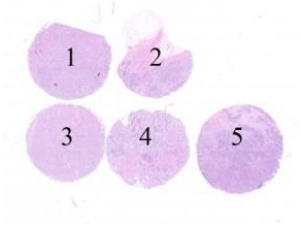
Assessment Run B17 2014

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/chr17 ratio**
1.Breast carcinoma	2+	2,4 – 2,8 (a)
2.Breast carcinoma	1-2+	1,3 – 1,7 (u)
3.Breast carcinoma	0-1+	1,0 – 1,3 (u)
4.Breast carcinoma	0-1+	1,2 – 1,6 (u)
5.Breast carcinoma	3+	> 6,0 (clusters) (a)



* 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 ZytoLight ® 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 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 4.
- Staining corresponding to score 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. 5.
- 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. 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 HER-2 gene non-amplified tumour no. 3 and/or 4 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 showing 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

289 laboratories participated in this assessment. 92% achieved a sufficient mark. Assessment marks for antibodies and detection systems are summarized in table 1 (see page 2).

Table 1. Assessment marks for **IHC systems and antibodies run B17, 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**	96	Ventana	90	2	1	3	96%	99%
CONFIRM™, rmAb clone **4B5, 790-4493**	47	Ventana	43	4	0	0	100%	100%
CONFIRM™, rmAb clone **4B5, 800-2996**	5	Ventana	5	0	0	0	100%	100%
HercepTest™ **SK001**	33	Dako	28	2	0	3	91%	94%
HercepTest™ **K5207**	10	Dako	7	1	0	2	80%	88%
HercepTest™ **K5204**	10	Dako	5	4	1	0	90%	100%
Oracle™ mAb clone **CB11, TA9145**	8	Leica	5	2	0	1	88%	88%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11**	1 1	Klinipath Monosan	0	2	0	0	-	-
rmAb clone **SP3**	14 1 1 1 1	Thermo/NeoMarkers Cell Marque ID Labs Zeta Corp Zytomed	8	6	0	4	78%	100%
pAb clone **A0485**	54	Dako	20	30	1	3	93%	91%
Unknown	1	Unknown	0	1	0	0	-	-
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor						
mAb clone **CB11, RTU-CB11**	3	Leica/Novocastra	0	2	1	0	-	-
rmAB clone **EP3, RMPD**	1	Diagnostics Biosystems	0	0	0	1	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	0	0	0	1	-	-
Total	289		211	56	4	18	-	-
Proportion			73%	19%	2%	6%	92%	-

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.

FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana): 90 of 96 (94%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in Cell Conditioning 1 (CC1), short, mild or standard in the BenchMark XT, GX or Ultra, 12 – 36 min. incubation of the primary Ab and in the majority of the protocols (n=87) Iview or UltraView was used as detection kit. Using these protocol settings 92 of 93 (99%) 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, short, mild or standard in the BenchMark XT, GX or Ultra, 16 – 32 min incubation time of the primary Ab and Iview or UltraView as detection kit. Using these protocol settings 46 of 46 (100%) laboratories produced a sufficient staining.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 5 of 5 (100%) protocols were assessed as optimal. Protocols with optimal result were typically based on HIER in CC1, short, mild and standard in the BenchMark XT, GX or Ultra, 12 – 32 min incubation time of the primary Ab and UltraView as detection kit. Using these protocol settings 5 of 5 (100%) laboratories produced a sufficient staining.

HercepTest™ pAb (SK001, Dako): 28 of 33 (85%) 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 30 of 32 (94%) laboratories produced a sufficient staining.

HercepTest™ pAb (K5207, Dako): 7 of 10 (70%) 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 and 30 min incubation of the primary Ab. Using these protocol settings 7 of 8 (88%) laboratories produced a sufficient staining.

HercepTest™ pAb (K5204, Dako): 5 of 10 (50%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 98 - 99°C for 40-60 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.

Oracle™ mAb clone **CB11** (TA9145, Leica): 5 of 8 (63%) protocols were assessed as optimal. Protocols with optimal result were based on HIER in Bond Epitope Retrieval Solution (BERS1) for 20-25 min and 30 min incubation of the primary Ab. Using these protocol settings 6 of 7 (86%) laboratories produced a sufficient staining.

Concentrated antibodies for laboratory developed (LD) assays
rmAb **SP3**: 8 of 18 (44%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako) (1/2)*, CC1 (BenchMark, Ventana) (4/6), BERS2 (Bond, Leica) (2/3) or Tris-EDTA/EGTA pH 9 (1/3). The rmAb clone SP3 was typically diluted in the range of 1:50-100 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 of 10 (100%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

pAb **A0485**: 20 of 54 (37%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS pH 6.1 (Dako) (10/22), TRS low pH 6.1, 3-in-1 (Dako) (3/4), TRS pH 9 (Dako) (2/4), CC1 (BenchMark, Ventana) (2/6), BERS1 (Bond, Leica) (1/6), Tris-EDTA/EGTA pH 9 (1/3) or Citrate pH 6 (1/3). The pAb A0485 was typically diluted in the range of 1:200-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 43 of 47 (91%) laboratories produced a sufficient staining.

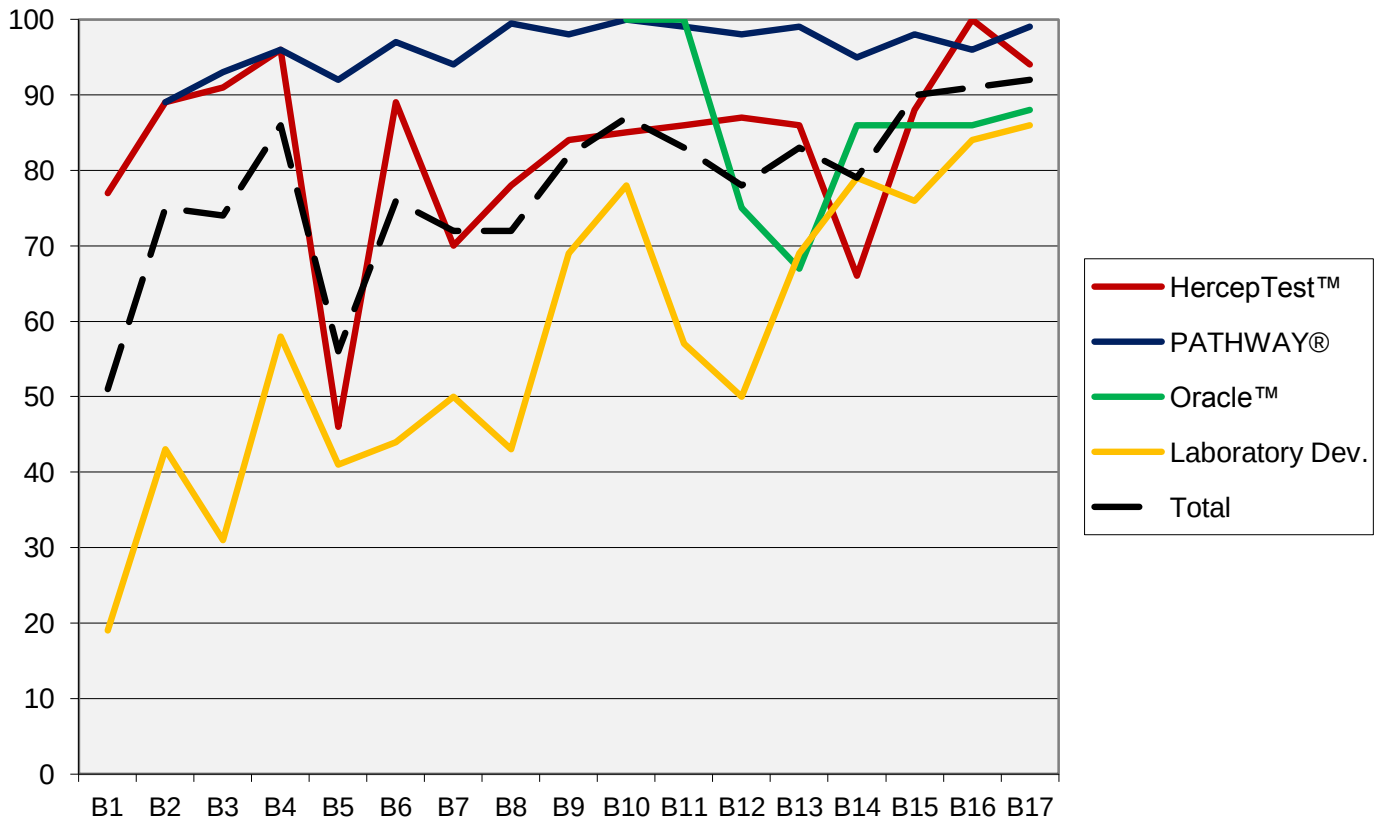
Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, the prominent feature of an insufficient HER-2 staining was a too weak and false negative staining reaction, which particularly and most critically was observed as a 0/1+ IHC reaction in the HER-2 gene amplified breast carcinoma 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 2,4 – 2,8) by ISH. A false negative staining reaction of the breast carcinoma no. 1 was seen in 82% of the insufficient results (18 of 22). The remaining insufficient results was characterized by either a false positive 3+ staining in the HER-2 non-amplified tumours or a poor signal-to-noise ratio, complicating interpretation.
False negative results were seen both in laboratory developed (LD) and FDA-/CE-IVD approved assays, while false positive results only were seen by the use of 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 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, all FDA-/CE-IVD approved HER-2 IHC systems provided a higher pass-rate compared to LD assays, see Fig. 1.
A consistent improvement of the pass rate (proportion of sufficient results) for LD has been seen from run B1 to B17 and the difference between the two types of assays has been reduced. In run B1 the pass rate for LD assays was 19% compared to 86% in the present run B17. However, the proportion of optimal staining results using LD assays were lower compared to FDA-/CE-IVD approved assays. Using a LD HER-2 assay 35% of the results were assessed as optimal (28 of 80); whereas the use of FDA-/CE-IVD approved HER-2 assays provided a proportion of optimal results of 88% (183 of 209). In this assessment almost 50% of results using a LD HER-2 assay were assessed as good, typically caused by a 2+ staining reaction in one or both of the HER-2 non-amplified tumours (no. 3 and 4) 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. See Fig. 2 for the proportion of results assessed as optimal and good using FDA/-CE-IVD and LD assays respectively.
The introduction of the updated 2013 ASCO/CAP IHC scoring guidelines, modifying the criteria for 2+ tumours, seemed to have an impact on the results. As an example, using these updated guidelines, incomplete membranous staining reaction in > 10% of the neoplastic cells now is considered as a 2+ staining reaction, while previously a complete membranous staining reaction was required to classify a tumour as 2+. The overall pass rate of 92% obtained in this assessment was the highest observed in the 17 NordiQC HER-2 IHC runs performed and significantly higher compared to the pass rate of 51% seen in the 1st run of HER-2 IHC in the NordiQC breast module. However as adjustmens of the assessment criteria accordingly to the ASCO/CAP guidelines (2007 and 2013) have been applied in the different runs a direct comparison is not possible.

The proportion of laboratories using LD IHC assays is relatively consistent. In this run, 28% of the participants (n=80) used LD assays compared to 28 - 31% in the last 6 assessments.

Figure 1. **Pass rate of 17 HER-2 IHC assessment in the NordiQC breast cancer module**

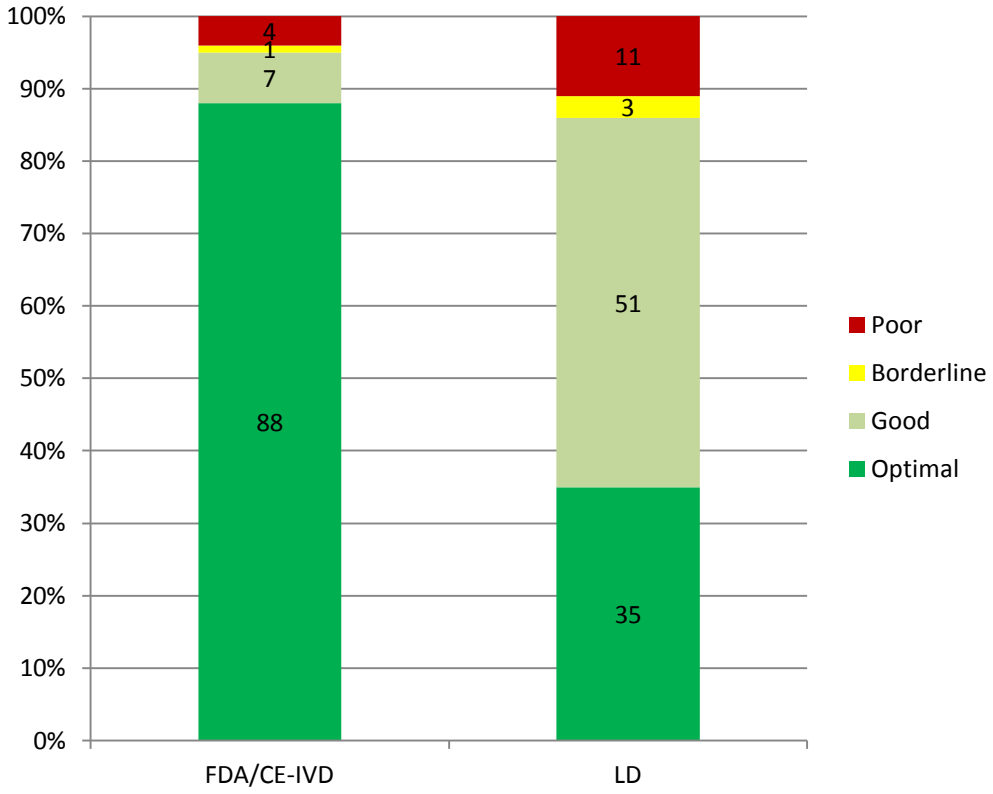


Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) on their stained sections. For 222 of the 247 laboratories (90%) responding, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group. Sufficient staining and interpretation in agreement with the NordiQC assessors were seen in 92% (215 of 233), which was comparable (95%) to the previous run B16. Insufficient staining and interpretation in concordance with the NordiQC assessor group was improved from 26% in run B16 to 50% (7 of 14 laboratories).

Conclusion
The overall pass rate increased to 92%, the highest observed in the 17 NordiQC HER-2 IHC runs performed.
The FDA-/CE-IVD approved HER-2 IHC assays PATHWAY® & CONFIRM™ rmAb clone 4B5 (Ventana), HercepTest™ (Dako) and Oracle™ (Leica) were in this assessment the most precise assays for the semi-quantitative IHC determination of HER-2 protein expression. A high proportion of laboratory developed assays was less precise and required 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.

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

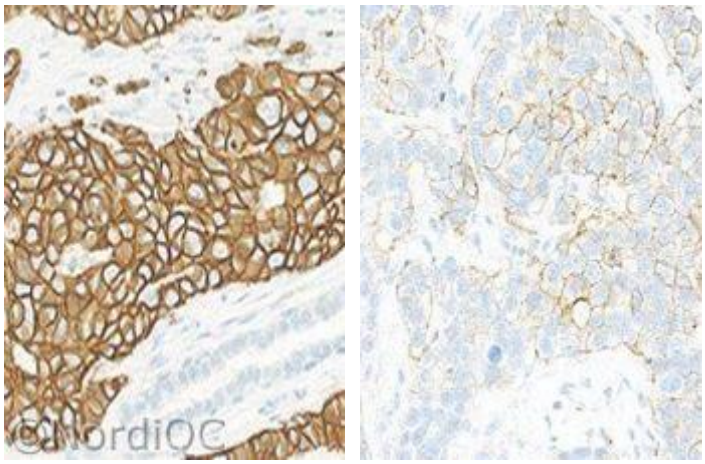


Fig 1a
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6,0.
> 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2,4 – 2,8.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

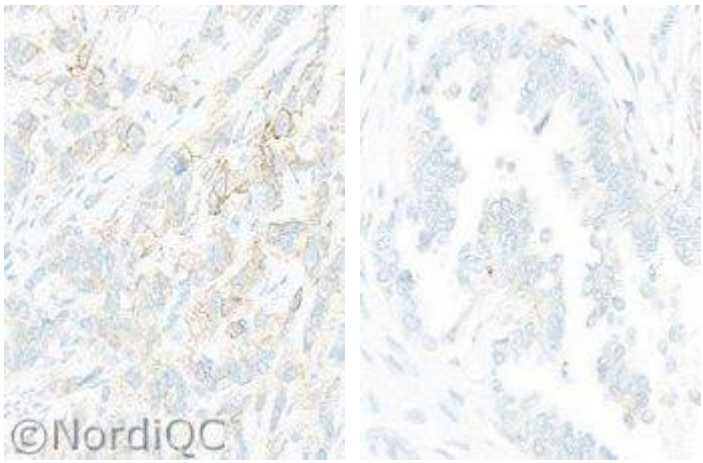


Fig 1b
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2/chr17 of of 1,3 – 1,7.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 1,2 – 1,6.
< 10% of the neoplastic cells show a membranous staining reaction corresponding to 0.

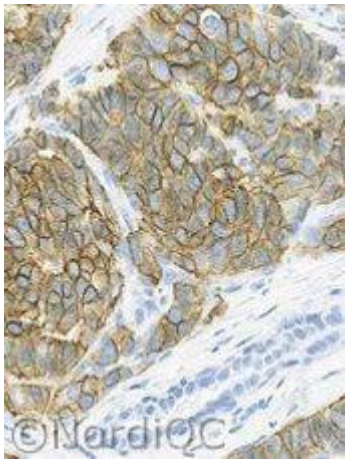
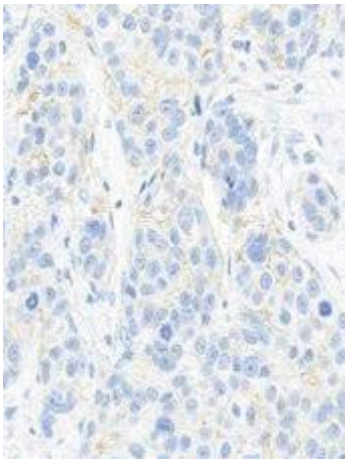



Fig 2a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6,0. > 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Insufficient and false negative staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2,4 – 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.

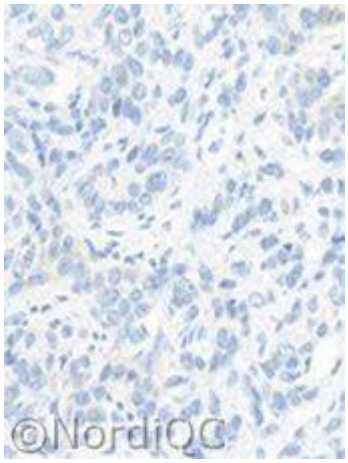
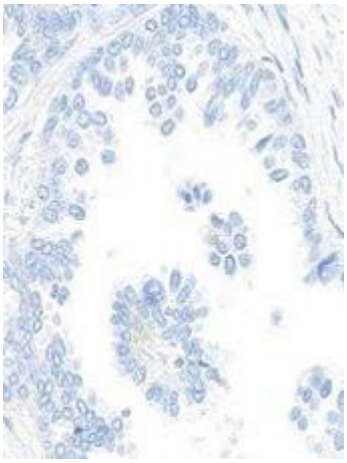



Fig 2b
Left: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2/chr17 of 1,3 – 1,7.
< 10% of the neoplastic cells show a membranous staining reaction corresponding to 0.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 1,2 – 1,6.
< 10% of the neoplastic cells show a membranous staining reaction corresponding to 0.

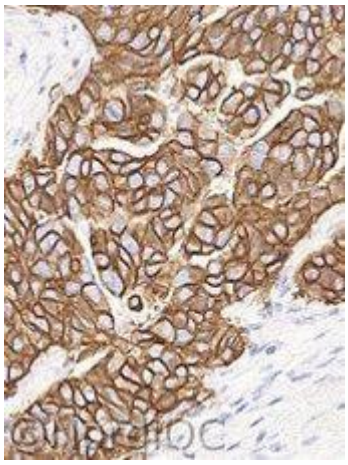
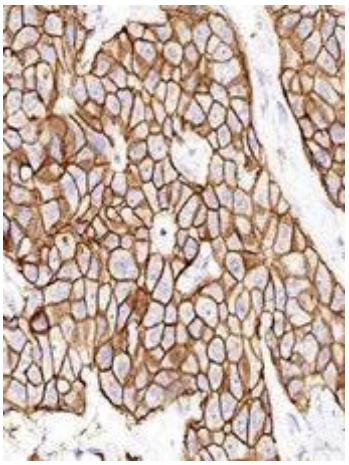



Fig 3a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6,0. > 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2,4 – 2,8.
> 10% of the neoplastic cells show a strong and complete membranous staining corresponding to 3+. Also compare with Figs. 3b, same protocol.

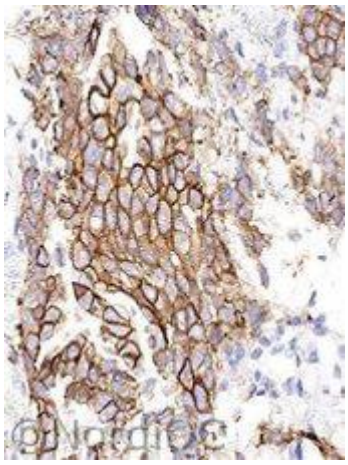
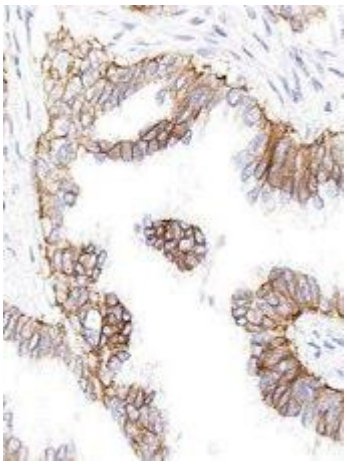



Fig 3b
Left: Insufficient and false positive staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2/chr17 of 1,3 – 1,7. > 10% of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 1,2 – 1,6.
> 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/MV/LE 02-04-2014