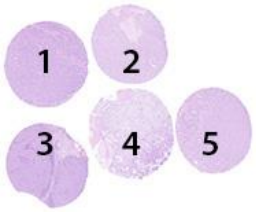
Assessment Run B27 2019

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	0-1+	1.1 – 1.3 (unamplified)
2. Breast carcinoma, no. 2	3+	> 6.0 (clusters) (amplified)
3. Breast carcinoma, no. 3	2+	1.5 – 1.8 (unamplified)
4. Breast carcinoma, no. 4	2+	3.1 – 3.7 (amplified)
5. Breast carcinoma, no. 5	3+	> 6.0 (clusters) (amplified)



* 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 2018 ASCOP guidelines:

Score 0	No staining is observed **or** membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells.
Score 1+	Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells.
Score 2+	Weak to moderate complete membrane staining observed in >10% of tumor cells.
Score 3+	Circumferential membrane staining that is complete, intense, and in >10% of tumor cells*.

**Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.*

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

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

Staining was assessed as **good**, if (1) the HER2 gene amplified tumours no. 2 and 5 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. 1 showed a 2+ reaction and the other breast carcinomas showed the expected reaction pattern.

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 B27	337
Number of laboratories returning slides	325 (96%)

One laboratory stained the HER2 IHC slide with ER and was not included in the results below.

Results: 324 laboratories participated in this assessment and 86% 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 B27, HER2 IHC**

FDA approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	191	Ventana/Roche	177	4	2	8	95%	95%
PATHWAY® rmAb clone **4B5, 790-2991[4]**	2	Ventana/Roche	1	-	-	1	-	-
rmAb clone **4B5, 790-4493**	14	Ventana/Roche	12	1	-	1	93%	92%
HercepTest™ **SK001**	24	Dako/Agilent	21	-	1	2	88%	87%
HercepTest™ **SK001[4]**	4	Dako/Agilent	3	1	-	-	-	-
Oracle™ mAb clone **CB11, TA9145**	9	Leica	7	-	-	2	78%	-
Oracle™ mAb clone **CB11, TA9145[4]**	1	Leica	-	-	-	1	-	-
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	2	2	-	2	67%	-
mAb clone **C1F7**	1	Celnovte	1	-	-	-	-	-
rmAB clone **EP1045Y**	1	ThermoFisher Scientific	1	-	-	-	-	-
pAb, A0485	44	Dako/Agilent	33	1	2	8	77%	77%
rmAb clone **SP3**	9 6 3 1	ThermoFisher Scientific Cell Marque Zytomed Spring Biosystems	5	-	1	13	26%	50%
rmAb clone **EP3**	3	Cell Marque Diagnostic BioSystems	1	1	-	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 **EP3, 237R-17/18**	1	Cell Marque	1	-	-	-	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	1	-	-	-	-	-
Total	324		268	10	6	40	-	-
Proportion			83%	3%	2%	12%	86%	-

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 used on a different platform than it was developed for.

Detailed Analysis
FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): 177 of 191 (93%) 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 or UltraView with or without UltraView/iView Amplification Kit or OptiView as detection kit. Using these protocol settings, 171 of 181 (95%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **4B5** (790-4493, Ventana/Roche): 12 of 14 (86%) stains were assessed as optimal. Protocols with optimal results were based on HIER in CC1 (efficient heating time 24-48 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView or OptiView as detection system. Using these protocol settings, 12 of 13 (95%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): 21 of 24 (88%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 10-40 min. in a water bath or PT Link and 20-30 min. incubation of the primary Ab. Using these protocol settings, 20 of 23 (92%) 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**	54/61 (89%)	53/61 (87%)	127/130 (98%)	124/130 (95%)
Ventana BenchMark XT, GX, Ultra rmAb 4B5, **790-4493**	2/2 (100%)	2/2 (100%)	11/12 (92%)	10/12 (83%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	18/21 (86%)	18/21 (86%)	3/3	3/3
Leica Bond MAX, III Oracle™ mAb CB11 **TA9145**	4/4	4/4	3/5	3/5

* 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: 33 of 44 (75%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH (Dako) (10/13*), TRS pH 6.1 (3-in-1) (Dako) (11/16), TRS pH 9 (3-in-1) (Dako) (4/6), TRS High pH (Dako) (2/2), CC1 (Ventana) (2/2), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/2), Novocastra Epitope Retrieval Solutions pH 6 (Leica) (1/1) or citrate buffer (1/2). The pAb A0485 was typically diluted in the range of 1:100-1,500 with either a 2-layer detection system (25/35) or a 3-layer detection system (8/9). Using these protocol settings, 34 of 44 (77%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP3**: 5 of 19 (26%) protocols were assessed as optimal. Optimal protocols were based on HIER using TRS High pH (Dako) (1/4), TRS pH 9 (3-in-1) (Dako) (1/2), BERS2 (Leica) (2/8) or CC1 (Ventana) (1/4). The rmAb clone SP3 was diluted 1:40-80 with either a 2 layer detection system (3/8) or 3-layer detection system (2/11). Using these protocol settings, 5 of 10 (50%) laboratories produced a sufficient staining result.

Table 3 summarizes the overall proportion of optimal staining results when using the two 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**	4/6**	11/16 (69%)	2/2	10/13 (77%)	2/2	-	2/2	-
rmAb clone **SP3**	1/2	-	1/4	-	1/4	-	2/8	-

* 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. This pattern was seen in 33 of 46 (72%) of the insufficient results. Virtually all laboratories were able to demonstrate HER2 3+ staining reaction in the tissue cores no. 2 and 5. False negative staining results were particularly and most critically observed as 0/1+ IHC reaction in the HER2 gene amplified breast carcinoma (tumour no. 4). This tumour 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 a high level of HER2 gene amplification (ratio 3.1-3.7) by FISH.
The remaining insufficient results were based on either false positive staining results (6/46) or poor signal-to-noise ratio/impaired morphology/technical issue (7/46).

Insufficient staining results were seen in both laboratory developed (LD) assays and FDA-/CE-IVD approved assays. For LD assays the prevalent features of insufficient results were 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. 94% (224 of 238) used optimal protocol settings, of which 94% (210 of 224) obtained a sufficient staining result.

The Ventana PATHWAY® HER2 IHC assay was increasingly modified by the participants. The most common modification observed was prolonged incubation of the primary Ab. 119 laboratories incubated for ≥20 min and 98% (117 of 119) obtained a sufficient result.
12 laboratories applied OptiView as detection system and not UltraView or iView as recommended by Ventana, 82% (10 of 12) with optimal results. In contrast, internal studies previously performed in the NordiQC reference laboratory indicated a less 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.

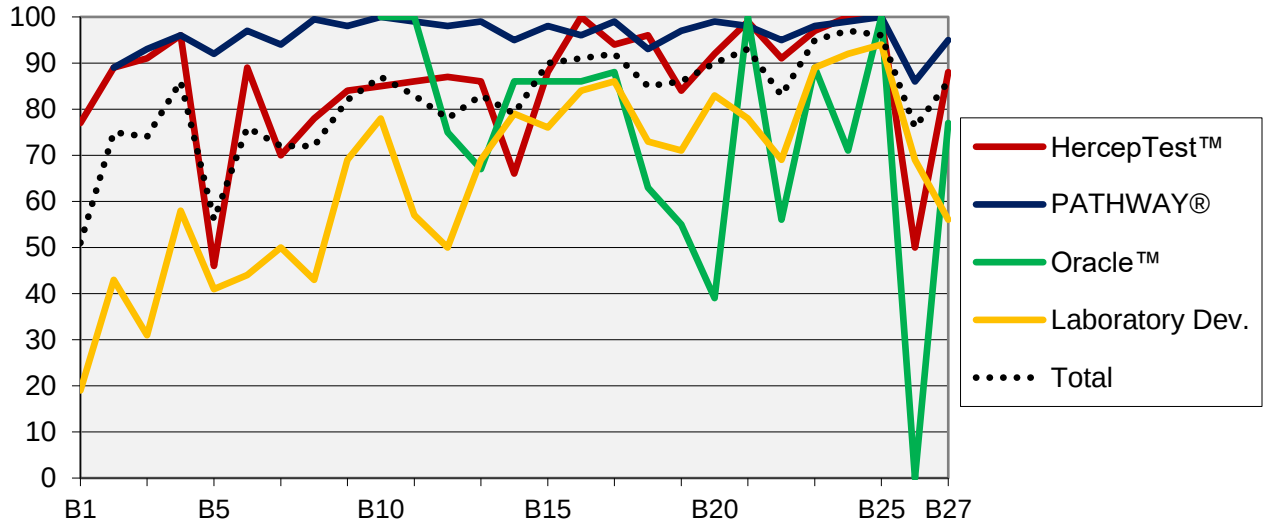
The Dako HercepTest™ assay provided a pass rate of 88% (21 of 24). Using the recommended protocol settings from Dako, a pass rate of 86% (18 of 21) was obtained. Three laboratories modified the protocol adjusting HIER time, all with optimal results.

In this HER2 assessment, LD assays provided a significant lower pass rate of 65% (56 of 86) compared to the FDA-/CE-IVD approved assays. pAb A0485 from Dako was the most successful concentrate. If optimal protocol settings was applied, a pass rate of 77% was obtained. The mAb clone CB11 provided an overall pass rate 67%.

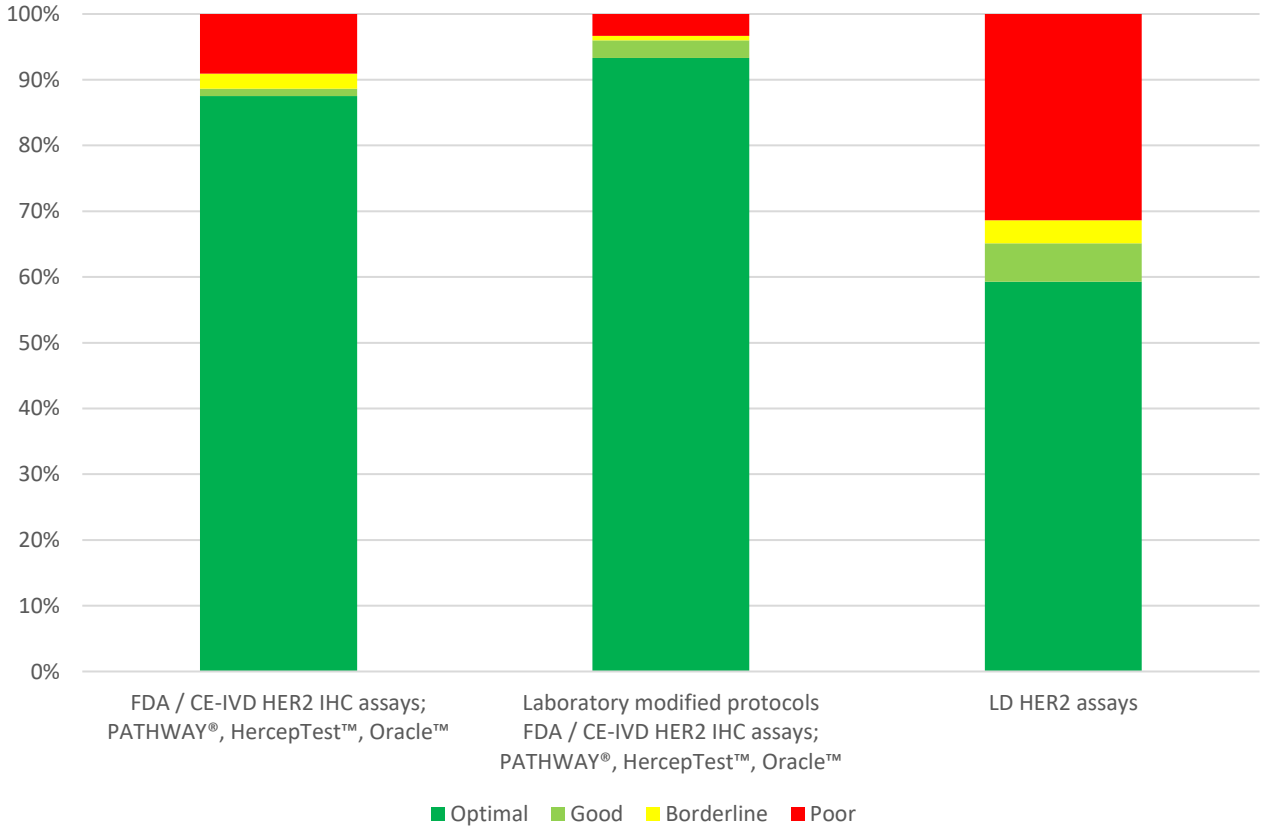
In this assessment, the FDA-/CE-IVD approved HER2 IHC assay PATHWAY® was the most successful and provided a high pass rate superior to both HercepTest™ and Oracle™, from Dako and Leica respectively, and LD assays as illustrated in Graph 1.
The proportion of laboratories using FDA-/CE-IVD approved HER2 IHC assays and LD assays is very consistent. In this run, 27% of the participants (n=86) used LD assays compared to 23-31% in the last 15 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 Ventana PATHWAY® and Dako HercepTest™ 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). As shown in Graph 2, LD HER2 assays both provided a reduced proportion of sufficient

results but also a shift from optimal to good, typically caused by 2+ staining reaction in the HER2 non-amplified tumour (no. 1) expected to show a 0/1+ staining reaction. The staining reaction of 2+ in this tumour would not directly lead to a wrong diagnosis but require an additional ISH test due to the less precise IHC result.

Graph 1. **Pass rates of 27 HER2 IHC assessments in the NordiQC breast cancer module**



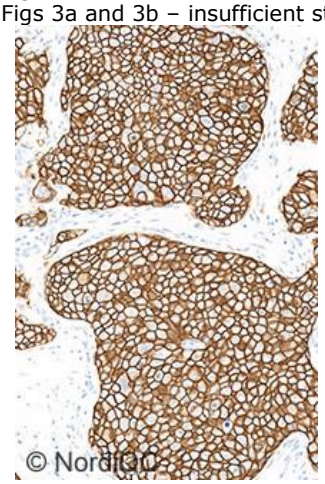
Graph 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**



Scoring consensus B27
Laboratories were requested to submit scores (0, 1+, 2+, 3+) of their own HER2 stained slides. This was done by 85% (276 of 324) of the participants returning slides.
For 211 of the 276 (76%) responding participants, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group using the ASCO/CAP 2018 interpretation guidelines. This was similar to run B26, where 71% of the scores were in consensus with the NordiQC assessor group. Among laboratories with sufficient staining, 78% (185 of 236) of interpretations were in agreement with the NordiQC assessors. Among participants with insufficient staining, 65% were in consensus with the NordiQC assessor group (26 of 40).

Conclusion
The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/CONFIRM™** rmAb clone 4B5 (Ventana) and **HercepTest™** (Dako) were in this assessment the most precise assays for the semi-quantitative IHC determination of HER2 protein expression. 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.

Figs 1a and 1b – optimal staining results, same protocol
Figs 2a and 2b – insufficient staining results - false negative, same protocol – inefficient HIER
Figs 3a and 3b – insufficient staining results – false positive, same protocol – using OptiView as detection system.



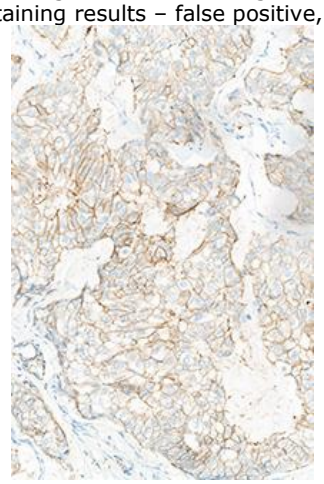
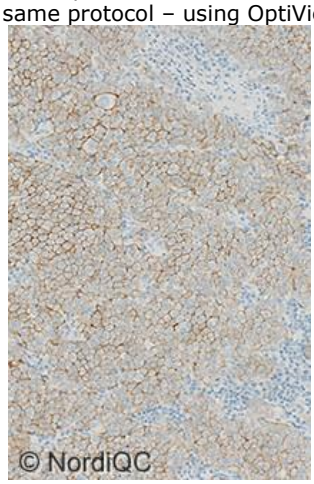


Fig 1a.
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of 3.1-3.7.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



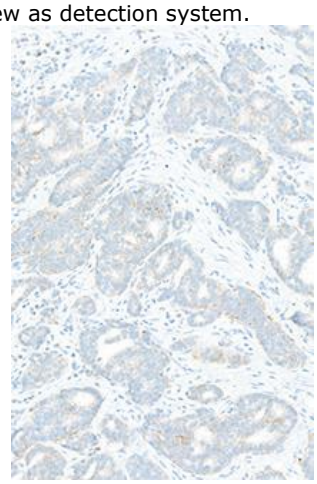
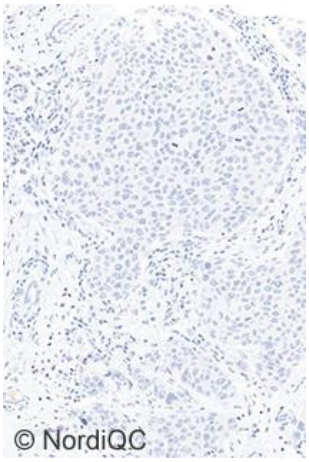


Fig 1b.
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr17 of 1.5-1.8.
> 10% of the neoplastic cells show a weak-moderate membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a HER-2 / chr17 ratio of 1.3-1.5.
< 10% of the neoplastic cells show a faint, incomplete membranous staining reaction corresponding to 0.



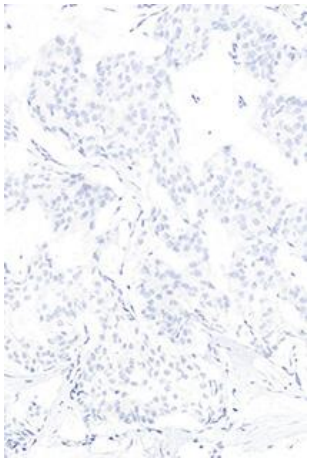
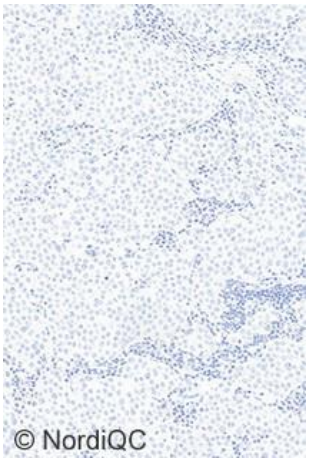


Fig 2a.
Left: Insufficient staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / chr17 of > 6.0.
Virtually all neoplastic cells are negative corresponding to 0.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of 3.1-3.7
Virtually all neoplastic cells are negative corresponding to 0.



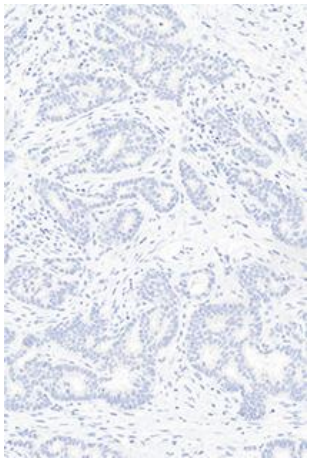
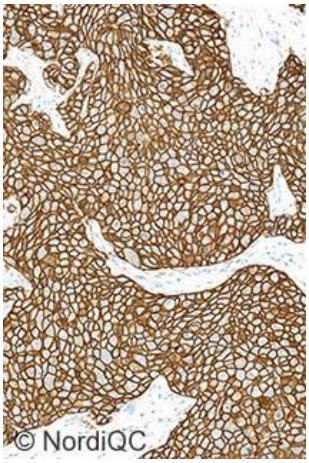


Fig 2b.
Left: Insufficient Staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr17 of 1.5-1.8.
Virtually all neoplastic cells are negative corresponding to 0.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a HER-2 / chr17 ratio of 1.3-1.5.
Virtually all neoplastic cells are negative corresponding to 0.



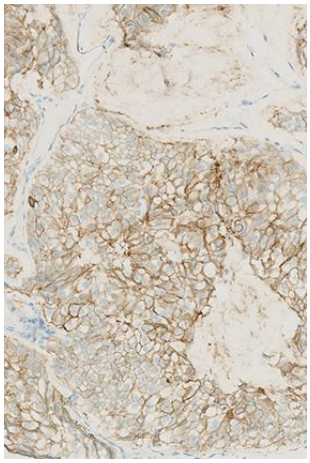
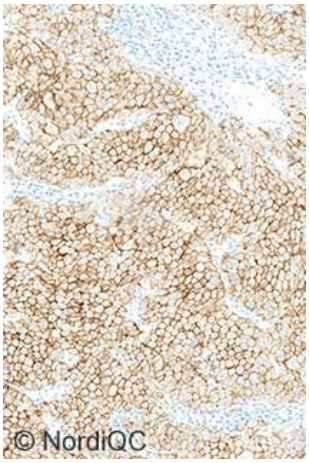


Fig 3a.
Left: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr17 of 3.1-3.7
> 10% of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+.



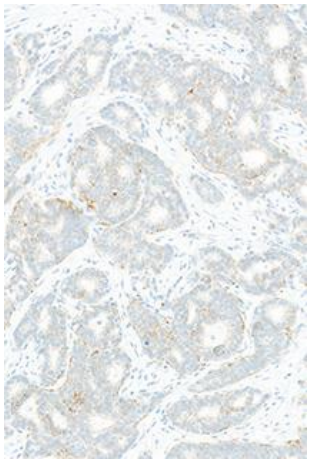


Fig 3b.
Left: Insufficient and false positive staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr17 of 1.5-1.8.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a HER-2 / chr17 ratio of 1.3-1.5.
> 10% of the neoplastic cells show a weak membranous staining reaction corresponding to 1+.

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