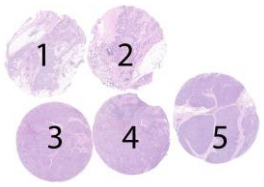
Assessment Run B22 2016

HER-2 IHC

Material

The slide to be stained for HER-2 comprised the following 5 tissues:

	IHC: HER-2 Score* (0, 1+, 2+, 3+)	FISH: HER-2 gene/chr 17 ratio**
1.Breast carcinoma	2+	2.3 – 2.7 (amplified)
2.Breast carcinoma	1-2+	1.3 – 1.7 (unamplified)
3.Breast carcinoma	0-1+	1.1 – 1.5 (unamplified)
4.Breast carcinoma	3+	> 6.0 (clusters) (amplified)
5.Breast carcinoma	0-1+	0.9 – 1.2 (unamplified)



* HER-2 immunohistochemical score (see table below) as achieved using three FDA approved kits and antibodies (HercepTest™ Dako, Oracle™ Leica and PATHWAY® Ventana) in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 ratios achieved using Zyto*Light*® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)

All carcinomas had been 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 HER-2 staining as **optimal** were:

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

Staining was assessed as **good**, if (1) the HER-2 gene amplified tumour no. 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 HER-2 gene non-amplified tumour no. 3 and/or 5 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 B22	410
Number of laboratories returning slides	387 (94%)

Results: 387 laboratories participated in this assessment and 84% achieved a sufficient mark. Assessment marks for HER-2 IHC assays and HER-2 antibodies are summarized in table 1.

Table 1. **Assessment marks for IHC assays and antibodies run B22, HER-2 IHC**

FDA approved HER-2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	194	Ventana/Roche	165	18	1	10	94%	95%
CONFIRM™, rmAb clone **4B5, 790-4493**	16	Ventana/Roche	14	2	0	0	100%	100%
HercepTest™ **SK001**	47	Dako/Agilent	33	3	0	11	77%	90%
HercepTest™ **K5207**	2	Dako/Agilent	2	0	0	0	-	-
HercepTest™ **K5204**	5	Dako/Agilent	1	2	0	2	60%	-
Oracle™ mAb clone **CB11, TA9145**	9	Leica	1	4	0	4	56%	100%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **B24**	1	Nordic Biosite	0	0	0	1	-	-
mAb clone **CB11**	12 1	Leica/Novocastra Biogenex	0	3	6	4	-	-
mAb clone **UMAB36**	7	ZSBio	2	4	0	1	86%	80%
rmAb clone **EP1045Y**	1	Thermo/NeoMarkers	1	0	0	0	-	-
rmAb clone **EP3**	2 1	Biocare PathnSitu	1	1	0	1	-	-
rmAb clone **SP3**	16 4 2 1 1	Thermo/NeoMarkers Zytomed Cell Marque Immunologic Spring Bioscience	15	3	0	6	75%	81%
pAb clone **A0485**	60	Dako	36	8	0	16	73%	76%
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **EP3, AN726**	1	Biogenex	0	1	0	0	-	-
rmAb clone **EP3, 237R**	1	Cell Marque	0	1	0	0	-	-
rmAb clone **EP3, RMPD049R**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostics	1	0	0	0	-	-
Ab clone **MXR001, RMA-0701**	1	Maixin	1	0	0	0	-	-
Total	387		273	51	7	56	-	-
Proportion			71%	13%	2%	14%	84%	-

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.

Detailed Analysis
FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana): 165 of 183 (90%) 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 8-64 min.) in BenchMark XT, GX or Ultra, 8–60 min. incubation of the primary Ab and iVIEW, UltraView or OptiView as detection kit. Using these protocol settings, 183 of 193 (95%) laboratories produced a sufficient staining result (optimal or good).

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 14 of 16 (88%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 24-64 min.) in BenchMark XT, GX or Ultra, 16–44 min. incubation of the primary Ab and iVIEW, UltraView or OptiView as detection kit. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako): 33 of 47 (70%) protocols were assessed as optimal. Protocols with optimal results were typically 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, 27 of 30 (90%) laboratories produced a sufficient staining result.

HercepTest™ pAb (K5207, Dako): 2 of 2 (100%) protocols were assessed as optimal. Protocols with optimal result were typically 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, 2 of 2 (100%) laboratories produced a sufficient staining result.

Oracle™ mAb clone **CB11** (TA9145, Leica): 1 of 9 (11%) protocols was assessed as optimal.
The protocol with an optimal result was based on HIER in Bond Epitope Retrieval Solution 2 for 30 min. and 15 min. incubation of the primary Ab. These protocol settings were only used by one laboratory.

Concentrated antibodies for laboratory developed (LD) assays

mAb **UMAB36**: 2 of 7 (29%) protocols were assessed as optimal. Optimal protocols were based on HIER using Target Retrieval Solution (TRS) Low (Dako) (1/1)* or Tris-EDTA/EGTA pH 9 (1/4). The mAb clone UMAB36 was diluted 1:120. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient staining result.

rmAb **EP1045Y**: One protocol with an optimal result was based on HIER in pressure cooker using Tris-EDTA/EGTA pH 9. The rmAb clone EP1045Y was diluted 1:100 and used with Gene Tech GK6005 as detection system.

rmAb **EP3**: One protocol with an optimal result was based on HIER in pressure cooker using Tris-EDTA/EGTA pH 9. The rmAb clone EP3 was diluted 1:100 and used with NovoLink RE7280-K as detection system.

rmAb **SP3**: 15 of 24 (63%) protocols were assessed as optimal. These were based on HIER using TRS pH 9 (3-in-1) (Dako) (3/3), Cell Conditioning 1 (CC1) (BenchMark, Ventana) (3/7), Bond Epitope Retrieval Solution 2 pH 9 (BERS2, Leica) (7/7) or Citrate pH 6 (2/3). The rmAb clone SP3 was diluted in the range of 1:40-200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 17 of 21 (81%) laboratories produced a sufficient staining result.

pAb **A0485**: 36 of 60 (55%) protocols were assessed as optimal. These protocols were based on HIER using either TRS low pH 6.1 (Dako) 12/27), TRS pH 9 (3-in-1) (Dako) (10/12), CC1 (BenchMark, Ventana) (4/5), BERS1 (Bond, Leica) (3/4), BERS2 (Bond, Leica) (1/2), Citrate pH 6 (1/4) or unknown (5/6).
pAb A0485 was typically diluted in the range of 1:150-1,600 depending on the total sensitivity of the protocol employed. Using these protocol settings, 44 of 58 (76%) laboratories produced a sufficient staining result.

Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, insufficient HER-2 staining results were characterized by too weak or false negative staining reactions. This was particularly and most critically observed as 0/1+ IHC reaction in the low level HER-2 gene amplified breast carcinoma tissue core no. 1. This tumour was categorized as IHC 2+ in the NordiQC reference laboratories using the three FDA/CE-IVD HER-2 IHC assays (PATHWAY® (Ventana), HercepTest™ (Dako) and Oracle™ (Leica)) showing a low level of HER-2 gene amplification (ratio 2.3–2.7) by ISH. False negative staining reaction of the breast carcinoma no. 1 was seen in 83% of the insufficient results (52 of 63).
The remaining insufficient results were typically characterized by a poor signal-to-noise ratio, impaired morphology, excessive cytoplasmic staining reaction complicating the interpretation or false positive 3+ IHC staining in the HER-2 non-amplified tumours.
False negative results were seen both in laboratory developed (LD) and FDA-/CE-IVD approved assays, while false positive results only were seen in LD assays.
False negative results were, for the LD assays, typically caused by a too low analytical sensitivity of the protocol applied (e.g. too low concentration of the primary Ab, too short incubation time of the primary Ab and/or insufficient HIER).
For the FDA-/CE-IVD approved systems, used according to the official package inserts for the respective systems, no single cause for insufficient and false negative staining reactions could be identified from the protocols submitted.
In this assessment, several FDA-CE-IVD approved systems (but especially the Dako HercepTest™ system SK001 for Autostainer 48 Link) were used off-label on other staining platforms than the intended such as

the Leica Bond or Dako Omnis. This is problematic since the intended use is violated and would require an extended internal validation. In total, SK001 was performed off-label by 10 laboratories who, grouped together, obtained a pass rate 60%, significantly lower than protocol settings applied in compliance with the vendor recommendations (90%).

The Ventana PATHWAY® /CONFIRM™ HER2 IHC assay was also increasingly used off-label by participants applying OptiView as detection system and not UltraView or iView as recommended by Ventana. This assessment revealed no impact on the analytical sensitivity and specificity. In contrast, internal studies previously performed in the NordiQC reference laboratory indicate a less accurate and robust HER2 IHC assay if UltraView was substituted by OptiView for the PATHWAY® /CONFIRM™ HER2 IHC assay. OptiView will typically amplify the sensitivity of the IHC system 3-4x compared to level seen for UltraView, if all other parameters are maintained. Consequently, if OptiView is applied then other parameters of the assay (e.g incubation time, primary Ab or HIER settings) must be adjusted to provide the correct level of sensitivity. In the experience of NordiQC, this can as mentioned cause the assay to be less accurate and robust.

In this assessment, the FDA-/CE-IVD approved HER-2 IHC assays from Ventana and Dako, PATHWAY® /CONFIRM™ and HercepTest™, respectively, provided a higher pass-rate superior to LD assays as illustrated in Fig. 1. PATHWAY®/CONFIRM™ has provided a consistently high pass rate throughout all HER-2 IHC runs in NordiQC. The FDA/CE-IVD approved system Oracle™, Leica has shown an inferior consistency in the proportion of sufficient results in the latest NordiQC HER-2 IHC assessments. At present, no single cause for the inconsistency can be identified, and as only a relatively small number of participants used the Oracle™ system, no conclusions for the divergent pass rates can be drawn. In this run, the insufficient results for Oracle™ were characterized by false negative results in the breast carcinoma tissue core no. 1 with low-level HER2 amplification and expected 2+ score. In this context, it has to be emphasized that the material circulated has been processed according to the recommendations given by ASCO/CAP both concerning fixation time in 10% NBF, but also other central fixation and tissue processing conditions. The level of HER-2 protein expression throughout the circulated material is extensively validated by NordiQC and is in addition verified by FISH. Approximately every 50th slide is stained by NordiQC to serve as reference for the expected level of HER-2 IHC expression.

In this assessment the proportion of laboratories using LD assays increased compared to the last run. In this run, 32% of the participants (n=124) used LD assays compared to 23% in run B21. LD assays were defined as using a concentrated Ab format, a single RTU format to be validated by the individual laboratory or using a HER2 IHC FDA/CE-IVD approved assay off-label as Dako HercepTest™ on non-Dako Autostainer platform.

Despite an overall improvement of the pass rate for LD HER-2 assays from run B1 to B22, the pass rate and proportion of optimal results is still inferior to the FDA/CE-IVD approved systems as PATHWAY® /CONFIRM™ and HercepTest™. In general, the two FDA-/CE-IVD approved HER-2 assays provided a proportion of optimal results of 92% (234 of 254), whereas only 49% of LD HER-2 assays were assessed as optimal (61 of 124). As shown in Fig. 2, LD HER-2 assays both provided a reduced proportion of sufficient results (mainly related to a false negative staining reaction), but also a shift from optimal to good. The reduced performance was typically characterized by a poor signal-to-noise ratio and/or a 2+ staining reaction in one or both of the HER-2 non-amplified tumours (no. 3 and 5) expected to show a 0/1+ staining reaction. The staining reaction of 2+ in these tumours would not directly lead to a wrong diagnosis but require an additional ISH test due to the less precise IHC result.

The overall pass rate of 83% obtained in this assessment was slightly reduced compared to the level obtained in the previous run B21, which mainly was related to the increased use of less successful validated LD assays.

Figure 1. **Pass rates of HER-2 IHC assessments in the NordiQC breast cancer module**

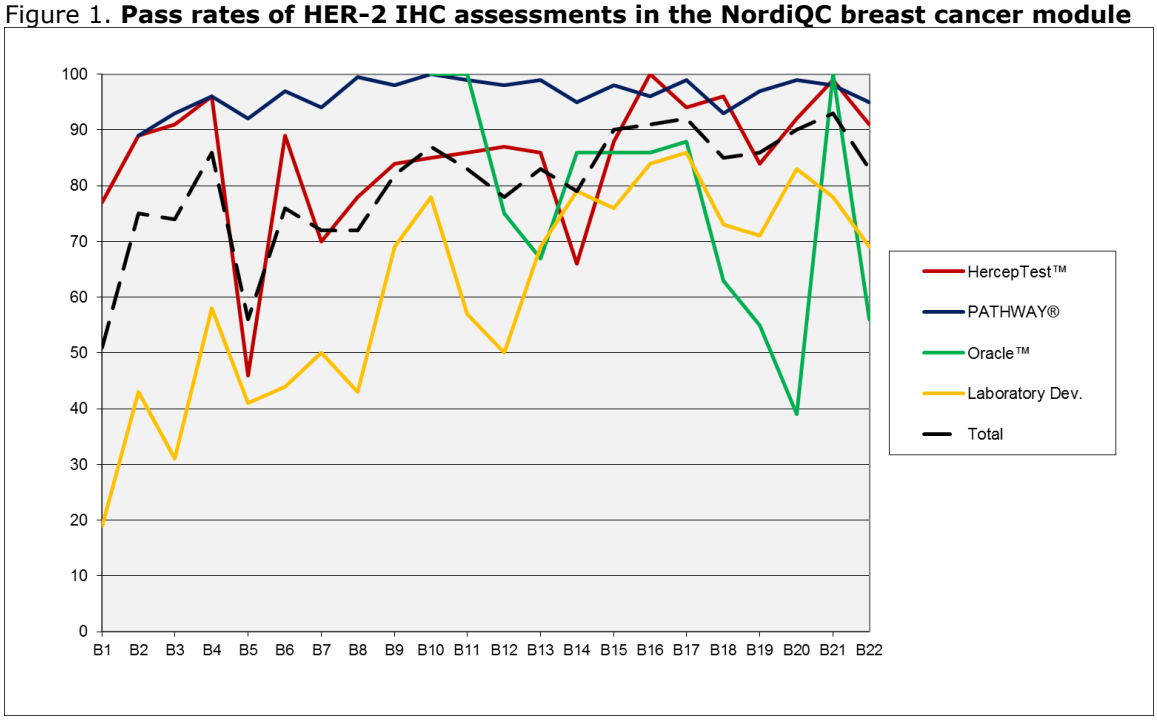
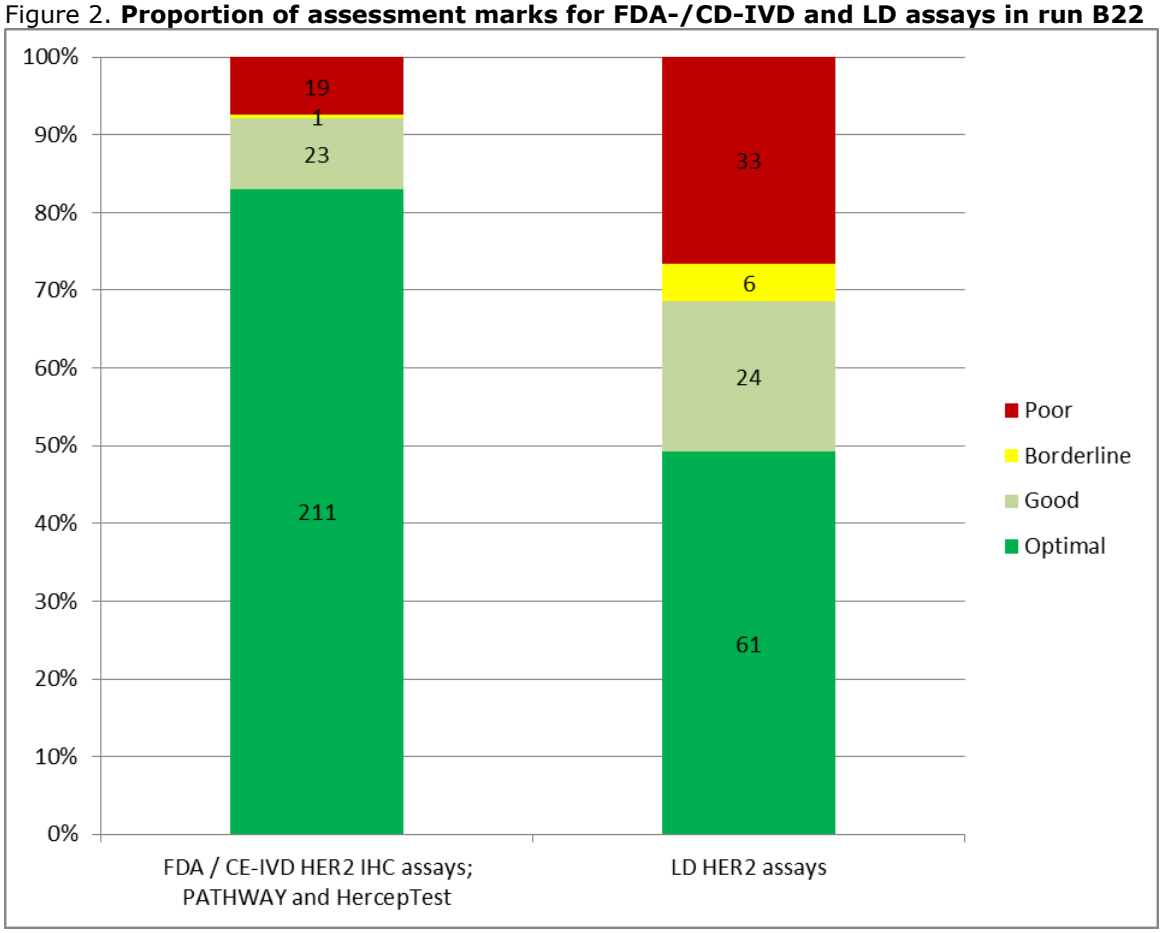


Figure 2. **Proportion of assessment marks for FDA-/CD-IVD and LD assays in run B22**



Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) of their stained sections. This was done by 84% (327 of 387) of the participants. For 282 of the 327 (86%) 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 a small decline compared to the level of scoring consensus (92%) observed in run B21. Among laboratories with sufficient staining, 92% (256 of 277) of interpretations were in agreement with the NordiQC assessors. Interpretation in concordance with the NordiQC assessor group was seen in 52% (26 of 50) among participants with insufficient staining. Typically, tumour core no. 1 was interpreted as 2+ by the laboratory, but 0-1+ by the NordiQC assessor group.

Conclusion
The FDA-/CE-IVD approved HER-2 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 HER-2 protein expression. Laboratory developed assays produced a lower pass-rate and were less precise for the HER-2 status, requiring additional ISH tests for final evaluation.
Inclusion of 2+ tumours with and without HER-2 gene amplification in control material is essential to evaluate precision and performance stability of the IHC HER-2 assays.
An overall reduced pass rate was observed compared to the performance in the latest run B21, which mainly was caused by extended use of laboratory developed assays and off-label use of HER2 IHC approved assays.

Figs. 1a and 1b – optimal staining results, same protocol
Figs. 2a and 2b – insufficient staining results - false negative, same protocol
Figs. 3a and 3b – insufficient staining results - false positive, same protocol

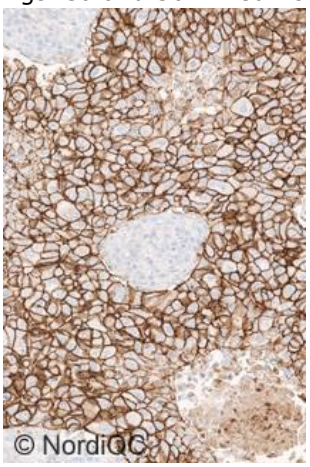


Fig. 1a
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.

Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of 2.3 – 2.7.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

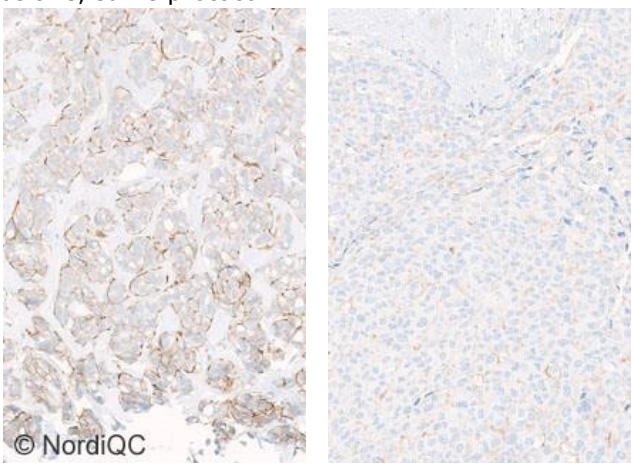
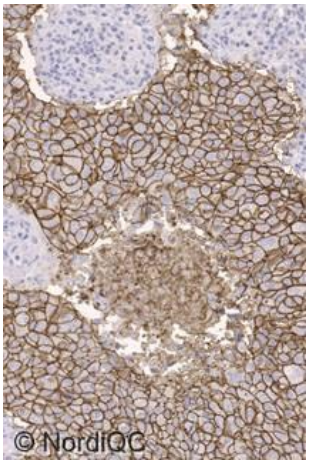


Fig. 1b
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 1.3 – 1.7.
> 10% of the neoplastic cells show a weak to moderate membranous staining reaction corresponding to 2+.

Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chromosome 17 ratio of 0.9– 1.2.
> 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 1+.



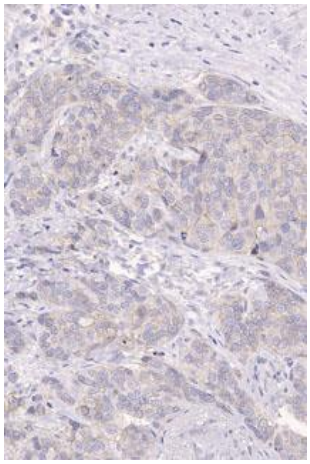
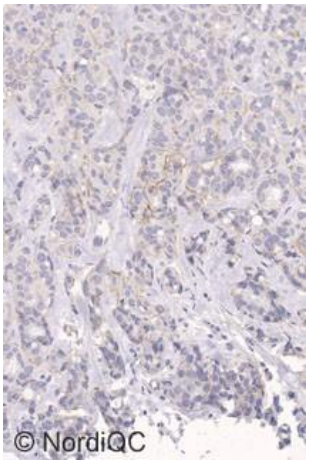


Fig. 2a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

Right: Insufficient and false negative staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of 2.3 – 2.7.
> 10% of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be reflexed to ISH.



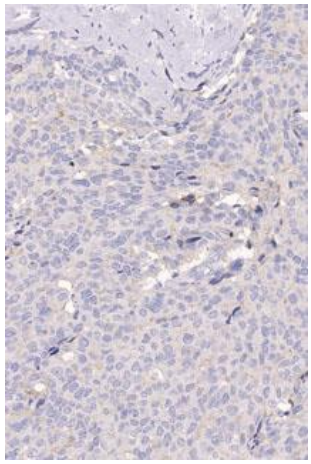
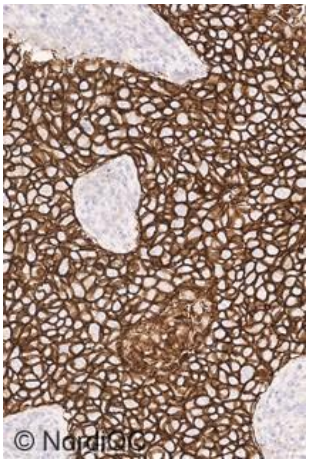


Fig. 2b
Left: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 1.3 – 1.7.
> 10% of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chromosome 17 ratio of 0.9 – 1.2.
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.



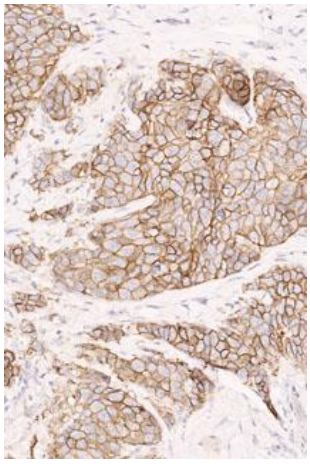
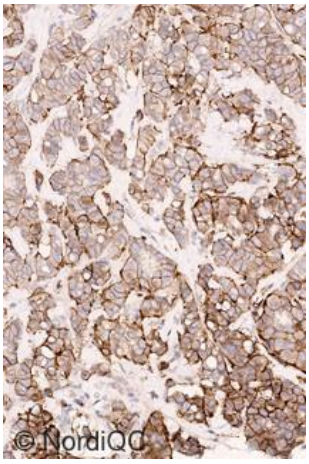


Fig. 3a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of 2.3 – 2.7.
> 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 2+.



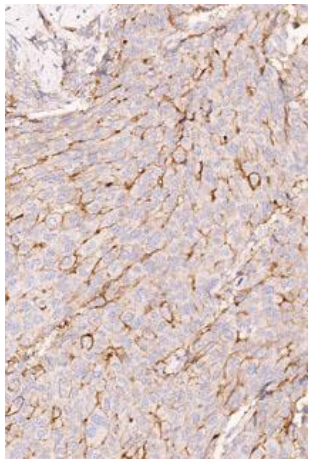


Fig. 3b
Left: Insufficient and false positive staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 1.3 – 1.7.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chromosome 17 ratio of 0.9 – 1.2.
> 10% of the neoplastic cells show a weak to moderate membranous staining reaction corresponding to 2+.

SN/LE/MV/RR 04.12.2016