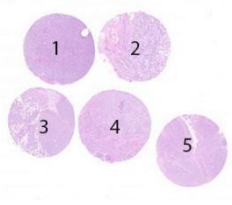
Assessment Run B18 2014

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/chr 17 ratio**
1.Breast carcinoma	2-3+	2.3 – 2.8 (a)
2.Breast carcinoma	0-1+	1.0 – 1.3 (u)
3.Breast carcinoma	1-2+	1.3 – 1.6 (u)
4.Breast carcinoma	3+	> 6.0 (clusters) (a)
5.Breast carcinoma	0-1+	1.0 – 1.3 (u)



* HER-2 immunohistochemical score (see table below) as achieved by using the 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) and Inform HER-2 Dual colour ISH (Ventana). u = unamplified, a = amplified.

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 > 10% of the tumour cells.
Score 2+	A weak to moderate circumferential incomplete membrane staining is observed in > 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 > 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. 2 and 5.
- Staining corresponding to score 1+ or 2+ in carcinoma no. 3.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 1.
- Staining corresponding to score 3+ in carcinoma no. 4.
- No or only a weak cytoplasmic reaction that did not interfere with the interpretation.

A 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. 2 and/or 5 showed a 2+ reaction and the other breast carcinomas showed the expected reaction pattern.

A 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.

A staining was assessed as **poor** in case of a false negative staining (e.g., the 3+ tumour and 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).

Results

335 laboratories participated in this assessment. 85% achieved a sufficient mark. Assessment marks for antibodies and detection systems are summarized in table 1.

Table 1. Assessment marks for **IHC systems and antibodies run B18, 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**	122	Ventana	108	2	1	11	90%	92%
CONFIRM™, rmAb clone **4B5, 790-4493**	47	Ventana	44	0	0	3	94%	93%
CONFIRM™, rmAb clone **4B5, 800-2996**	3	Ventana	3	0	0	0	-	-
HercepTest™ **SK001**	35	Dako	33	0	0	2	94%	97%
HercepTest™ **K5207**	10	Dako	8	0	0	2	80%	88%
HercepTest™ **K5204**	12	Dako	10	2	0	0	100%	100%
Oracle™ mAb clone **CB11, TA9145**	10	Leica	4	2	2	2	60%	63%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11**	5 1 1	Leica/Novocastra Cell Marque Klinipath	5	2	0	0	100%	100%
mAb clone **EP1045Y**	1	Thermo/NeoMarkers	0	0	0	1	-	-
rmAb clone **SP3**	14 2 1 1	Thermo/NeoMarkers Zytomed Cell Marque Zeta Corp	5	4	0	9	50%	88%
pAb clone **A0485**	61	Dako	44	6	4	7	82%	87%
Unknown	1	Unknown	1	0	0	0	-	-
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor						
mAb clone **CB11, RTU-CB11**	4	Leica/Novocastra	2	0	1	1	-	-
mAb clone **CB11**	1	Cell Marque	0	0	1	0	-	-
mAb clone **EP1045Y**	1	Thermo/NeoMarkers	0	0	0	1	-	-
rmAB clone **EP3, AN726**	1	BioGenex	1	0	0	0	-	-
rmAB clone **EP3, RMPD**	1	Diagnostics Biosystems	0	0	0	1	-	-
Total	335		268	18	9	40	-	-
Proportion			80%	5%	3%	12%	85%	-

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): 108 of 122 (90%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in Cell Conditioning 1 (CC1) mild or standard in the BenchMark XT, GX or Ultra, 12 – 32 min. incubation of the primary Ab and in the majority of the protocols (n=87) iView or UltraView as detection kit. Using these protocol settings 92 of 100 (92%) laboratories produced a sufficient staining result (optimal or good).

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 43 of 47 (91%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 mild or standard in the BenchMark XT, GX or Ultra, 16 – 32 min. incubation of the primary Ab and iView or UltraView as detection kit. Using these protocol settings 42 of 45 (93%) laboratories produced an optimal staining result.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 3 of 3 protocols were assessed as optimal. Protocols with optimal result were typically based on HIER in CC1 mild and standard in the BenchMark XT or Ultra, 16 – 32 min. incubation of the primary Ab and UltraView as detection kit

HercepTest™ pAb (SK001, Dako): 33 of 35 (94%) 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 20-30 min. incubation of the primary Ab. Using these protocol settings 28 of 29 (97%) laboratories produced an optimal staining result.

HercepTest™ pAb (K5207, Dako): 8 of 10 (80%) 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 7 of 8 (88%) laboratories produced an optimal staining result.

HercepTest™ pAb (K5204, Dako): 10 of 12 (83%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97 - 99°C for 40-45 min in a water bath or PT Link, 30-40 min. incubation of the primary Ab. Using these protocol settings 8 of 8 (100%) laboratories produced a sufficient staining result.

Oracle™ mAb clone **CB11** (TA9145, Leica): 4 of 10 (40%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in Bond Epitope Retrieval Solution (BERS1) for 25 min and 30 min incubation of the primary Ab. Using these protocol settings 5 of 8 (63%) laboratories produced a sufficient staining result.

Concentrated antibodies for laboratory developed (LD) assays
mAb **CB11**: 5 of 7 (71%) protocols were assessed as optimal. Optimal protocols were based on HIER using either CC1 (BenchMark, Ventana) (2/2)*, Tris-EDTA/EGTA pH 9 (1/1), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/1). The mAb clone CB11 was typically diluted in the range of 1:70-600 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 (100%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb **SP3**: 5 of 18 (28%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako) (2/4), CC1 (BenchMark, Ventana) (1/4) or Tris-EDTA/EGTA pH 9 (1/3). The rmAb clone SP3 was typically diluted in the range of 1:40-50 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 of 8 (88%) laboratories produced a sufficient staining.

pAb **A0485**: 44 of 61 (72%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH 6.1 (Dako) (22/26), TRS pH 9 (Dako) (6/11), CC1 (BenchMark, Ventana) (7/9), BERS1 (Bond, Leica) (6/8), Citrate pH 6 (2/4) or Tris-EDTA/EGTA pH 9 (1/2). The pAb A0485 was typically diluted in the range of 1:150-1:900 depending on the total sensitivity of the protocol employed. Using these protocol settings 46 of 53 (87%) laboratories produced a sufficient staining.

Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, the prominent features of insufficient HER-2 staining were too weak or false negative staining reaction. This was particularly and most critically observed as 0/1+ IHC reaction in the HER-2 gene amplified breast carcinoma core no. 1. This tumour was shown to be IHC 2+ in the NordiQC reference laboratories using the three FDA/CE-IVD HER-2 IHC assays, PATHWAY® (Ventana), HercepTest™ (Dako) and Oracle™ (Leica) and showed a low level of HER-2 gene amplification (ratio 2.3 – 2.8) by ISH. False negative staining reaction of the breast carcinoma no. 1 was seen in 80% of the insufficient results (39 of 49). The remaining insufficient results were typically characterized by a poor signal-to-noise ratio, complicating the interpretation, or by a false positive 3+ 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. The weak and false negative results were for the LD assays typically caused by a too low 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 no single cause for insufficient and false negative staining reactions could be identified from the submitted protocols.

In this assessment, the FDA-/CE-IVD approved HER-2 IHC systems from Ventana and Dako, PATHWAY® /CONFIRM™ or HercepTest™, respectively provided a high pass-rate superior to LD assays as illustrated in

Fig. 1. For unexplained reasons the FDA/CE-IVD approved system Oracle™, Leica showed a noticeable decline in the proportion of sufficient results in this run. At present, no plausible cause for the decline could be identified, and as only a relatively small number of participants have used the Oracle™ system, no conclusions could be drawn.
Despite relative consistent improvement of the pass rate for LD HER-2 assays from run B1 to B18, the pass rate and proportion of optimal results still is inferior to the FDA/CE-IVD approved systems as PATHWAY® /CONFIRM™ and HercepTest™. Using FDA-/CE-IVD approved HER-2 assay a proportion of optimal results of 88% (210 of 239) was observed, whereas only 60% of LD HER-2 assays were assessed as optimal (58 of 96). As shown in Fig. 2, LD HER-2 assays both provided a higher proportion of insufficient results but also an increased number of results assessed as good, typically caused by 2+ staining reaction in one or both of the HER-2 non-amplified tumours (no. 2 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 proportion of laboratories using LD assays is relatively consistent. In this run, 29 % of the participants (n=96) used LD assays compared to 28 - 31 % in the last 7 assessments.

The overall pass rate of 85% obtained in this assessment was slightly lower than the pass rates (proportion of sufficient results) seen in the two latest NordiQC HER-2 IHC runs performed. In this run many new laboratories (n=72) participated for the first time. A slight difference regarding the pass rates was observed for the laboratories participating in the HER2-IHC assessment for the first time compared to the laboratories also participating in the latest assessment run B17, 2014: For the laboratories participating for the first time the pass rate was 74% (53 out of 72 laboratories), whereas the pass rate was 89% (233 out of 263 laboratories) for the laboratories participating in both runs.

Figure 1. **Pass rates of 18 HER-2 IHC assessments in the NordiQC breast cancer module**

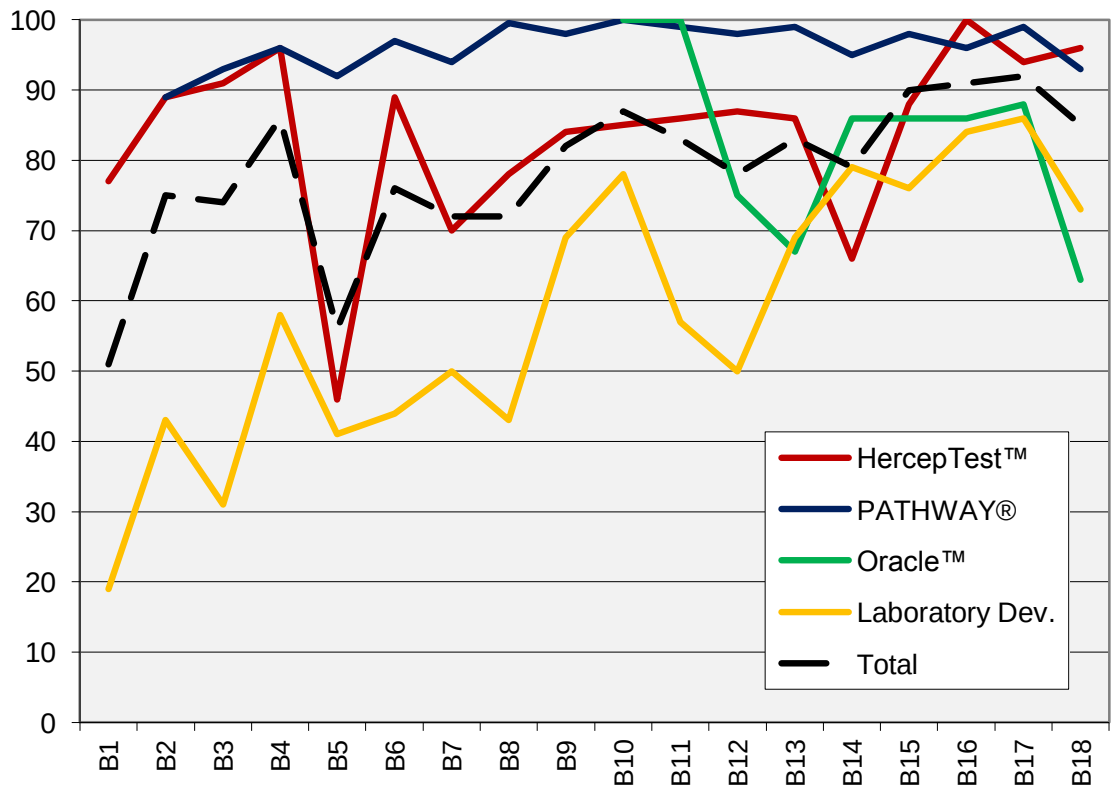
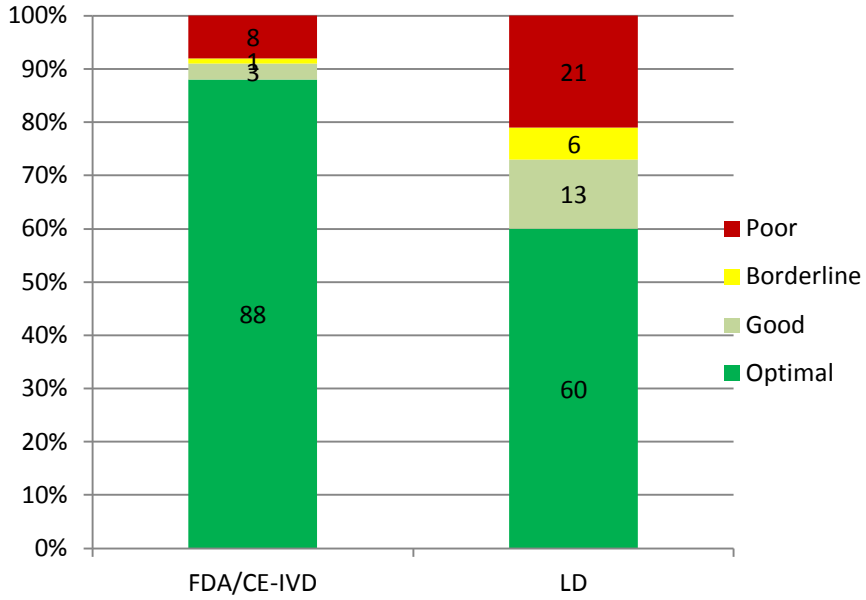


Figure 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**



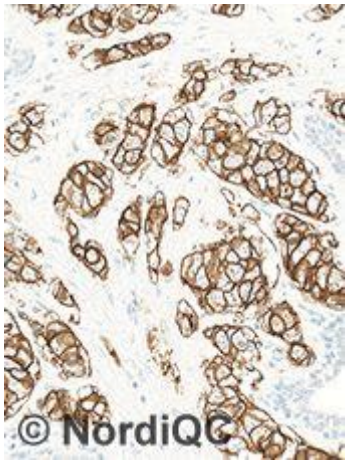
Scoring consensus

The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) on their stained sections. For 213 of the 285 laboratories (75%) responding, 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.
A sufficient staining result and interpretation by the laboratories in agreement with the NordiQC assessor group was seen in 74% (183 of 247) of cases. An insufficient staining result but interpretation in concordance with the NordiQC assessor group was seen in 79% (30 of 38). It was difficult to conclude on the scoring consensus, as many laboratories used the ASCO/CAP 2007 guidelines, which in particular affected the consensus agreement for laboratories obtaining an optimal mark. Many of these laboratories interpreted tumour no. 1 as 1+, whereas the NordiQC assessor group evaluated the result as 2+ in this tumour. Typically, moderate incomplete membranous staining reaction was seen in > 10% of the neoplastic cells and thus most likely to be scored as 1+ by the 2007 guidelines and 2+ by the 2013 guidelines. The tumour was HER-2 gene amplified, low-level, ranging from 2.3-2.8.

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 was less precise requiring an additional ISH test for final evaluation.
Inclusion of 2+ tumours with and without HER-2 gene amplification is essential as control material to evaluate the precision of the IHC HER-2 performance and the robustness of the protocols used by the participants.

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



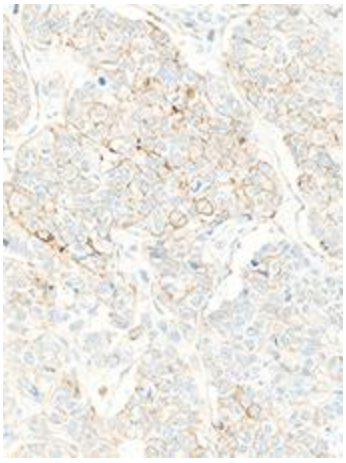
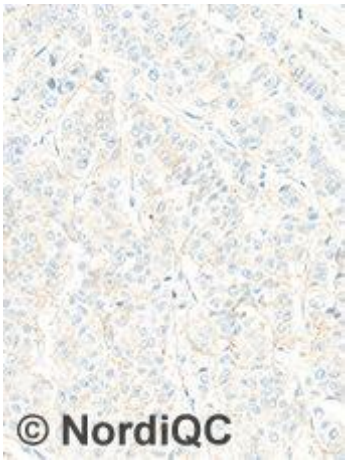


Fig. 1a
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr 17 of > 6.0.
> 10 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / chr 17 of 2.3 – 2.8.
> 10 % of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



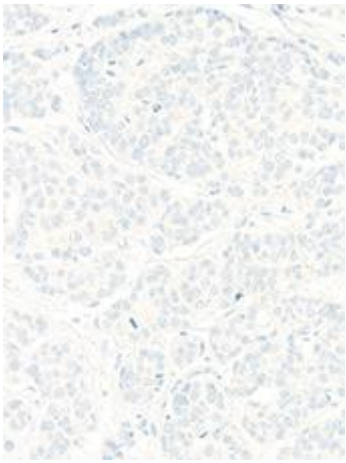
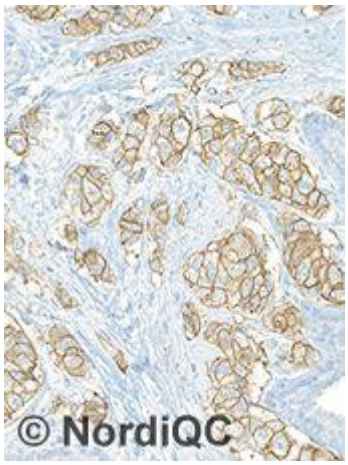


Fig. 1b
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr 17 of of 1.3 – 1.6.
> 10 % of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+.

Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / chr 17 ratio of 1.0– 1.3.
< 10 % of the neoplastic cells show a membranous staining reaction corresponding to 0.



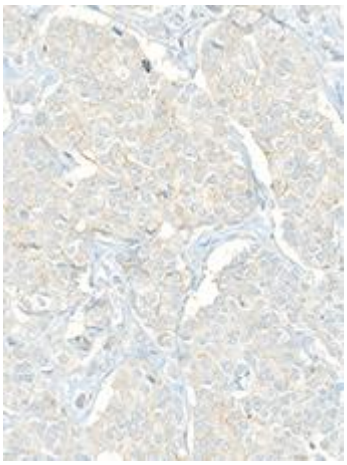
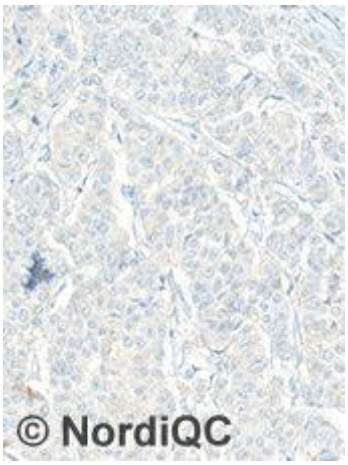


Fig. 2a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr 17 of > 6.0.
> 10 % of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+.

Right: Insufficient false negative staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / chr 17 of 2.3 – 2.8.
> 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 referred to ISH.



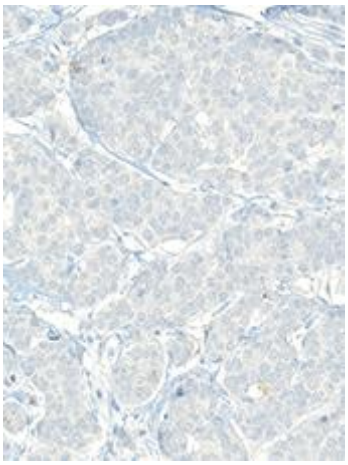
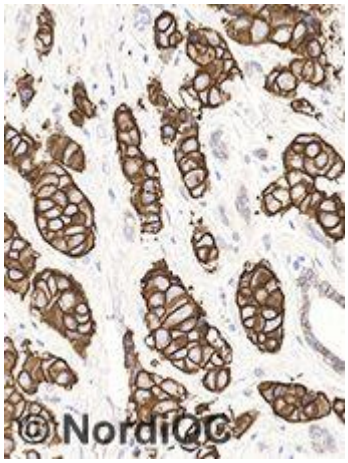


Fig. 2b
Left: Staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr 17 of 1.3 – 1.6.
< 10 % of the neoplastic cells show a membranous staining reaction corresponding to 0.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / chr 17 ratio of 1.0 – 1.3.
< 10 % of the neoplastic cells show a membranous staining reaction corresponding to 0.



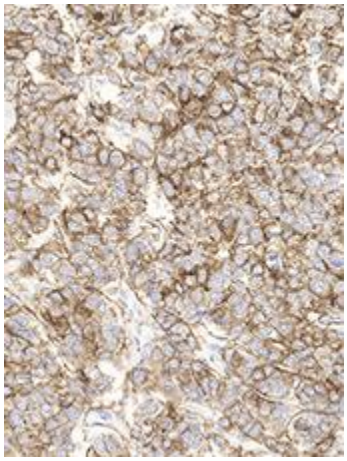
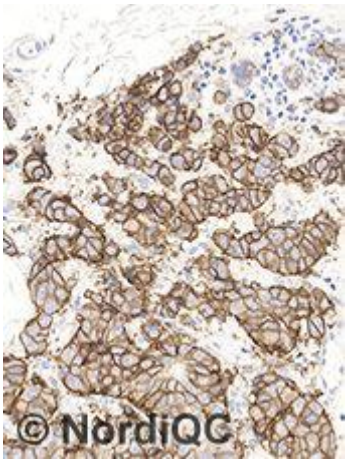


Fig. 3a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / chr 17 of > 6.0.
> 10 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / chr 17 of 2.3 – 2.8.
> 10 % of the neoplastic cells show a moderate membranous staining reaction corresponding to 2+. An excessive granular cytoplasmic staining reaction complicates the interpretation.

Also compare with Figs. 3b, same protocol.



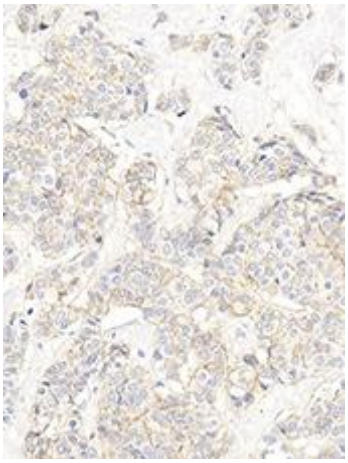


Fig. 3b
Left: Insufficient false positive staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / chr 17 of 1.3 – 1.6.
> 10 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

Right: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / chr 17 ratio of 1.0 – 1.3.
> 10 % of the neoplastic cells show a moderate incomplete membranous staining reaction corresponding to 2+. The HER-2 status must be further evaluated by ISH.

SN/RR/LE 21-11-2014