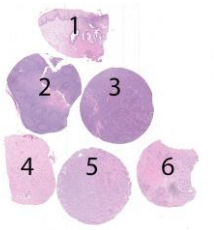
Assessment Run B20 2015
Progesterone Receptor (PR)

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	Negative	Negative
3.	Breast carcinoma	Negative	Negative
4.	Breast carcinoma	40 - 60%	Weak to strong
5.	Breast carcinoma	60 - 80%	Weak to strong
6.	Breast carcinoma	80 - 100%	Moderate to strong



*PR-positivity and intensity as characterized by NordiQC reference laboratories using the mmAb clone 16 (Leica/Novocastra)

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 above) of neoplastic cells in the breast ductal carcinomas no. 4, 5 & 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 mmAb 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 & 6 or 2)
If a distinct nuclear staining reaction was seen in ≥ 10% of 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 B20	525
Number of laboratories returning slides	491(94%)

Results
491 laboratories participated in this assessment. Two used an inappropriate Ab. Of the remaining 489 laboratories, 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:
- Protocols based on rmAb clone 1E2 giving a false positive result (no single reason was identified)
- Too low concentration of the primary Ab
- Insufficient HIER – too short efficient heating time

Conclusion
The mmAb clones **16**, **PgR 636** and **PgR 1294** were in this assessment the most successful antibodies. Irrespective of the clone applied, efficient HIER and careful calibration of the primary antibody were mandatory for optimal performance. In general, Ready-To-Use systems for these three Abs showed a slightly superior pass rate compared to laboratory developed assays.
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.
Both in this and previous runs for PR, rmAb Abs clones 1E2 and SP2 provided false positive results. For this reason, it is highly advisable to include both PR negative breast tumours and well characterized PR negative tissues as tonsil in the validation 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 B20**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **16**	48 1 1	Leica/Novocastra Biocare Vector	39	7	3	1	92%	96%
mAb clone cocktail **16 + SAN27**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **1A6**	5	Leica/Novocastra	2	1	0	2	-	-
mAb clone **PgR 636**	68	Dako	48	15	4	1	93%	94%
mAb clone **PgR 1294**	17	Dako	13	4	0	0	100%	100%
rmAb clone **SP2**	5	Thermo/NeoMarkers	2	2	0	1	-	-
rmAb clone **SP42**	1	Zytomed	1	0	0	0	-	-
rmAb clone **Y85**	1 1	Cell Marque Thermo/NeoMarkers	1	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **16 PA0312**	13	Leica/Novocastra	11	2	0	0	100%	100%
mAb clone **16 MAD-000670QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb **PgR 636 IR/IS068**	78	Dako	60	15	1	2	96%	96%
mAb clone **PgR 1294 K4071/SK310**	4	Dako	1	3	0	0	-	-
mAb clone **PR88 AM328-5ME**	1	Biogenex	0	0	1	0	-	-
rmAb clone **1E2 790-2223/4296**	239	Ventana	85	98	53	3	77%	88%
rmAb clone **SP2 Kit-0013**	1	Maixin	1	0	0	0	-	-
rmAb clone **SP2 RM-9102**	1	Thermo/NeoMarkers	0	0	1	0	-	-
pAb E2071	1	Spring Bioscience	0	0	0	1	-	-
Total	489		266	149	63	11	-	
Proportion			54%	31%	13%	2%	85%	

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

Detailed analysis of PR, run B20
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mmAb 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 pH 6.1 (Dako) (3/4), Cell Conditioning 1 (CC1, Ventana) (6/10), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (13/13), BERS1 (Leica) (10/11), Tris-EDTA pH 9 (3/4) or Citrate pH 6 (1/1) as retrieval buffer. The mmAb was typically diluted in the range of 1:50-1:800, depending on the total sensitivity of the protocol employed. Using these protocol settings 44 of 46 (96%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mmAb clone **1A6**: Protocols with optimal results were based on HIER using BERS1 (Leica) (1/2) or Citrate pH 6 as retrieval buffer. The mmAb was diluted 1:10-40. Using these protocol settings 2 of 2 (100%) laboratories produced a sufficient staining result.

mmAb clone **PgR 636**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako) (11/11), TRS pH 9 (Dako) (11/11), TRS pH 6.1 (Dako) (4/4), CC1 (Ventana) (1/2), BERS2 (Leica) (7/7), BERS1 (Leica) (4/4), Tris-EDTA pH 9 (5/5), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (4/8) as retrieval buffer. The mmAb was typically diluted in the range of 1:50-1:700, depending on the total sensitivity of the protocol employed. Using these protocol settings 63 of 67 (94%) laboratories produced a sufficient staining result.

mmAb clone **PgR 1294**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako) (1/2), TRS pH 9 (Dako) (9/12), CC1 (Ventana) (1/1), Tris-EDTA pH 9 (1/1) or Citrate pH6 (1/1) as retrieval buffer. The mmAb was typically diluted in the range of 1:50-1:1.500, depending on the total sensitivity of the protocol employed. Using these protocol settings 17 of 17 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP2**: Protocols with optimal results were both based on HIER using Tris-EDTA pH 9 (1/3) or Citrate pH6 (1/2) as retrieval buffer. The mmAb was diluted in the range of 1:100-1:150. Using these protocol settings 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP42**: One protocol with an optimal result was based on HIER using Citrate pH 6 as retrieval buffer. The rmAb was diluted 1:200 using a 3-step polymer based detection system.

rmAb clone **YR85**: One protocol with an optimal result was based on HIER using TRS (Dako) pH 9 (1/1) as retrieval buffer. The rmAb was diluted 1:25 using a 2-step polymer based detection system.

Ready-To-Use antibodies and corresponding systems
mAb clone **16**, product no. **PA0312**, Leica, Bond Max, Bond III:
Protocols with an optimal result were based on HIER using BERS1 pH 6 or BERS2 pH 9 (Bond, Leica) (efficient heating time 15-30 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 12 of 12 (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) or TRS pH 9 (efficient heating time 10-20 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 69 of 72 (96%) laboratories produced a sufficient staining result.

mAb clone **PgR 1294** (prod. no SK310/K4071, Dako):
One protocol with an optimal result was based on HIER in a Pressure Cooker using Citrate pH 6, 30 min. incubation of the primary Ab and EnVision (SK310/K4071) as detection system. Using these protocol settings 2 of 2 (100%) laboratories produced a sufficient staining result.

rmAb clone **1E2** product no. **790-2223/4296,** Ventana, BenchMark GX, XT/Ultra:
Protocols with optimal result were typically based on HIER using Cell Conditioning 1 (efficient heating time 32-64 min.), 8-24 min. incubation of the primary Ab and IView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. Using these or similar protocol settings 92 of 105 (88%) laboratories produced a sufficient staining result.

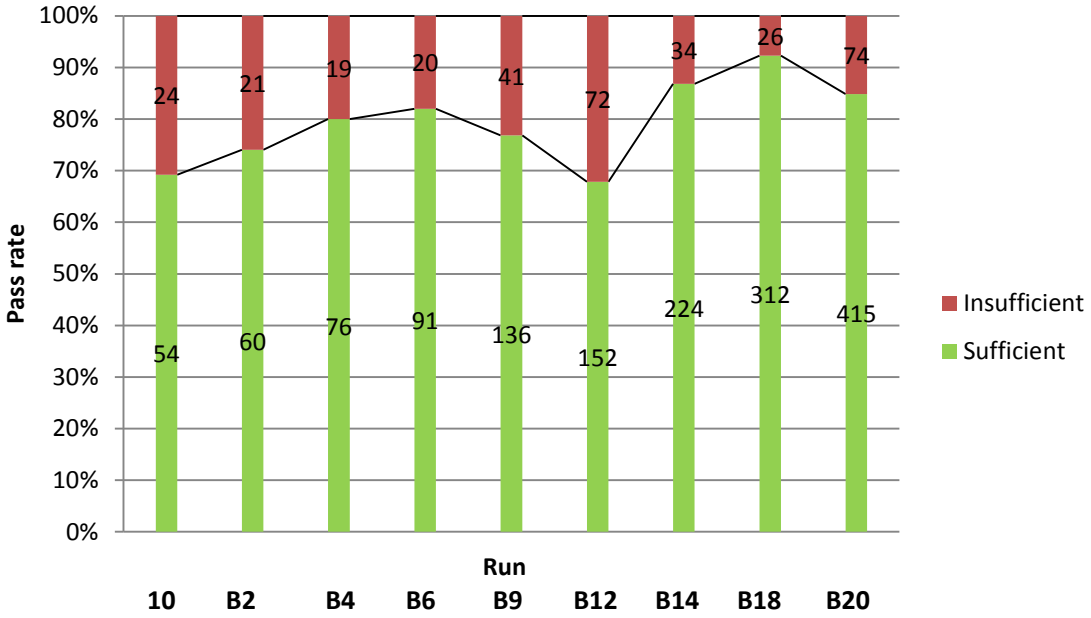
rmAb clone **SP2**, product no. **Kit-0013,** Maixin, manual staining:
One protocol with an optimal result was based on HIER using Citrate buffer pH 6 (efficient heating time 2 min. at 120°C), 60 min. incubation of the primary Ab. and KIT-5230 (Maixin) as detection system.

Comments
In this NordiQC assessment B20 for PR the prevalent features of an insufficient staining result were either a generally too weak staining reaction or a false positive staining reaction.
A too weak staining reaction was seen in 24% of the insufficient results (18 out of 74 laboratories). Virtually all laboratories were able to demonstrate PR in the breast carcinoma tissue core no. 6 and in stromal cells of the uterine cervix, whereas demonstration of PR in the breast carcinomas no. 4 in which at least a weak nuclear staining reaction of 40% of the neoplastic cells was expected, was more challenging and required a carefully calibrated protocol.
Using a laboratory developed (LD) assay the two most widely used antibodies, mmAb clones 16 and PgR 636 were both successful and provided a high number of sufficient and optimal results on all 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. 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 Ready-To-Use (RTU) systems for mmAb clone 16 (Leica) and clone PgR 636 (Dako) both provided a slightly improved pass rate compared to LD assays using same clones – see table 1.
Optimal result could be obtained both by the official 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.

An aberrant and false positive staining reaction was seen in 70% of the insufficient results (52 of 74 laboratories) and was mainly characterized by a 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) and rmAb clone SP2.
As seen in table 1, the Ventana RTU system provided a result typically evaluated as either optimal, good or borderline. For the Ventana RTU system it was observed, that the vast majority of participants, that failed this assessment due to false positive staining result, used a laboratory modified protocol compared to the official recommendation given in the package insert by Ventana.
Typically a reduced HIER time (e.g. 20-32 min. in CC1) and a prolonged incubation time of the primary antibody were applied compared to the official recommendation given in the package insert. This was the case in 30 of the 51 insufficient results. 12 insufficient results were based on a prolonged incubation time of the primary Ab and 7 insufficient results were based on a protocol using OptiView as detection system – only UltraView and iView are officially recommended.
In total only 36 laboratories used the Ventana RTU system based on the RTU format of rmAb clone 1E2 according to the official recommendations. 94% (34 protocols) were evaluated as sufficient and 6% insufficient (caused by the aberrant false positive staining reaction of tonsil). This clearly indicates that modification of the protocol and in particular increasing the sensitivity enhances the risk of a false positive result for PR when using rmAb clone 1E2. However the presented data also confirms the observations generated in previous NordiQC assessments for PR that the rmAb clone 1E2, despite using official and best practice protocol settings provides a higher rate of false positive results e.g. compared to the mmAb clones 16 and PgR 636.

Performance history
This was the ninth NordiQC assessment of PR. A small decrease in the proportion of sufficient results was seen compared to the previous runs, as shown in figure 1:

Figure 1 – pass rate in the NordiQC assessments for PR



The decrease of the number of sufficient results was mainly related to the material circulated and not to the increase of many laboratories participating for the first time. Exactly same pass rate of 85% were thus observed for the laboratories participating in the PR assessment for the first time (n=168) compared to the laboratories also participating in the latest assessment run B18, 2014.

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.

1. Yaziji H, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008 Dec;16(6):513-20. PubMed PMID: 18931614.

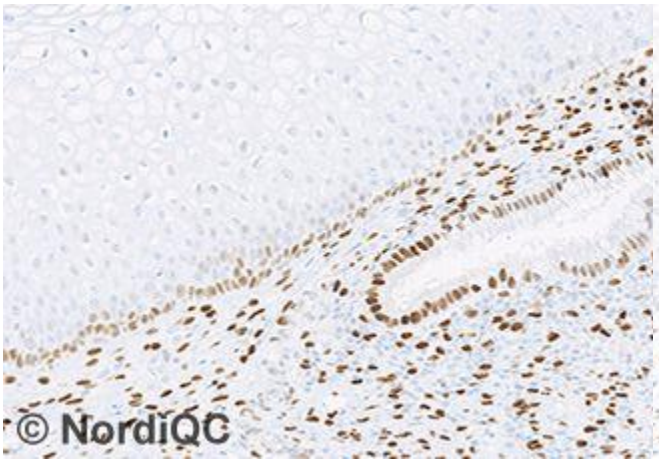


Fig. 1a
Optimal staining for PR of the uterine cervix using the mmAb clone 16 optimally calibrated at a titre of 1:50, efficient HIER for 48 min. in CC1 pH 8,5 using a 3-step multimer based detection system (OptiView, Ventana). The vast majority of basal squamous epithelial cells show a weak to moderate nuclear staining reaction, whereas the majority of columnar epithelial cells and stromal cells show a moderate to strong nuclear staining reaction.

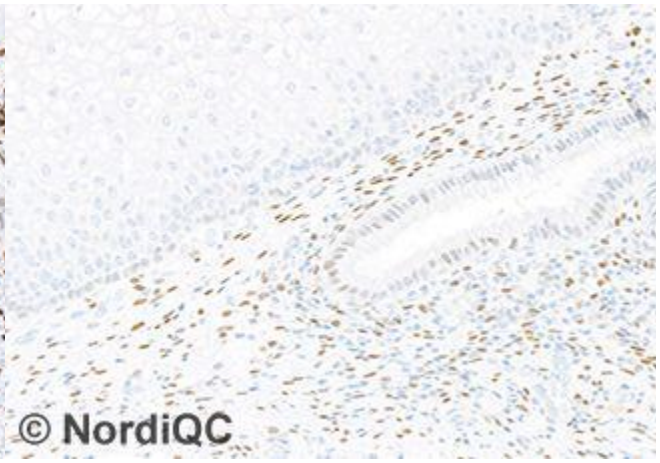


Fig. 1b
Insufficient staining for PR of the uterine cervix, using the mmAb clone 16 with protocol settings giving a too low sensitivity - same field as in Fig. 1a.
The stromal cells are demonstrated, but the squamous and columnar epithelial cells are virtually negative. Also compare with Figs. 2b - 3b – same protocol.

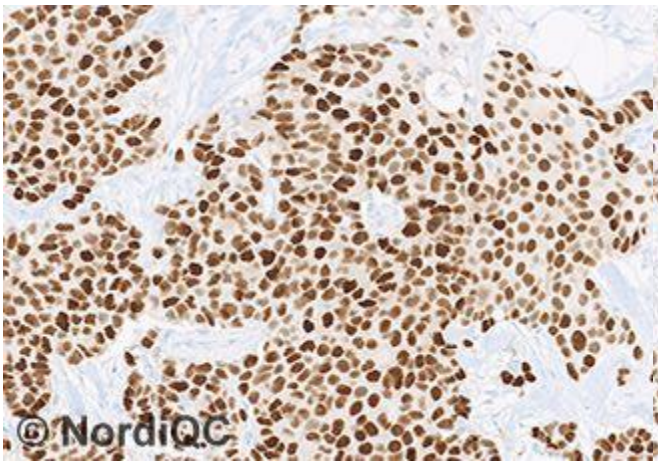


Fig. 2a
Optimal staining for PR of the breast carcinoma no. 6 with 80 - 100% cells positive using same protocol as in Fig. 1a.
A moderate to strong nuclear staining reaction is seen. A weak cytoplasmic staining reaction in the neoplastic cells is seen, but no background staining.

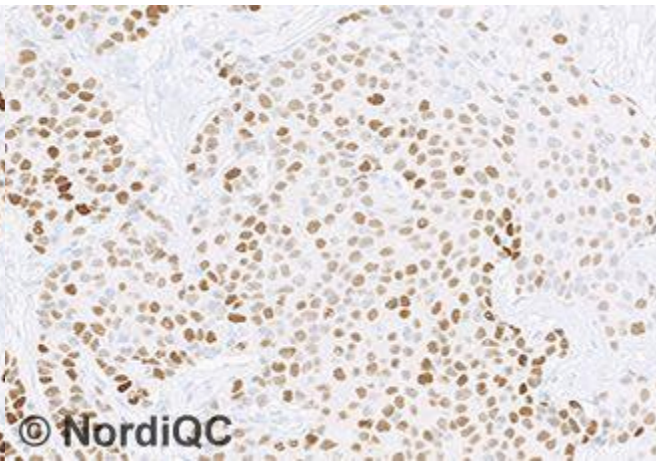


Fig. 2b
Staining for PR of the breast carcinoma no. 5 with 80 - 100% cells positive using same protocol as in Fig. 1b. - same field as in Fig. 2a.
A weak to strong distinct nuclear staining reaction in virtually all neoplastic cells is seen.
However also compare with Fig. 3b – same protocol.

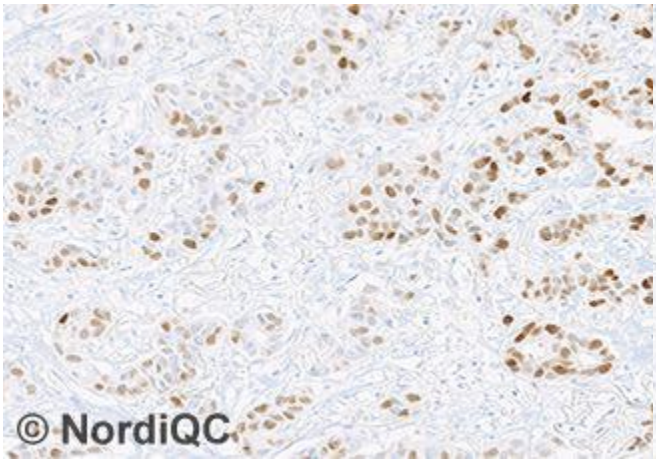


Fig. 3a
Optimal staining for PR of the breast carcinoma no. 4 with 40 - 60% cells positive using same protocol as in Figs. 1a - 2a.
The PR positive cells are easily recognized and the appropriate proportion of cells is demonstrated.

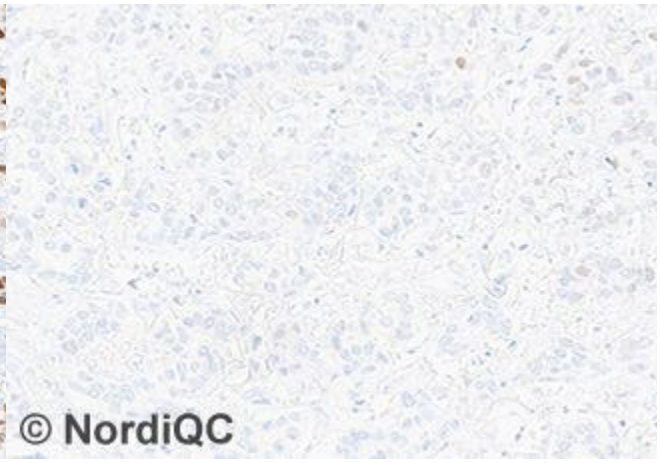


Fig. 3b
Insufficient staining for PR of the breast carcinoma no. 4 with 40 - 60% cells positive using same protocol as in Figs. 1b – 2b – same field as in Fig. 3a.
Only dispersed cells are demonstrated and a significant reduced proportion of cells are identified compared to the level expected.

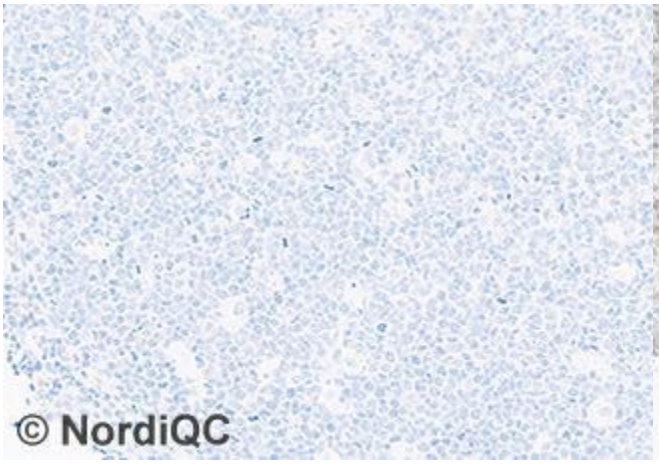


Fig. 4a
Optimal staining for PR of tonsil using same protocol as in Figs. 1a - 3a.
No nuclear staining reaction is seen.
This staining pattern was consistently seen for protocols based on mmAb clone 1A6, 16, PgR 636 and PgR 1294 irrespective of protocol settings applied.

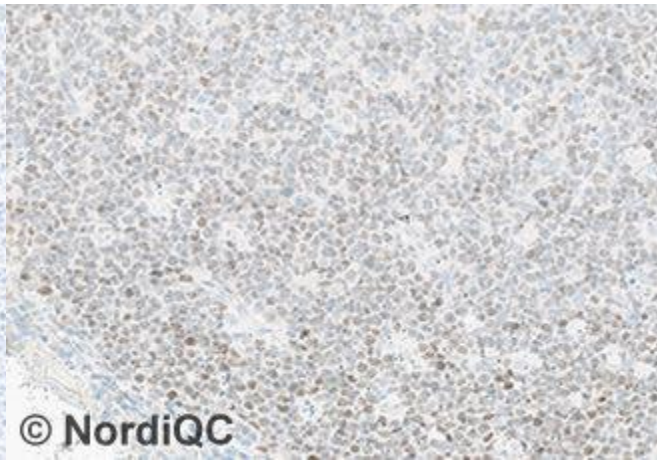


Fig. 4b
Insufficient staining for PR of tonsil – same field as in Fig. 4a.
The majority of germinal cells show a weak to moderate and aberrant false positive nuclear staining reaction.
This aberrant staining reaction was only seen for rmAb clones 1E2 (RTU, Ventana) and SP2.
For rmAb clone 1E2 prolonged antibody incubation time in combination with a reduced HIER time compared to the recommendations provided by Ventana seemed to enhance the aberrant staining pattern. In case of aberrant positive nuclear staining reaction in tonsil and otherwise an expected staining pattern in the other tissues was seen, the result was evaluated as borderline. In case also an aberrant and false positive staining in the PR negative breast carcinoma was observed, the result was assessed as poor – see Fig. 5b.

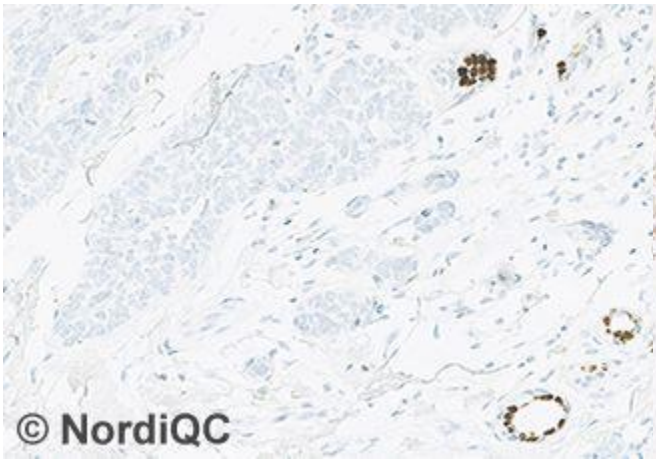


Fig. 5a
Optimal staining for PR of the breast carcinoma no. 3 expected to be negative using same protocol as in Figs. 1a - 4a.
No nuclear staining reaction in the neoplastic cells is seen. The PR status was tested and confirmed by different Abs and protocol settings in the NordiQC reference laboratories.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

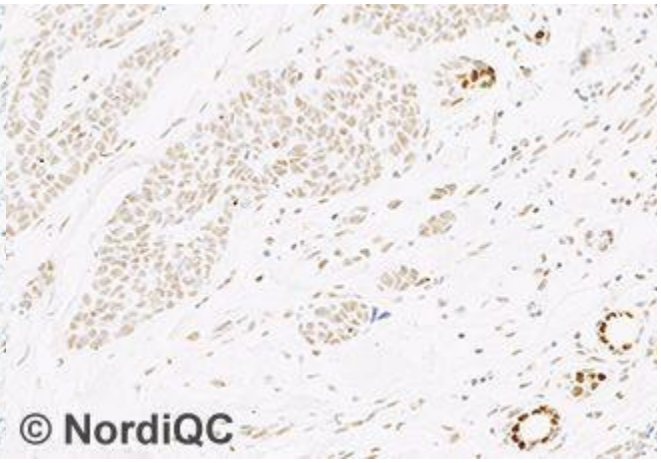


Fig. 5b
Insufficient staining for PR of the breast ductal carcinoma no. 3 expected to be negative – same field as in Fig. 5a.
Virtually all neoplastic cells show a weak to moderate and aberrant false positive nuclear staining reaction.
The protocol was based on the rmAb clone SP2, using HIER in an alkaline buffer and a 2-step polymer based detection system.

SN/LE/MV/RR 11.12.2015