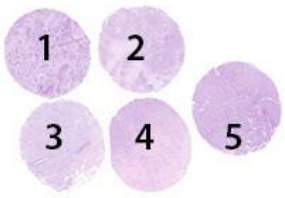
Assessment Run B26 2018

HER2 IHC

Material

The slide to be stained for HER2 comprised the following 5 materials:

	IHC: HER2 Score* (0, 1+, 2+, 3+)	FISH: HER2 gene/chr 17 ratio**
1. Breast carcinoma, no. 1	2+	1.1 – 1.3 (unamplified)
2. Breast carcinoma, no. 2	3+	> 6.0 (clusters) (amplified)
3. Breast carcinoma, no. 3	2+	3.0 – 3.2 (amplified)
4. Breast carcinoma, no. 4	3+	> 6.0 (clusters) (amplified)
5. Breast carcinoma, no. 5	0-1+	0.9 – 1.1 (unamplified)



* HER2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits and antibodies, HercepTest™ (Dako) and PATHWAY® (Ventana), in NordiQC reference laboratories.
** HER2 gene/chromosome 17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)

All carcinomas were fixed for 24 - 48 h in 10% neutral buffered formalin.

IHC scoring system according to the 2013 ASCO/CAP guidelines

Score 0	No staining is observed or incomplete membrane staining is observed in ≤ 10% of the tumour cells.
Score 1+	A faint perceptible and incomplete membrane staining is observed in more than 10% of the tumour cells.
Score 2+	A weak to moderate circumferential incomplete membrane staining is observed in more than 10% of the tumour cells or an intense circumferential complete membranous staining in ≤ 10% of the tumour cells.
Score 3+	An intense circumferential complete membrane staining is observed in more than 10% of the tumour cells.

Criteria for assessing a HER2 staining as **optimal** were:

- Staining corresponding to score 0 or 1+ in carcinomas no. 5.
- Staining corresponding to score 1+ or 2+ in carcinoma no. 1.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 3.
- Staining corresponding to score 3+ in carcinoma no. 2 and 4.
- No or only weak cytoplasmic reaction that did not interfere with the interpretation.

Staining was assessed as **good**, if (1) the HER2 gene amplified tumour no. 2 and 4 showed a 2+ reaction and the other breast carcinomas showed reaction pattern as described above (equivocal 2+ IHC staining should always be analyzed by ISH according to the ASCO/CAP guidelines) ***or*** (2) the HER2 0/1+ gene non-amplified tumour no. 5 showed a 2+ reaction and the other breast carcinomas showed the expected reaction pattern ***or*** (3) the HER2 2+ gene non-amplified tumor no. 1 showed a 0 reaction.

Staining was assessed as **borderline,** if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive counterstaining or excessive retrieval hampering the interpretation.

Staining was assessed as **poor** in case of a false negative staining (e.g., the 3+ tumour or the 2+ tumour with gene amplification showed a 0 or 1+ reaction) or a false positive staining (e.g., the 0/1+ tumors and the 2+ tumour without gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B25	354
Number of laboratories returning slides	335 (95%)

One laboratory stained the HER2 IHC slide with an ISH-assay. Data will not be included the results below.

Results: 334 laboratories participated in this assessment and 76% achieved a sufficient mark (optimal or good). Assessment marks for IHC HER2 assays and HER2 antibodies are summarized in Table 1.

Table 1. Assessment marks for **IHC assays and antibodies run B26, HER2 IHC**

FDA approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	192	Ventana/Roche	161	5	-	26	86%	86%
PATHWAY® rmAb clone **4B5, 790-2991**[4]	3	Ventana/Roche	2	-	-	1	-	-
rmAb clone **4B5, 790-4493**	21	Ventana/Roche	18	-	-	3	86%	90%
HercepTest™ **SK001**	26	Dako/Agilent	13	-	-	13	50%	59%
HercepTest™ **SK001**[5]	5	Dako/Agilent	3	1	-	1	80%	-
Oracle™ mAb clone **CB11, TA9145**	7	Leica	-	-	-	7	0%	-
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **BSR44**	1	Nordic Biosite	1	-	-	-	-	-
mAb clone **CB11**	5 1	Leica/Novocastra Biogenex	3	1	1	1	67%	100%
rmAB clone **EP1045Y**	1	ThermoFisher Scientific	1	-	-	-	-	-
pAb, A0485	43	Dako/Agilent	18	4	2	19	51%	55%
rmAb clone **RM228**	1	RevMAB Bioscience	1	-	-	-	-	-
rmAb clone **SP3**	10 5 2 2 1	ThermoFisher Scientific Cell Marque Zytomed Spring Biosystems Invitrogen	13	4	-	3	85%	94%
rmAb clone **EP3**	1 1	Cell Marque Diagnostic BioSystems	2	-	-	-	-	-
rmAb clone **A24-V**	1	DB Biotech	-	-	1	-	-	-
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11, PA0983**	1	Leica	1	-	-	-	-	-
Ab clone **MXR001, RMA-0701**	1	Maixin	1	-	-	-	-	-
rmAb clone **SP3, 237R-17/18**	2	Cell Marque	-	-	-	2	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	-	-	-	1	-	-
Total	334		238	15	4	77	-	-
Proportion			71%	4%	1%	23%	76%	-

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.
4) RTU system developed for the Roche/Ventana's fully automated systems (BenchMark) but used by laboratories on different platforms (e.g. Leica Bond)
5) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Leica Bond and Dako Omnis)

Detailed Analysis
FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): 161 of 192 (84%) protocols were assessed as optimal. Protocols with optimal results were typically based on heat induced epitope retrieval (HIER) in Cell Conditioning 1 (CC1) (efficient heating time 16-64 min.) on BenchMark XT, GX or Ultra, 12-60 min. incubation of the primary Ab and iView, UltraView with or without UltraView/iView Amplification Kit or OptiView as detection kit. Using these protocol settings, 156 of 181 (86%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **4B5** (790-4493, Ventana/Roche): 18 of 21 (86%) stains were assessed as optimal. Protocols with optimal results were based on HIER in CC1 (efficient heating time 24-64 min.) on BenchMark XT, GT or Ultra, 12-40 min. incubation of the primary Ab and iView, UltraView or OptiView as detection system. Using these protocol settings, 18 of 20 (90%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): 13 of 26 (50%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 40 min. in a water bath or PT Link and 30 min. incubation of the primary Ab. Using these protocol settings, 13 of 22 (59%) laboratories produced a sufficient staining result.

Table 2 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 systems changing basal protocol settings. Only protocols performed on the specific IHC stainer device are included.

Table 2. **Comparison of pass rates for vendor recommended and laboratory modified protocols**

CDx assay	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra PATHWAY® rmAb 4B5 **790-2991**	42/55 (76%)	40/55 (73%)	124/137 (91%)	121/137 (88%)
Ventana BenchMark XT, GX, Ultra rmAb 4B5, **790-4493**	3/6 (50%)	3/6 (50%)	15/15 (100%)	15/15 (100%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	13/22 (59%)	13/22 (59%)	0/4	0/4
Leica Bond MAX, III Oracle™ mAb CB11 **TA9145**	0/4	0/4	0/3	0/3

* 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.

Concentrated antibodies for laboratory developed (LD) assays
pAb, A0485: 18 of 43 (42%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH 6.1 (Dako) (5/11*), TRS pH 9 (3-in-1) (Dako) (3/7), TRS High pH (Dako) (2/2), CC1 (Ventana) (3/3), BERS2 (Leica) (2/2) or unknown (1/3). The pAb A0485 was typically diluted in the range of 1:200-1,500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 22 of 40 (55%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP3**: 13 of 20 (65%) protocols were assessed as optimal. Optimal protocols were based on HIER using TRS High pH (Dako) (2/4), TRS pH 9 (3-in-1) (Dako) (1/2), BERS2 (Leica) (6/8) or CC1 (Ventana) (4/4). The rmAb clone SP3 was diluted 1:40-200 depending on the total sensitivity of the protocol employed. Using this protocol setting, 16 of 17 (94%) laboratories produced a sufficient staining result.

mAb clone **CB11**: 3 of 6 (50%) protocols were assessed as optimal. Optimal protocols were based on HIER using BERS2 (Leica) (1/1) or citrate buffer pH 6.0 (2/2). The mAb clone CB11 was diluted 1:100-300. Using these protocol settings, 3 of 3 laboratories produced a sufficient result.

Table 3 summarizes the overall proportion of optimal staining results when using the three most frequently used concentrated Abs on the most commonly used IHC stainer platforms.

Table 3. **Optimal results for HER2 for the most commonly used antibodies as concentrate 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
pAb clone **A0485**	3/7** (43%)	2/13 (15%)	2/2	5/11 (45%)	3/3	-	2/2	-
rmAb clone **SP3**	1/2	-	2/4	-	4/4	-	6/8 (75%)	-
mAb clone **CB11**	-	-	-	-	-	-	1/1	0/2

* 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)

Comments
In this assessment, the insufficient results were typically characterized by a false negative staining reaction, 75 of 81 (93%). Virtually all laboratories were able to demonstrate a HER2 3+ staining reaction in the tissue cores no. 2 and 4. Demonstration of HER2 in tissue core no. 3, was much more difficult and challenged the diagnostic sensitivity of Abs and protocols applied. Tissue core no. 3 was categorized as IHC 2+ in the NordiQC reference laboratories using two FDA/CE-IVD HER2 IHC assays: PATHWAY® (Ventana) and HercepTest™ (Dako) and showed HER2 gene amplification (ratio 3.0 – 3.2) by FISH.
6% (5 of 81) of the insufficient results were caused by poor signal-to-noise ratio or excessive background complicating interpretation. One laboratory had a contamination with a nuclear marker, most likely ER, which interfered with the interpretation of HER2. No false positive membranous staining reaction were seen.

Insufficient staining results were seen in both laboratory developed (LD) assays and FDA-/CE-IVD approved assays. For LD assays the prevalent feature for insufficient results were caused by too low concentration of the primary Ab or insufficient HIER.
For the FDA-/CE-IVD approved assays no general cause of insufficient staining results could be identified. 91% (223 of 246) used optimal protocol settings, of which 84% (187 of 223) obtained a sufficient staining result.

The Ventana PATHWAY® HER2 IHC assay was increasingly modified by the participants. The most common modification observed was a prolonged incubation time of the primary Ab. 119 laboratories incubated for ≥20 min and 91% (108 of 119) obtained a sufficient result.
12 laboratories applied OptiView as detection system and not UltraView or iView as recommended by Ventana, all with optimal results. In contrast, internal studies previously performed in the NordiQC reference laboratory indicated a less precise and robust HER2 IHC assay if UltraView was substituted by OptiView. OptiView will typically amplify the analytical sensitivity of the IHC system 3-4 times compared to UltraView. Consequently if OptiView is applied, the HER2 IHC assay must be adjusted at other parameters e.g incubation time of the primary Ab or HIER settings to provide the analytical sensitivity level validated by Ventana, which, as mentioned, can cause a less precise and robust assay.

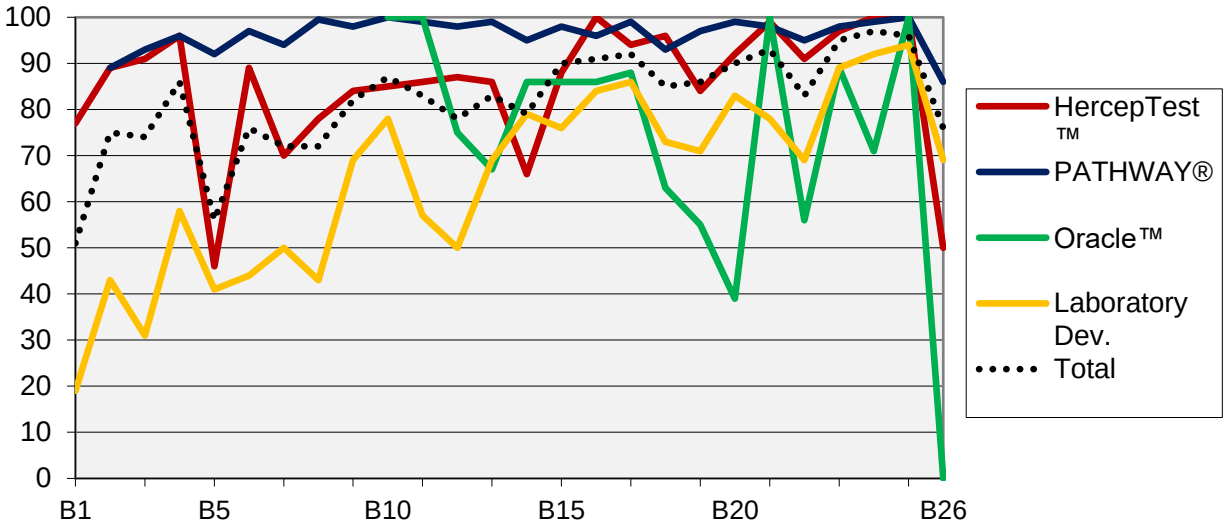
The Dako HercepTest™ assay provided the lowest pass rate, 50% (13 of 26), since run B5. Four laboratories modified the protocol unsuccessfully. Using the recommended protocol from Dako, a pass rate of 59% (13 of 22) was obtained. No obvious cause for the low pass rate could be identified since no lot-to-lot variation was detected.

In this HER2 assessment also the LD assays provided a lower pass rate, 64% (56 of 88), than in the previous runs. rmAb clone SP3 was overall the most successful clone. If optimal protocol settings was applied, a pass rate of 94% was obtained. The mAb clone CB11 provided an overall pass rate 67%, and if applying optimal protocol settings, a 100% pass rate was obtained.

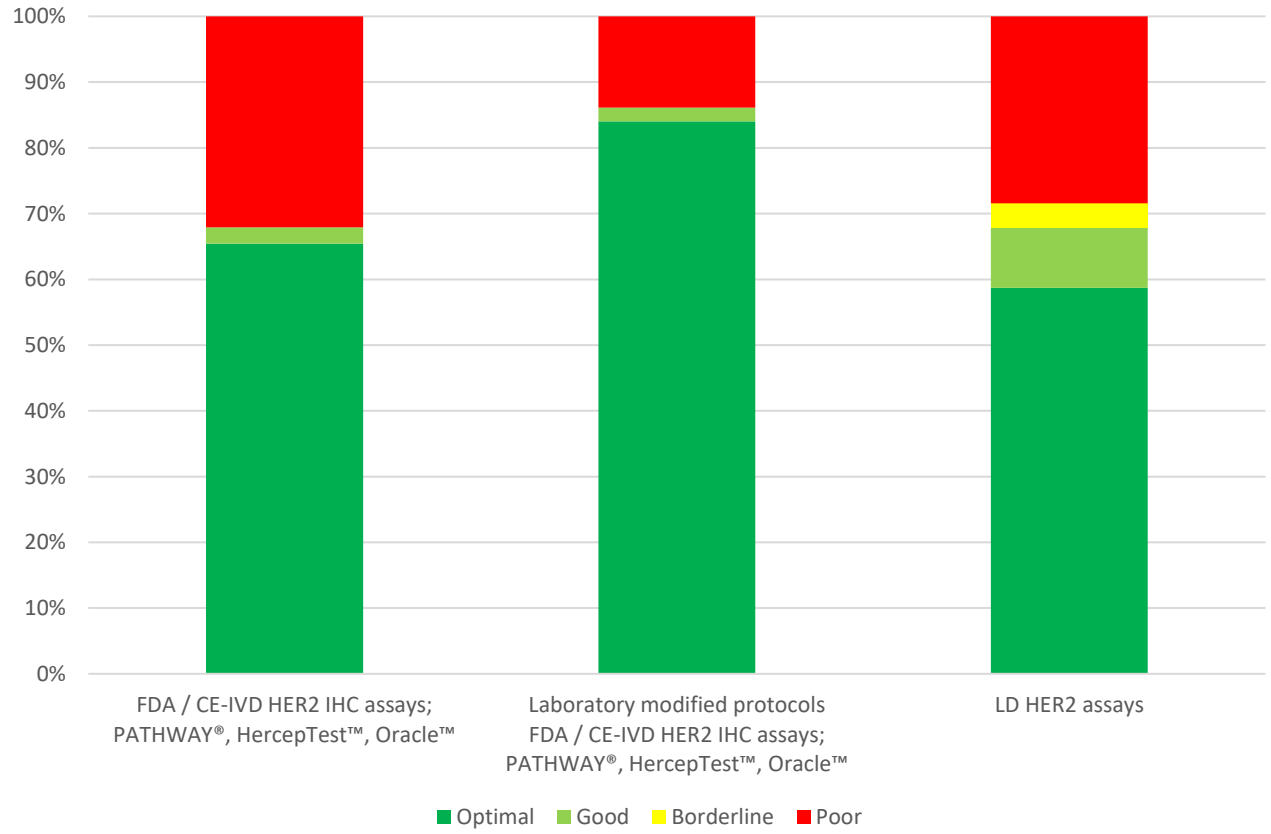
In this assessment, the FDA-/CE-IVD approved HER2 IHC assay PATHWAY® was the most successful and provided a high pass rate superior to both HercepTest™ and Oracle™, from Dako and Leica respectively, and LD assays as illustrated in Graph 1.
The proportion of laboratories using FDA-/CE-IVD approved HER2 IHC assays and LD assays is very consistent. In this run, 26% of the participants (n=88) used LD assays compared to 23-31% in the last 14 assessments.

The laboratory modified protocols obtained both a higher pass rate and an increased number of optimal results, compared to laboratories using vendor recommended protocols for the FDA/IVD-CE approved assays as illustrated in Graph 2. However, despite the encouraging results, modifications must be meticulously validated by the end-users on a large cohort of breast carcinomas (n=100, ASCO/CAP 2013 guidelines).

Graph 1. **Pass rates of 26 HER2 IHC assessments in the NordiQC breast cancer module**



Graph 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**

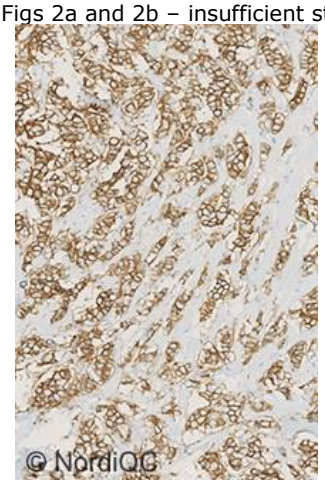


Performance history
The overall pass rate of 76% obtained in this assessment is a significantly decrease compared to previous assessments.
The decrease of sufficient results can be caused by many factors. The circulated material, especially tissue core no. 3, 2+ with gene amplification, challenged the diagnostic sensitivity of the Abs and protocols applied on a higher level than in the previous assessments, and might be the main reason for the low pass rate.
No obvious cause to the decreased pass rate could be identified in the submitted data. Day-to-day variation of the performance – due to e.g. period since last maintenance, fading Abs or reagents, could be the reason for the different scores for similar protocol settings.

Scoring consensus B26
Laboratories were requested to submit scores (0, 1+, 2+, 3+) of their own HER2 stained slides. This was done by 86% (289 of 335) of the participants returning slides.
For 204 of the 289 (71%) responding participants, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group using the ASCO/CAP 2013 interpretation guidelines. This was similar to run B25, where 71% of the scores were in consensus with the NordiQC assessor group. Among laboratories with sufficient staining, 66% (147 of 223) of interpretations were in agreement with the NordiQC assessors. Typically, the laboratories had interpreted the core no 3. (2+) as 1+. Among participants with insufficient staining, 86% were in consensus with the NordiQC assessor group (57 of 66).

Conclusion
The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/CONFIRM™** rmAb clone 4B5 (Ventana) was in this assessment the most precise assay for the semi-quantitative IHC determination of HER2 protein expression. **HercepTest™** (Dako), **Oracle**™ (Leica) and Laboratory developed assays produced a lower pass-rate and were less precise for the HER2 status, requiring an additional ISH test for final evaluation. Inclusion of 2+ tumours with and without HER2 gene amplification in the control material for both EQA and internal quality control is essential to evaluate precision and performance stability of the IHC HER2 assays used by laboratories. Tissues included in this run challenged many laboratories and is close to the diagnostic sensitivity of most HER2 assays.

Figs 1a and 1b – optimal staining results, same protocol
Figs 2a and 2b – insufficient staining results - false negative, same protocol



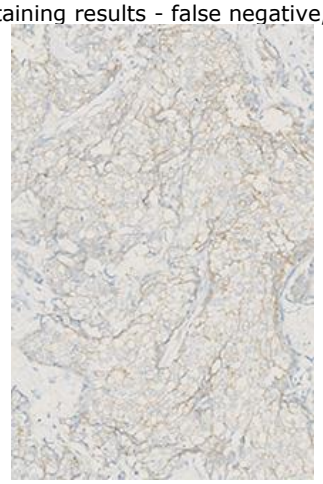
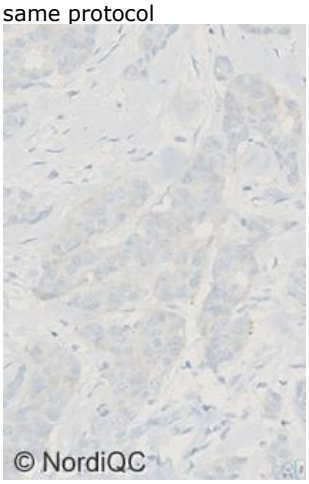


Fig 1a.
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr17 of 3.0 – 3.2.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



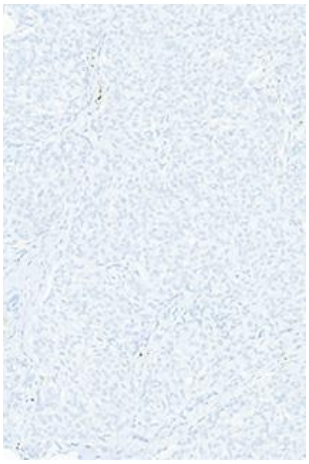
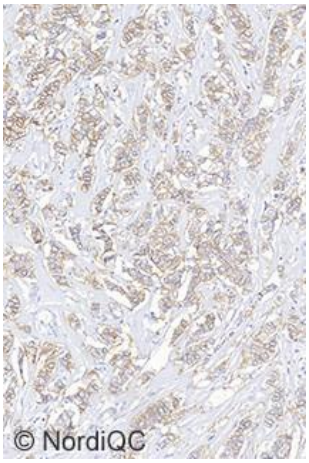


Fig 1b.
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / chr17 of 1.1 – 1.3.
> 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / chr17 ratio of 0.9 – 1.1.
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.



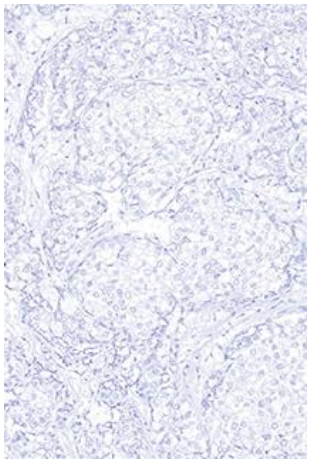
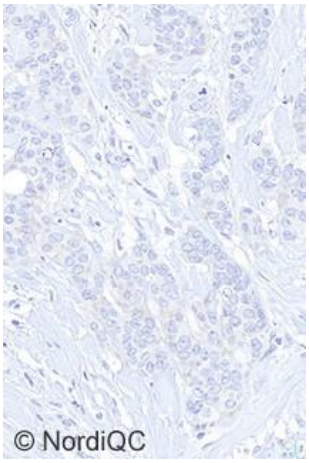


Fig 2a.
Left: Insufficient staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of > 6.0.
> 10% of the neoplastic cells show faint membranous staining corresponding to 2+
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of 3.0 – 3.2
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.



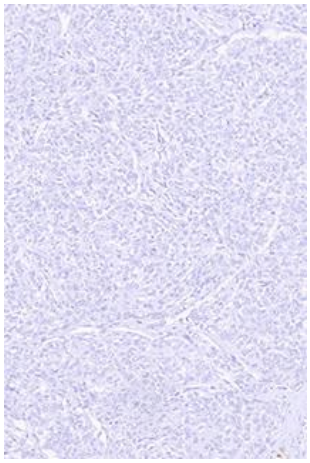


Fig 2b.
Left: Insufficient Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / chr17 of 1.1 – 1.3.
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / chr17 ratio of 0.9 – 1.1.
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.

HLK/LE/MV/RR 08.12.2018