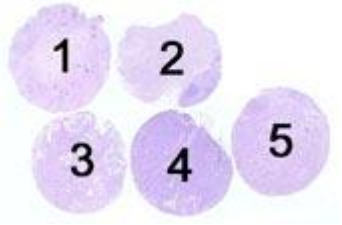
Assessment Run B11 2011
HER2 IHC

Material

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

	IHC	ISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2/chr17 ratio****
1. Breast ductal carcinoma	0	1.1 – 1.3
2. Breast ductal carcinoma	1+	1.2 – 1.4
3. Breast ductal carcinoma	1+ - 2+	1.4 – 1.6
4. Breast ductal carcinoma	2+	2.4 – 2.9
5. Breast ductal carcinoma	3+	> 6.0, clusters



*HER-2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits and antibodies (HercepTest™, Dako & PATHWAY®, Ventana) in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 ratio as achieved by using HER-2 FISH pharmDX™ Kit, Dako and INFORM™ HER-2 Dual colour ISH, 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 immunohistochemical staining marked as score 0 or 1+ in the breast ductal carcinomas no. 1 & 2.
- A clear and unequivocal immunohistochemical staining marked as score 1+ or 2+ in the breast ductal carcinoma no 3.
- A clear and unequivocal immunohistochemical staining marked as score 2+ or 3+ in the breast ductal carcinoma no 4.
- A clear and unequivocal immunohistochemical 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

209 laboratories participated in this assessment. 83 % achieved a sufficient mark. In table 1 the antibodies (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**	**Borderline**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
PATHWAY® rmAb clone **4B5, 790-2991**	56	Ventana	55	0	0	1	98 %	98 %
CONFIRM™, rmAb clone **4B5, 800-2996**	30	Ventana	28	2	0	0	100 %	100 %
HercepTest™ **SK001**	27	Dako	22	2	0	3	89 %	96 %
HercepTest™ **K5204**	6	Dako	3	1	0	2	67 %	75 %
HercepTest™ **K5207**	17	Dako	15	0	1	1	88 %	94 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	8	Leica	7	1	0	0	100 %	100 %
Abs for in-house HER-2 systems, concentrated Ab	**N**	**Vendor**	**Optimal**	**Good**	**Borderline**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
pAb clone **A0485**	35	Dako	16	6	3	10	63 %	63 %
mAb clone **CB11**	5 1 1 1	Leica/Novocastra BioGenex Monosan NeoMarkers	2	1	2	3	38 %	60 %
mAb clone **e-2-4001+3B5**	1	NeoMarkers	0	1	0	0	-	-
rmAb clone **SP3**	14 2 1 1 1	NeoMarkers Zytomed Master Diagnostica Spring Vector	8	2	0	9	53 %	64 %
rmAb clone **EP1045Y**	1	Epitomics	1	0	0	0	-	-
Abs for in-house HER-2 systems, RTU Ab								
mAb clone **CB11, AM134-5ME**	1	BioGenex	0	0	0	1	-	-
Total	209		157	16	6	30	-	-
Proportion			75 %	8 %	3 %	14 %	83 %	-

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 approved systems

PATHWAY® rmAb clone **4B5** (Ventana): 55 out of 56 (98 %) protocols 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 54 out of 55 (98%) laboratories produced a sufficient staining (optimal or good).

CONFIRM™ rmAb clone **4B5** (Ventana): 28 out of 30 (93 %) protocols 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 in the range of 8 – 60 min. and either iView or UltraView was used as the detection kit. Using these protocol settings 30 out of 30 (100 %) laboratories produced a sufficient staining.

HercepTest™ SK001 (Dako): 22 out of 27 (81 %) protocols were assessed as optimal. The protocols giving an 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 an incubation time of 20-30 min in the primary Ab. Using these protocol settings 23 out of 24 (96 %) laboratories produced a sufficient staining.

HercepTest™ K5204 (Dako): 3 out of 6 (50 %) protocols were assessed as optimal. The protocols giving an 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 an incubation time of 20-30 min in the primary Ab. Using these protocol settings 3 out

of 4 (75 %) laboratories produced a sufficient staining.

HercepTest™ K5207 (Dako): 15 out of 17 (88 %) protocols were assessed as optimal. The protocols giving an optimal result were typically based on HIER in HercepTest™ epitope retrieval solution at 95 - 99°C for 40 min in a water bath or PT Link and an incubation time of 30 min in the primary Ab. Using these protocol settings 15 out of 16 (94 %) laboratories produced a sufficient staining.

CE IVD approved systems

Oracle™ (Leica) mAb clone **CB11**: 7 out of 8 (88 %) protocols were assessed as optimal. The protocols giving an optimal result were based on HIER in Bond Epitope Retrieval Solution (BERS) 1 for 25 min. and an incubation time for 15-30 min. of the primary Ab and using Refine as the detection system. Using these protocol settings 8 out of 8 (100 %) laboratories produced a sufficient staining.

Abs for in-house systems

pAb **A0485**: 16 out of 35 (46 %) protocols were assessed as optimal. All protocols giving an optimal staining were based on HIER using either Target Retreival Solution (TRS) low pH 6.1 (Dako) (10/22)*, Tris-EDTA/EGTA pH 9 (3/4), Citrate pH 6 (1/3), TRS pH 9 (Dako) (1/1) or CC2 (BenchMark, Ventana) (1/1). The pAb A0485 was typically diluted in the range of 1:200-1:700 depending on the total sensitivity of the protocol employed. Using these protocol settings 19 out of 30 (63 %) laboratories produced a sufficient staining.
*(number of optimal results/number of laboratories using this buffer)

mAb **CB11**: 2 out of 8 protocols (25 %) 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 (1/4). 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 5 (60 %) laboratories produced a sufficient staining.

rmAb **SP3**: 8 out of 19 (42 %) protocols were assessed as optimal. The optimal protocols were based on HIER using either Tris-EDTA/EGTA pH 9 (2/6), BERS 1 (Bond, Leica) (1/1), BERS 2 (Bond, Leica) (1/2), CC1 (BenchMark, Ventana)(1/3), EDTA/EGTA pH8 (1/2) or Citrate pH 6 (2/4) as HIER buffer. The rmAb clone SP3 was typically diluted in the range of 1:40-200 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 14 (64 %) laboratories produced a sufficient staining.

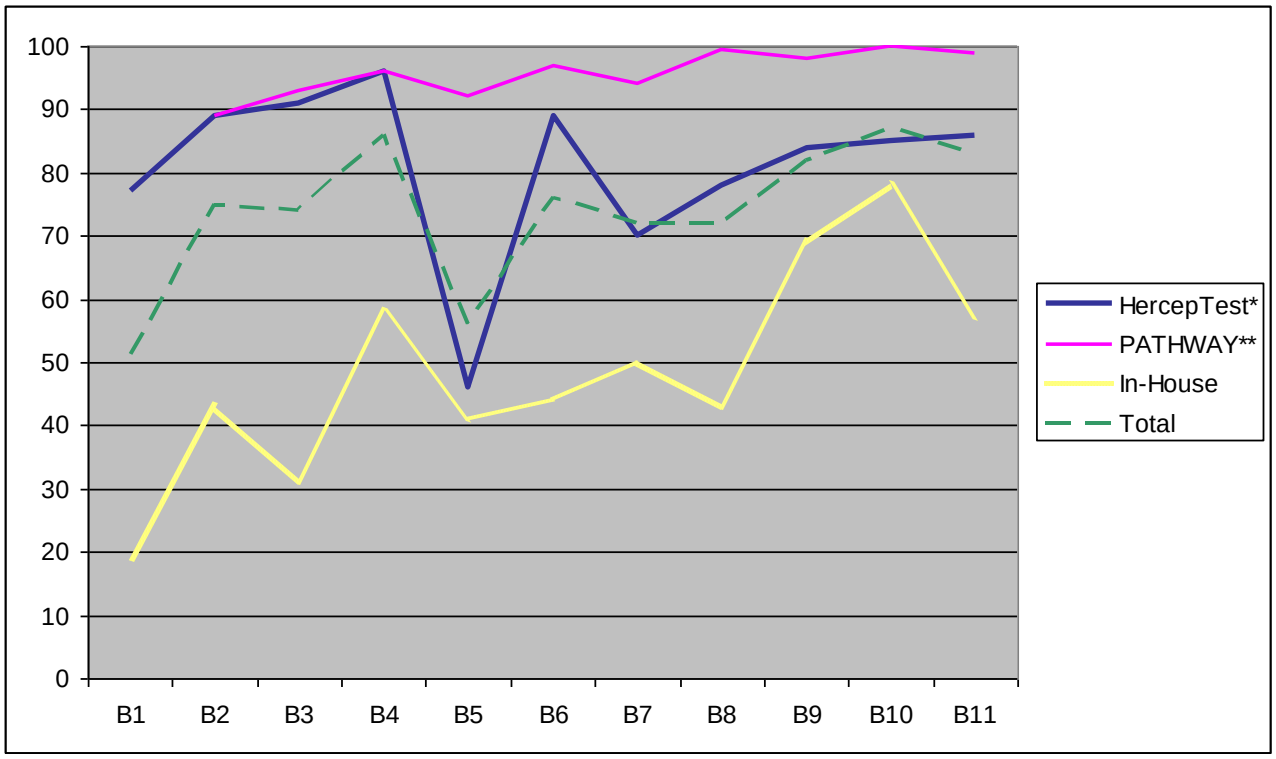
rmAb **EP1045Y**. The protocol giving an optimal staining was based on HIER in a pressure cooker using Diva Decloaker pH 6.2 (Biocare), an incubation time for 45 min. in the primary Ab diluted 1:50 and using MACH4, Biocare as the detection system.

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 critical 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 2.4 – 2.9) by ISH. The weak or false negative reactions were seen in 29 out of the 36 insufficient results (81%) whereas the remaining 7 (19%) insufficient results were characterized by a false positive staining and/or a poor signal-to-noise ratio in the 3 HER-2 non-amplified tumours, no. 1, 2 & 3. The weak or false negative results were seen both with in-house protocols and Ready-To-Use (RTU) systems as HercepTest™, Dako, while the false positive results were only seen when an in-house protocol 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 use of a RTU Ab not applied within a system for which this product was calibrated. Regarding the insufficient results for the 7 laboratories using HercepTest™, Dako, 4 laboratories did not follow the protocol guidelines from Dako, as e.g. the incubation time in the primary Ab was reduced, HIER was shortened or performed in a microwave oven instead of a calibrated water bath, whereas the remaining 3 protocols were performed according to the recommended protocol guidelines.
The false positive staining reactions were caused by use of a too sensitive protocol - e.g. a too concentrated format of the primary Ab - giving a continuous membranous staining of > 10 % of the neoplastic cells in the non-amplified tumours and in the normal epithelial cells of entrapped breast ductal glands.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a pass rate of 94 % (136 out of 144 laboratories), which was similar to the pass rates obtained in run B9 and B10. The pass rate for the in-house systems as a group was 57 % (37 out of 65 laboratories), which was a significant decrease compared to the pass rate of 78 % in run B10 and 69 % in run B9 for this group.

The use of in-house validated HER-2 assays is decreasing: In this run 31 % (65 laboratories) used an in-house HER-2 validated assay, compared to 37 % and 41 % in run B10 and B9, respectively.
This was the 11th NordiQC HER-2 assessment in the breast cancer module. As illustrated in Fig. 1, the two FDA approved systems PATHWAY®/CONFIRM™ (Ventana, rmAb clone 4B5) and HercepTest™ (Dako), have constantly given a superior pass rate compared to the in-house HER-2 assays. The average pass rate in the 11 consecutive runs has been 96 % for PATHWAY®/CONFIRM™ (Ventana, rmAb clone 4B5), 81 % for HercepTest™ (Dako) and 49 % for the in-house assays.

Fig. 1. **Pass rate through the 11 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+) on the stained sections. For 135 out of the 178 laboratories (76 %) 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 an interpretation in concordance with the NordiQC assessors was seen in 84 % (116 out of 138), which was an improvement from 78 % obtained in the two previous runs B9 and B10. An insufficient staining combined with an interpretation in concordance with the NordiQC assessor group was seen in 45 % (13 out of 29) of the laboratories.

Conclusion
The FDA approved HER-2 IHC systems PATHWAY®/CONFIRM™ rmAb clone 4B5 (Ventana), HercepTest™, Dako and the CE IVD labelled system Oracle™ (Leica), were in this assessment the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression. In-house validated assays gave a 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.

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

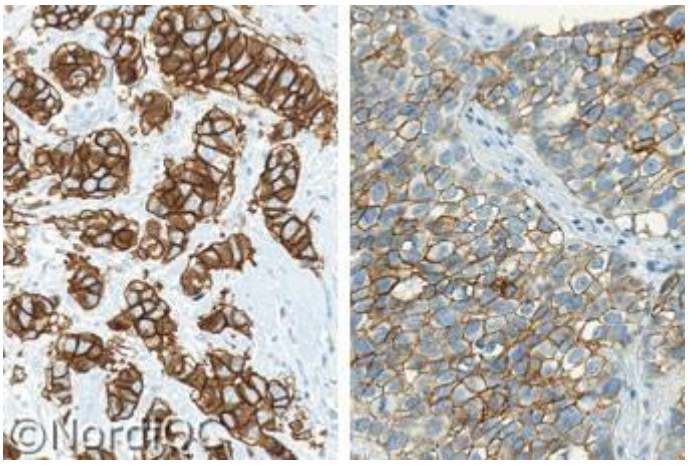


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.4 – 2.9.
> 10 % of the neoplastic cells show a moderate complete membranous staining corresponding to 2+.

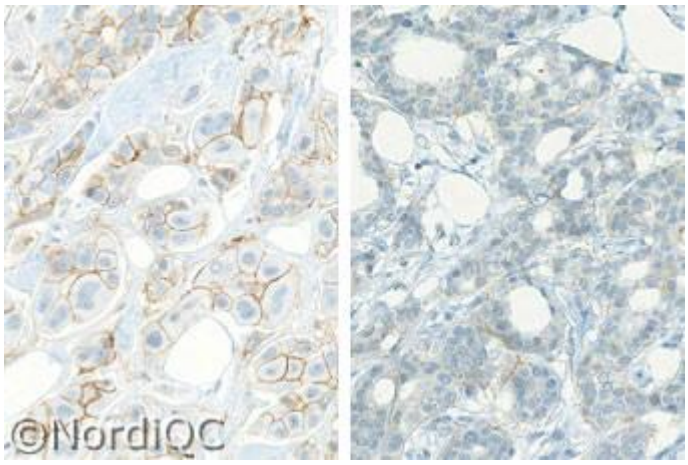


Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio of of 1.4 – 1.6.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.2 – 1.4.
The neoplastic cells show a faint membranous staining corresponding to 1+.

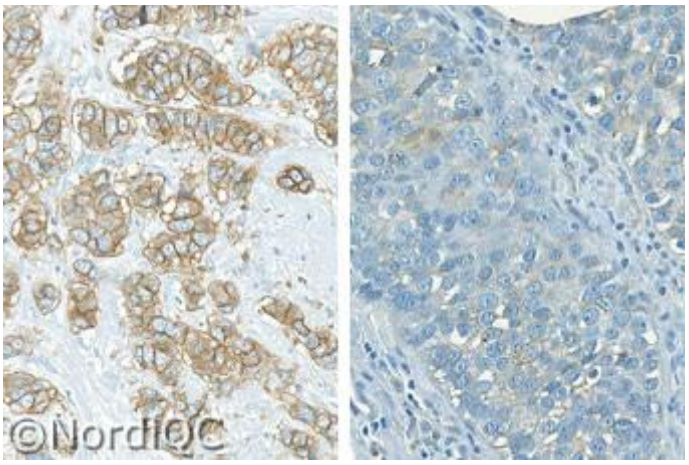


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.4 – 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.

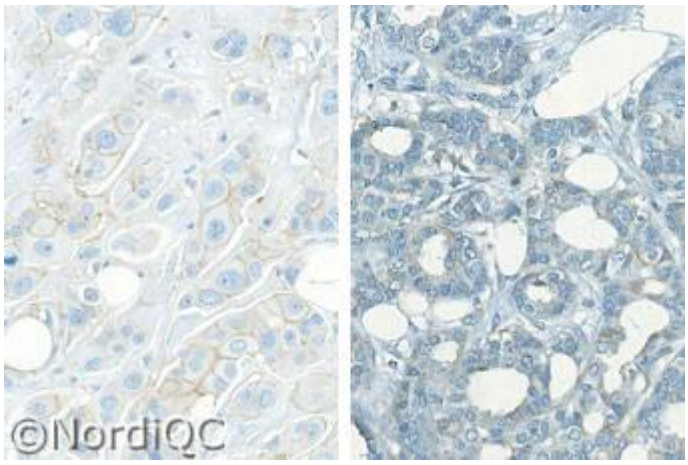


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio of 1.4 – 1.6.
> 10 % of the neoplastic cells show a moderate but incomplete membrane staining corresponding to 1+.

Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.2 – 1.4.
< 10 % of the neoplastic cells show a partial membranous staining corresponding to 0.

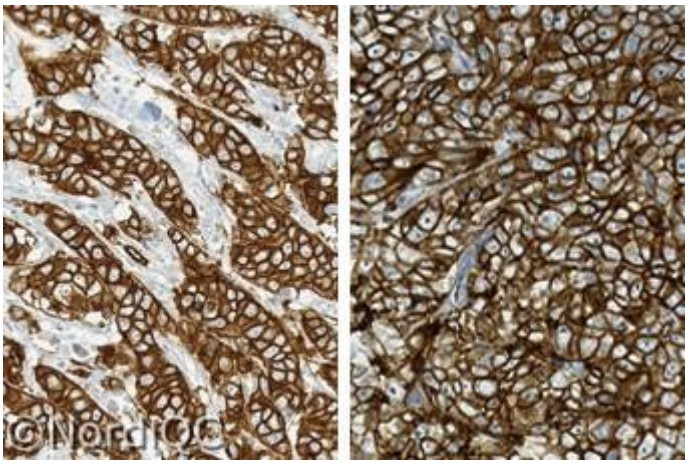


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.4 – 2.9. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+. Also compare the results in Figs. 3b left and right.

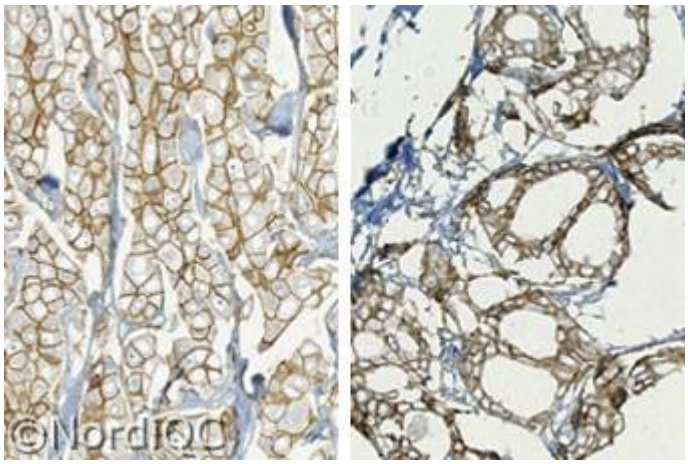


Fig. 3b
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio of 1.4 – 1.6. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+. The tumour was interpreted both by NordiQC and the laboratory as 3+, and thus false positive.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.2 – 1.4. > 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+. However, the interpretation is complicated due to excessive retrieval.

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