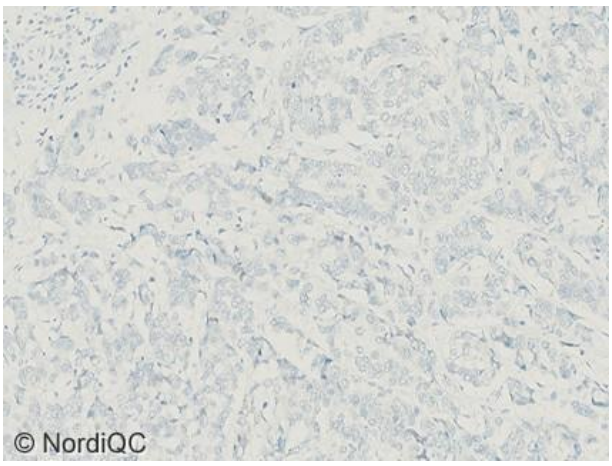


Fig. 5a
Optimal staining for PR of the breast carcinoma no. 3 expected to be negative using same protocol as in Figs. 1a-4a.
No nuclear staining reaction in the neoplastic cells is seen. The PR status was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories.

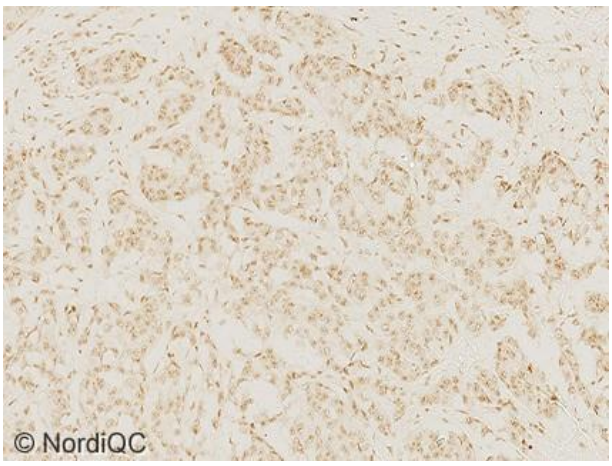


Fig. 5b
Insufficient staining for PR of the breast ductal carcinoma no. 3 expected to be negative – same field as in Fig. 5a.
Virtually all neoplastic cells show a weak and aberrant false positive nuclear staining reaction.
The protocol was based on the rmAb clone P21-S, using HIER in an alkaline buffer and a 2-step polymer based detection system. Using this protocol, all cells in tonsil was false positive as well.

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