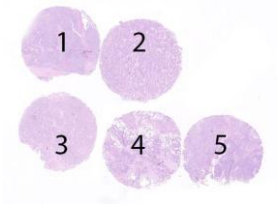
Assessment Run B19 2015

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	2-3+	2.3 – 2.8 (a)
2.Breast carcinoma	0-1+	0.9 – 1.3 (u)
3.Breast carcinoma	1-2+	1.2 – 1.5 (u)
4.Breast carcinoma	3+	> 6.0 (clusters) (a)
5.Breast carcinoma	0-1+***	1.2 – 1.5 (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)
*** scattered cells < 1% focally showed a moderate to strong complete membranous staining reaction.

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. 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 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. 2 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 B19	353
Number of laboratories returning slides	333 (94%)

Results: 333 laboratories participated in this assessment. 86% achieved a sufficient mark. Assessment marks for IHC HER-2 assays and HER-2 antibodies are summarized in table 1.

Table 1. **Assessment marks for IHC assays and antibodies run B19, 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**	118	Ventana	113	1	0	4	97%	99%
CONFIRM™, rmAb clone **4B5, 790-4493**	51	Ventana	47	2	0	2	96%	98%
CONFIRM™, rmAb clone **4B5, 800-2996**	2	Ventana	2	0	0	0	-	-
HercepTest™ **SK001**	40	Dako	30	3	0	7	83%	83%
HercepTest™ **K5207**	12	Dako	9	0	0	3	75%	78%
HercepTest™ **K5204**	13	Dako	7	4	0	2	85%	88%
Oracle™ mAb clone **CB11, TA9145**	11	Leica	0	6	1	4	55%	-
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11**	10 1 1 1	Leica/Novocastra Biogenex Cell Marque Klinipath	4	4	1	4	62%	71%
mAb clone **EP1045Y**	1	Thermo/NeoMarkers	1	0	0	0	-	-
rmAb clone **SP3**	16 2 1 1 1	Thermo/NeoMarkers Zytomed Cell Marque Immunologic Thermo/Pierce	12	3	1	5	71%	86%
pAb clone **A0485**	46	Dako	28	6	2	10	71%	78%
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11, RTU-CB11**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **CB11, 237M-18**	1	Cell Marque	0	0	0	1	-	-
rmAB clone **EP3, AN726-5M**	1	BioGenex	1	0	0	0	-	-
rmAB clone **SP3, MAD-000308QD**	1	Master Diagnostics	1	0	0	0	-	-
Ab **MXR001**	1	Maixin	1	0	0	0		
Total	333		256	29	5	43	-	-
Proportion			77%	9%	1%	13%	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.

Detailed Analysis
FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana): 113 of 118 (96%) 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 Iview or UltraView as detection kit. Using these protocol settings 100 of 104 (96%) laboratories produced a sufficient staining result (optimal or good).

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 47 of 51 (92%) 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, 12 – 32 min. incubation of the primary Ab and Iview or UltraView as detection kit. Using these protocol settings 44 of 45 (98%) laboratories produced a sufficient staining result.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 2 of 2 protocols were assessed as optimal. Both protocols were based on HIER in CC1 mild or standard in the BenchMark XT and Ultra, 16 – 32 min. incubation of the primary Ab and UltraView as detection kit.

HercepTest™ pAb (SK001, Dako): 30 of 40 (75%) 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 20 of 24 (83%) laboratories produced a sufficient staining result.

HercepTest™ pAb (K5207, Dako): 9 of 12 (75%) 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 9 (77%) laboratories produced an optimal staining result.

HercepTest™ pAb (K5204, Dako): 7 of 13 (54%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 98 - 99°C for 40-45 min in a water bath or PT Link and 30-40 min. incubation of the primary Ab. Using these protocol settings 7 of 8 (88%) laboratories produced a sufficient staining result.

Concentrated antibodies for laboratory developed (LD) assays
mAb **CB11**: 4 of 13 (31%) protocols were assessed as optimal. Optimal protocols were based on HIER using either CC1 (BenchMark, Ventana) (1/1)*, Bond Epitope Retrieval Solution 2 pH 9 (1/1), Target Retrieval Solution pH 9 (Dako) (1/2), or Citrate pH 6 (1/3). The mAb clone CB11 was diluted in the range of 1:40-1:1,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 7 (71%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb clone **EP1045Y**: One protocol with an optimal result was based on HIER using Dewax High pH (Thermo) as retrieval buffer. The rmAb was diluted 1:100 with Quanto (Thermo) as detection kit.

rmAb **SP3**: 12 of 21 (57%) protocols were assessed as optimal. Optimal protocols were based on HIER using either CC1 (BenchMark, Ventana) (4/5), Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako) (3/5), Bond Epitope Retrieval Solution 2 pH 9 (2/3), Tris-EDTA/EGTA pH 9 (1/4), Citrate pH 6 (1/2) or PATHCOM HIER buffer (1/1). The rmAb clone SP3 was typically diluted in the range of 1:40-100 depending on the total sensitivity of the protocol employed. Using these protocol settings 12 of 14 (86%) laboratories produced a sufficient staining (optimal or good).

pAb **A0485**: 28 of 46 (61%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH 6.1 (Dako) (13/17), TRS pH 9 (Dako) (7/8), CC1 (BenchMark, Ventana) (4/5), Bond Epitope Retrieval Solution 1 pH 6 (Bond, Leica) (2/7) or Citrate pH 6 (2/6). The pAb A0485 was typically diluted in the range of 1:150-1:1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 32 of 41 (78%) laboratories produced a sufficient staining.

Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, insufficient HER-2 staining result was characterized by a 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 established as a 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 89% of the insufficient results (42 of 48). 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 protocols submitted.

In this assessment, the FDA-/CE-IVD approved HER-2 IHC assays from Ventana and Dako, PATHWAY® /CONFIRM™ or HercepTest™, respectively, provided a higher pass-rate compared to LD assays as illustrated in Fig. 1. PATHWAY®/CONFIRM™ has shown to give a consistent high pass rate throughout all HER-2 IHC runs in NordiQC. The FDA/CE-IVD approved system Oracle™, Leica showed in both this and the

previous run B18 a noticeable decline in the proportion of sufficient results. At present, no single cause for the decline can be identified, and, as only a relatively small number of participants used the Oracle™ system, no conclusions can be drawn.

The proportion of laboratories using LD assays is relatively consistent. In this run, 26% of the participants (n=86) used LD assays compared to 28 - 31% in the last 8 assessments. Despite an overall improvement of the pass rate for LD HER-2 assays from run B1 to B19 has been achieved, the pass rate and proportion of optimal results still is inferior to the FDA/CE-IVD approved systems as PATHWAY® /CONFIRM™ and HercepTest™. In general, FDA-/CE-IVD approved HER-2 assays provided a proportion of optimal results of 83% (188 of 227), whereas only 56% of LD HER-2 assays were assessed as optimal (48 of 86). As shown in Fig. 2, LD HER-2 assays both provided a reduced proportion of sufficient results but also a shift from optimal to 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 overall pass rate of 86% obtained in this assessment was almost identical to the pass rate of 85% seen in the previous run B18. The number of participants and material composed for the two runs were virtually identical.

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

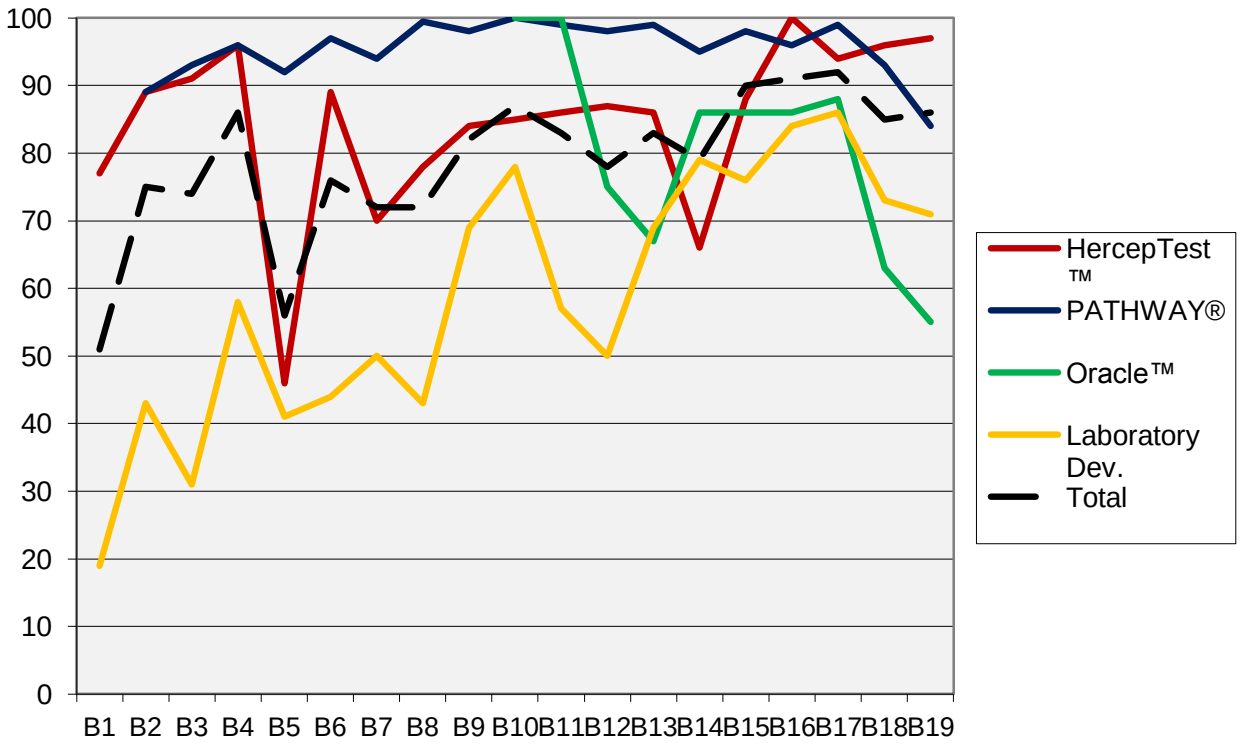
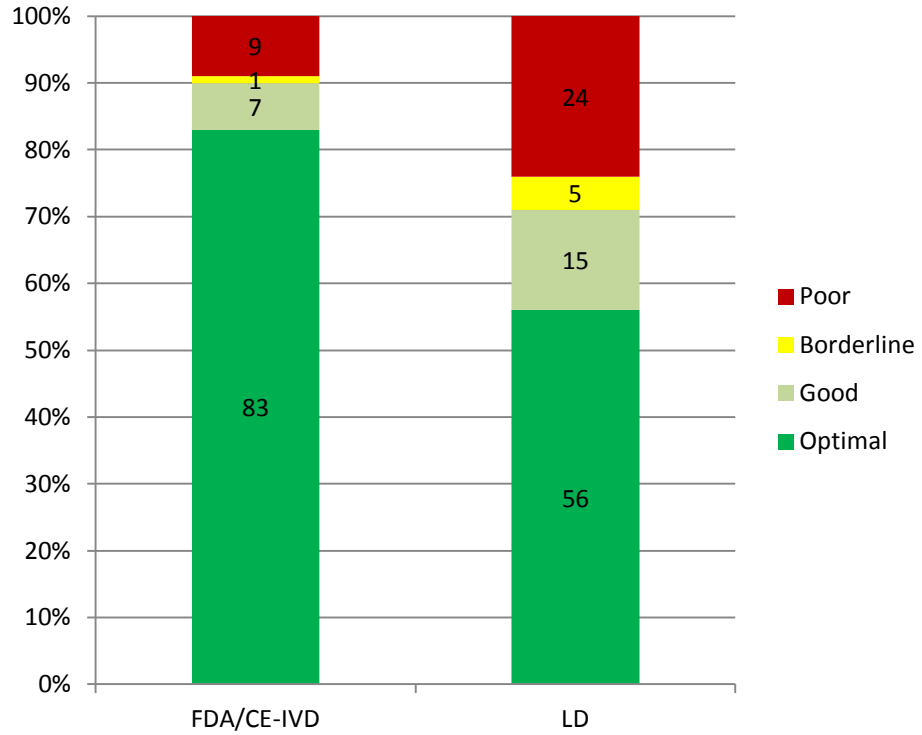


Figure 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**



Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) of their stained sections, which was effectuated by 90% (301 of 333) of participants. For 268 of the 301 participants (89%) 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, which was an improvement compared to the level of 75% observed in run B18. Sufficient staining and interpretation in agreement with the NordiQC assessors was seen in 94% (244 of 260). Insufficient staining and interpretation in concordance with the NordiQC assessor group was seen in 59% (24 of 41) of the participants. In general the scoring consensus was improved most likely due to implementation and use of the ASCO/CAP 2013 guidelines by the vast majority of the participating laboratories. However in case of an insufficient staining result consensus was only seen in 59%. Typically the tumour no. 1 was interpreted as 2+ by the laboratory, but 0-1+ by the NordiQC assessor group. This was to some extent extraordinary as the new HER-2 IHC guidelines have lowered the staining threshold for 2+ tumours and thus a higher level of consensus was expected.

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 an additional ISH test 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 used by laboratories.

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

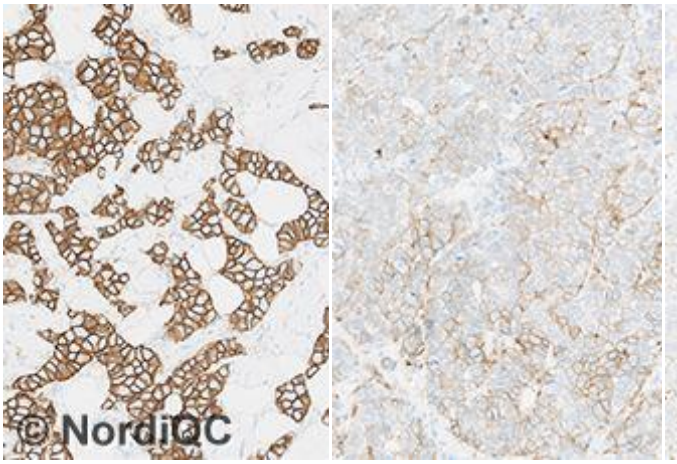


Fig 1a
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of > 6.0. > 10 % of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2.3 – 2.8. > 10 % of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

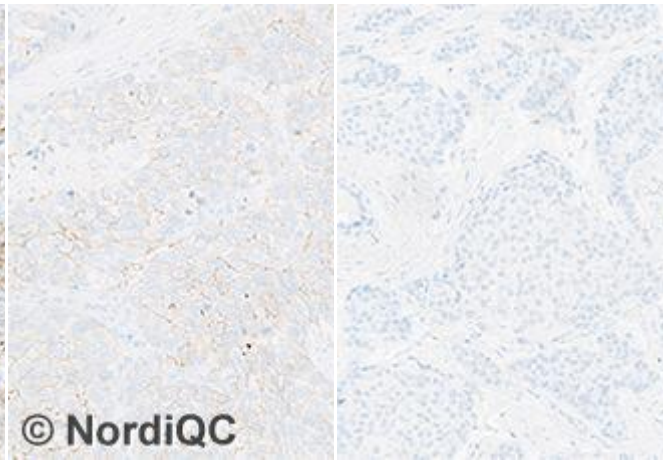


Fig 1b
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of of 1.2 – 1.5. > 10 % of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 0.9–1.3. < 10 % of the neoplastic cells show a membranous staining reaction corresponding to 0.

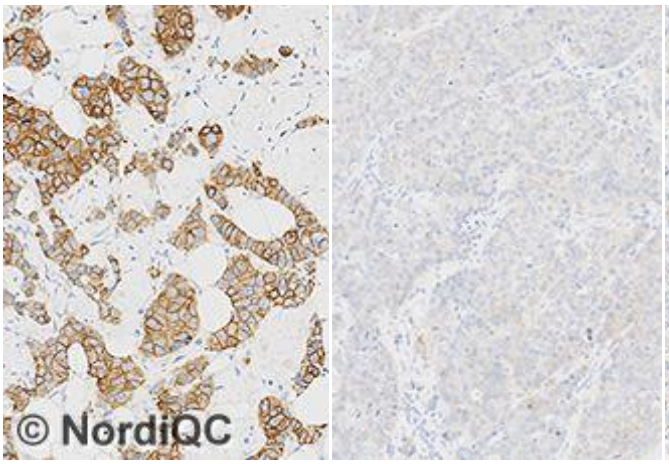


Fig 2a.
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of > 6.0. > 10 % of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Insufficient and false negative staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2.3 – 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.

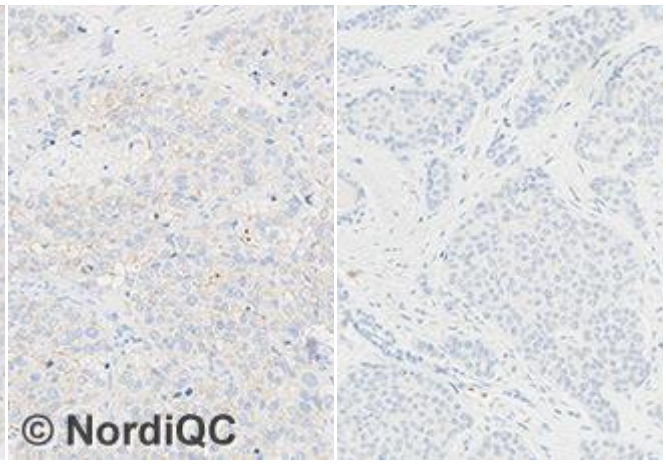


Fig 2b.
Left: Staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of 1.2 – 1.5. > 10 % of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 0.9 – 1.3. < 10 % of the neoplastic cells show a membranous staining reaction corresponding to 0.

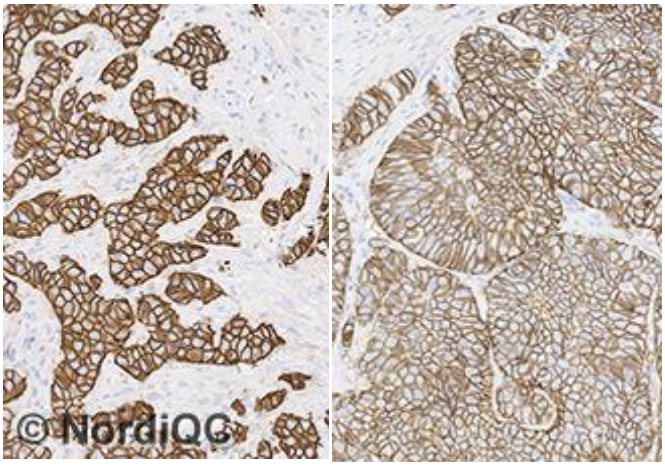


Fig 3a.
Left: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of > 6.0. > 10 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2/chr17 of 2.3 – 2.8. > 10 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

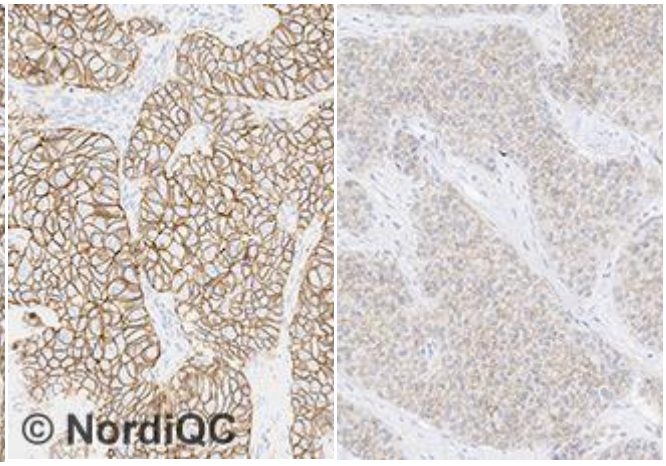


Fig 3b.
Left: Insufficient and false positive staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of 1.2 – 1.5. > 10 % of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 0.9 – 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/MV 29-3-2015