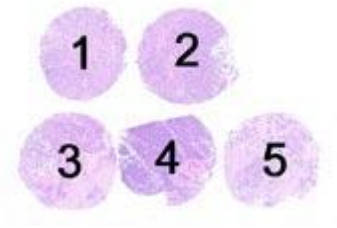
Assessment Run B12 2011
HER-2 IHC

Material

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

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2/chr17 ratio****
1. Breast ductal carcinoma	0[1]	1.1 – 1.3
2. Breast ductal carcinoma	1+	1.2 – 1.4
3. Breast lobular carcinoma	1+/2+	1.3 – 1.6
4. Breast ductal carcinoma	2+	2.5 – 2.9
5. Breast ductal carcinoma	3+	> 6.0, clusters



[1]Breast lobular carcinoma with focal component of ductal carcinoma HER-2 IHC score 3+
*HER-2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits/ antibodies (Abs), HercepTest™, Dako, and PATHWAY®, Ventana, in NordiQC reference laboratories.
**HER-2 gene/chromosome 17 Ratio achieved by HER-2 FISH pharmDX™ Kit, Dako, and Inform™ HER-2 Dual SISH, Ventana.

All carcinomas were fixed for 24 - 48 h in 10 % neutral buffered formalin.

IHC scoring system according to the guidelines given by ASCO/CAP:

Score 0	No staining is observed or cell membrane staining is observed in less than 10% of the tumour cells.
Score 1+	A faint perceptible membrane staining can be detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane.
Score 2+	A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells.
Score 3+	A strong complete membrane staining is observed in more than 30% of the tumour cells.

Criteria for assessing a HER-2 staining as optimal included:

- A clear and unequivocal staining marked as score 0 or 1+ in the breast ductal carcinomas no. 1 & 2.
- A clear and unequivocal staining marked as score 1+ or 2+ in the breast ductal carcinoma no 3.
- A clear and unequivocal staining marked as score 2+ or 3+ in the breast ductal carcinoma no 4.
- A clear and unequivocal staining marked as score 3+ in the breast ductal carcinoma no 5.
- No or only a weak cytoplasmic reaction that did not affect the interpretation of the true membranous HER-2 reaction.

A staining was assessed as good, if the HER-2 gene amplified tumour no. 5 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by ISH according to the ASCO/CAP guidelines and the national guidelines in Scandinavia) and the other breast carcinomas showed a reaction pattern as described above.

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+ and 2+ tumours without gene amplification showing a 3+ reaction).

Results:
232 laboratories participated in this assessment. 78 % achieved a sufficient mark. In table 1 the Abs used and marks are summarized.

Table 1. **The IHC systems/Abs used and the assessment marks given:**

FDA approved HER-2 systems	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
PATHWAY® rmAb clone **4B5, 790-2991**	63	Ventana	59	2	0	2	97 %	98 %
CONFIRM™, rmAb clone **4B5, 800-2996**	35	Ventana	35	0	0	0	100 %	100 %
HercepTest™ **SK001**	24	Dako	21	1	0	2	92 %	92 %
HercepTest™ **K5204**	4	Dako	1	1	1	1	-	-
HercepTest™ **K5207**	21	Dako	13	3	0	5	76 %	84 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	9	Leica	5	1	0	3	67 %	75 %
Abs for in-house HER-2 systems, conc. Ab.	**N**	**Vendor**	**Optimal**	**Good**	**Borderline**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
mAb clone **CB11**	3 1 1	Leica/Novocastra BioGenex Monosan	2	2	0	1	80 %	100 %
mAb clone **e-2-4001+3B5**	1	Thermo/NeoMarkers	0	0	0	1	-	-
rmAb clone **EP1045Y**	1	Epitomics	1	0	0	0	-	-
rmAb clone **SP3**	19 1 1 1	Thermo/NeoMarkers Master Diagnostica Spring Zytomed	5	5	1	11	45 %	70 %
pAb clone **A0485**	44	Dako	15	7	4	18	50 %	54 %
Abs for in-house HER-2 systems, RTU Ab.								
mAb clone **CB11, RTU-CB11**	3	Leica/Novocastra	1	0	1	1	-	-
Total	232		158	22	7	45	-	-
Proportion			68 %	10 %	3 %	19 %	78 %	-

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 systems

PATHWAY® rmAb clone **4B5** (Ventana): 59 out of 63 (94 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1), mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was in the range of 8 – 32 min. and either iView or UltraView was used as the detection kit. Using these protocol settings 59 out of 60 (98%) laboratories produced a sufficient staining (optimal or good).

CONFIRM™ rmAb clone **4B5** (Ventana): 35 out of 35 (100 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on HIER in CC1, mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was typically in the range of 8 – 40 min. and either iView or UltraView was used as the detection kit.

HercepTest™ SK001 (Dako): 21 out of 24 (88 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on HIER in HercepTest epitope retrieval solution (ERS) at 97 - 99°C for 40 min in a water bath or PT Link, an incubation time of 20-30 min. in the primary Ab and SK001 Visualization reagent for 20-30 min. Using these protocol settings 22 out of 24 (92 %) laboratories produced a sufficient staining.

HercepTest™ K5204 (Dako): 1 out of 4 stains was assessed as optimal. The protocol giving an optimal result was based on HIER in HercepTest ERS at 99°C for 40 min in a water bath, an incubation time of 30 min in the primary Ab and K5204 Visualization reagent for 30 min. Using these protocol settings 2 out of 3 laboratories produced a sufficient staining.

HercepTest™ K5207 (Dako): 13 out of 21 (62 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on HIER in HercepTest ERS at 96 - 99°C for 40 min in a water bath or PT Link, an incubation time of 30 min in the primary Ab and K5207 Visualization reagent for 30 min. Using these protocol settings 16 out of 19 (84 %) laboratories produced a sufficient staining.

Oracle™ (Leica) mAb clone **CB11**: 5 out of 9 (56 %) stains were assessed as optimal. The protocols giving an optimal result were based on HIER in Bond Epitope Retrieval Solution 1 (BERS1) for 25 min. and an incubation time for 30 min. of the primary Ab. Using these protocol settings 6 out of 8 (75 %) laboratories produced a sufficient staining.

Abs for in-house validated systems

mAb **CB11**: 2 out of 5 stains (40 %) were assessed as optimal. The protocols giving an optimal staining were based on HIER using CC1 (BenchMark, Ventana) (1/1)* or Tris-EDTA/EGTA pH 9 (1/2)*. The mAb CB11 was diluted in the range of 1:70-1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 laboratories produced a sufficient staining.
*(number of optimal results/number of laboratories using this buffer)

rmAb **EP1045Y**. The protocol giving an optimal staining was based on HIER in a pressure cooker using Diva Decloaker pH 6.2 (Biocare), an incubation time for 45 min. in the primary Ab. diluted 1:50 and using MACH4, Biocare as the detection system.

rmAb **SP3**: 5 out of 22 (23 %) stains were assessed as optimal. The optimal protocols were based on HIER using either Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (2/4), CC1 (BenchMark, Ventana) (1/3), EDTA/EGTA pH 8 (1/2) or Citrate pH 6 (1/7) as HIER buffer. The rmAb clone SP3 was typically diluted in the range of 1:25-50 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 out of 10 (70 %) laboratories produced a sufficient staining.

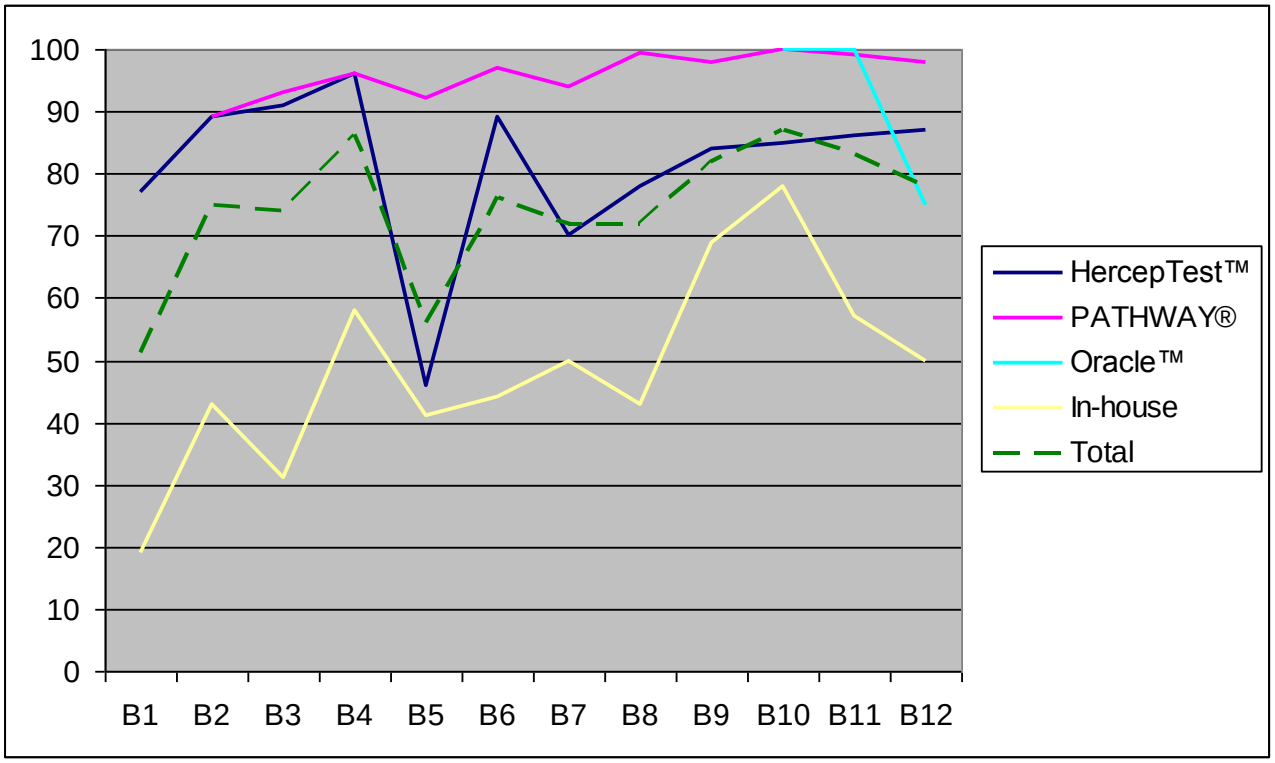
pAb **A0485**: 15 out of 44 (34 %) stains were assessed as optimal. All protocols giving an optimal staining were based on HIER using either Target Retrieval Solution (TRS) low pH 6.1 (Dako) (7/20), TRS pH 9 (Dako) (2/6), CC1 (BenchMark, Ventana) (1/3), Tris-EDTA/EGTA pH 9 (3/5), Citrate pH 6 (1/7) or EDTA/EGTA pH8 (1/1). The pAb A0485 was typically diluted in the range of 1:200-1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 22 out of 41 (54 %) laboratories produced a sufficient staining.

Comments
In this assessment and in concordance to the previous assessments for HER-2 IHC the prevalent feature of an insufficient HER-2 staining was a too weak or 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. 4. This tumour was shown to be IHC 2+ in the NordiQC reference laboratories using both HercepTest™, Dako and PATHWAY®, Ventana and showed a low level of HER-2 gene amplification (ratio 2.5 – 2.9) by ISH. The weak or false negative staining reactions were seen in 81 % of the insufficient results (42 out of the 52 laboratories), whereas 13 % (7 out of the 52 laboratories) were characterized by a a poor signal-to-noise ratio caused by an excessive cytoplasmic staining reaction hampering the interpretation and in 6 % (3 out of 52 laboratories) a false positive staining was seen one or more of the 3 HER-2 non-amplified tumours, no. 1, 2 & 3.
The false negative results and results with a poor signal-to-noise ratio were seen both with in-house validated assays and FDA-/CE-IVD approved systems, while the false positive results were only seen when an in-house validated assay was applied. The weak and false negative results were for the in-house systems typically related to a too low sensitivity of the protocol e.g. a too low concentration of the primary Ab, or a protocol based on a RTU Ab not applied within a system for which this product was calibrated.
For the FDA-/CE-IVD approved systems the false negative reactions could in part be related to the use of other protocol settings than recommended by the producers, e.g., too short incubation time in the primary Ab and detection system, but in a few cases no reason for the insufficient result could be identified.
The false positive staining reactions were caused by use of a too sensitive protocol - e.g., a too concentrated primary Ab - giving a continuous membranous staining of > 10 % of the neoplastic cells in the non-amplified tumours and in the normal epithelial cells entrapped in the tumour.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a pass rate of 91 % (142 out of 156 laboratories), which was comparable to the pass rate of 94 % obtained in run B11. The pass rate for the in-house validated assays as a group was 50 % (38 out of 76 laboratories), which was a decrease compared to the pass rate of 57 % in run B11 and 78 % in run B10 for this group.
The use of in-house validated HER-2 assays is still used by a considerable proportion of laboratories: In this run 33 % of the laboratories (76) used an in-house HER-2 validated assay, compared to 31 % and 37 % in run B11 and B10, respectively.

This was the 12th NordiQC HER-2 assessment in the breast cancer module. As illustrated in Fig. 1, the FDA-/CE-IVD approved systems such as PATHWAY®/CONFIRM™ (Ventana, rmAb clone 4B5), HercepTest™ (Dako) and Oracle™ (Leica), have constantly given superior pass rates compared to the in-house HER-2 assays. The average pass rate in the 12 consecutive runs has been 96 % for PATHWAY®/CONFIRM™ (Ventana, rmAb clone 4B5), 92 % for Oracle™ (last 3 runs; Leica) 82 % for HercepTest™ (Dako), and 49 % for the in-house assays.

Fig. 1. **Pass rates through the 12 HER-2 IHC assessments in the NordiQC breast module.**



*HercepTest™ code no. K5204, K5206, K5207 & SK001, Dako
**PATHWAY® & CONFIRM™, rmAb clone 4B5, Ventana

Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) of the stained sections. For 158 out of the 200 laboratories (79 %) responding, the scores on all the tissues in the multi-tissue sections given by the laboratories were in concordance with the scores given by the NordiQC assessor group. A sufficient staining combined with concordant scoring was seen in 89 % (138 out of 155), which was an increase from 84 % obtained in the previous run B11. An insufficient staining combined with an interpretation in concordance with the NordiQC assessor group was seen in only 44 % of the laboratories (20 out of 45).

Conclusion
The FDA-/CE-IVD approved HER-2 IHC systems PATHWAY®/CONFIRM™ rmAb clone 4B5 (Ventana), and HercepTest™ (Dako) were in this assessment the most reliable methods for the semi-quantitative IHC determination of the HER-2 protein expression. In-house validated assays gave a too high proportion of insufficient results, typically false negative. The inclusion of the 2+ tumours (from run B5 onwards) with and without HER-2 gene amplification is essential to evaluate the IHC HER-2 performance and the robustness of the protocols used by the participants. While scoring consensus is acceptable for laboratories with sufficient stains, it is markedly low for laboratories with insufficient stains, indicating that lack of laboratory proficiency and lack of training in HER-2 scoring is associated.

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 and excessive retrieval, same protocol

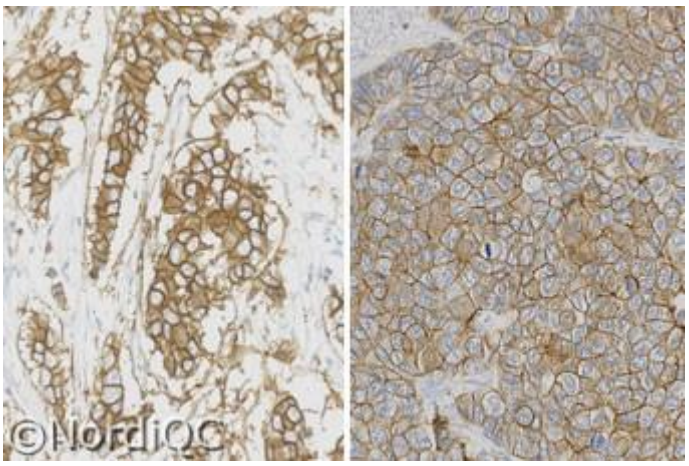


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/Chr. 17 ratio of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio of 2.5 – 2.9.
> 10 % of the neoplastic cells show a moderate complete membranous staining corresponding to 2+.

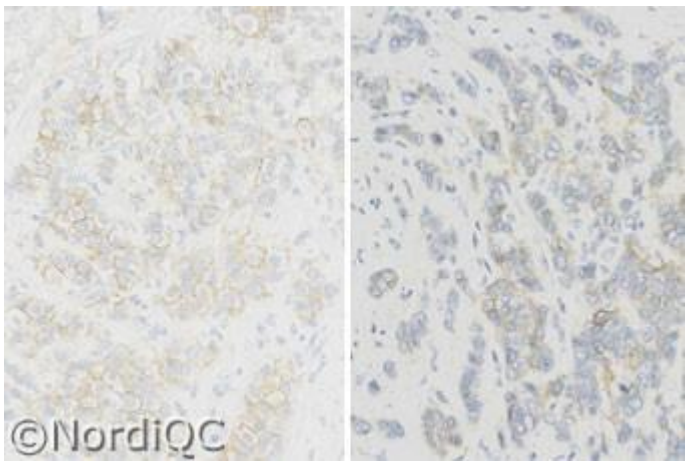


Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 with a a HER-2/Chr. 17 ratio of 1.3 – 1.6.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio of 1.2 – 1.4.
The neoplastic cells show a faint membranous staining corresponding to 1+.

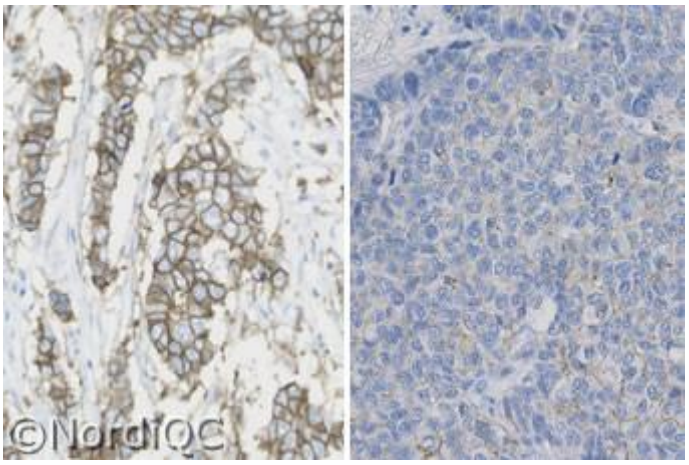


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a a HER-2/Chr. 17 ratio of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio of 2.5 – 2.9.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be referred to ISH.

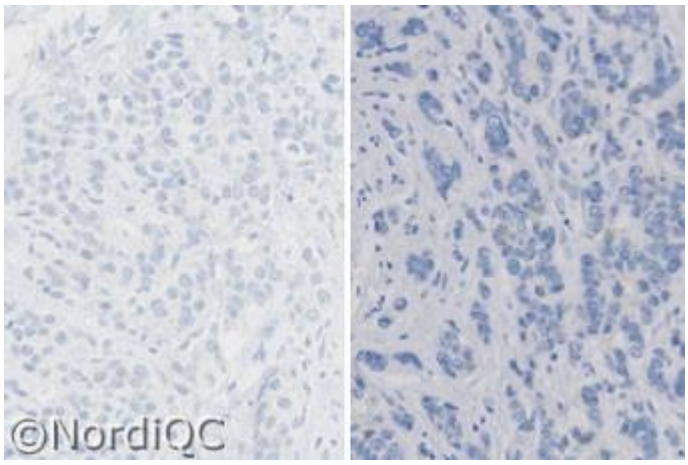


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/Chr. 17 ratio of 1.3 – 1.6.
> 10 % of the neoplastic cells show a moderate but incomplete membrane staining corresponding to 1+.

Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio of 1.2 – 1.4.
< 10 % of the neoplastic cells show a membranous staining corresponding to 1+.

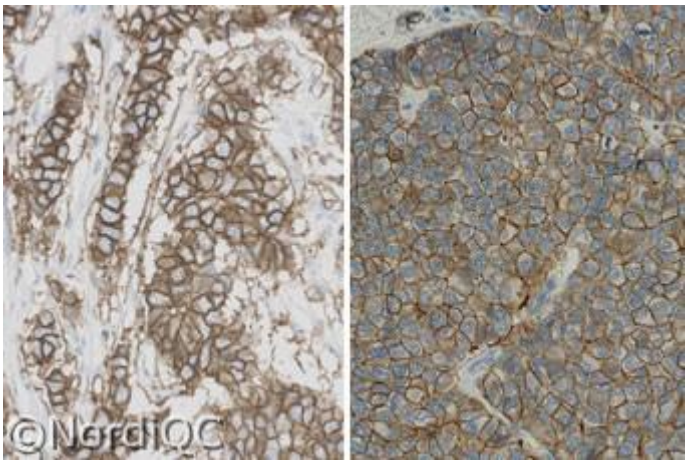


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/Chr. 17 ratio > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio of 2.5 – 2.9.
> 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+. Also compare the results in Figs. 3b left and right.

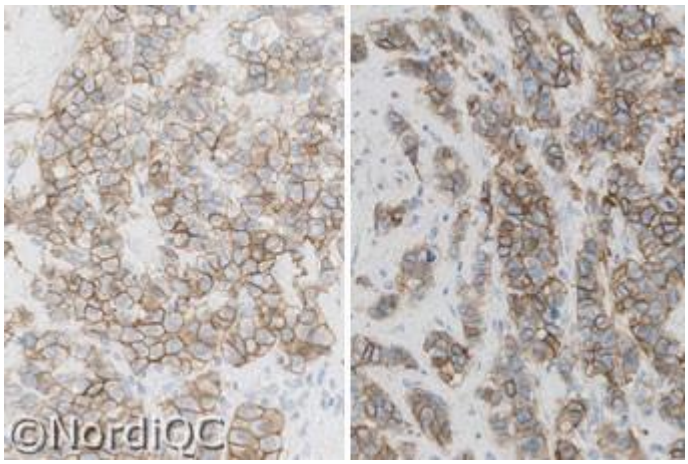


Fig. 3b
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/Chr.17 ratio of 1.3 – 1.6.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+. The tumour was interpreted both by NordiQC and the laboratory as 3+, and thus false positive.

Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio of 1.2 – 1.4.
> 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+. In the NordiQC reference laboratories this tumour was 1+.

SN/MV/LE 6-12-2011