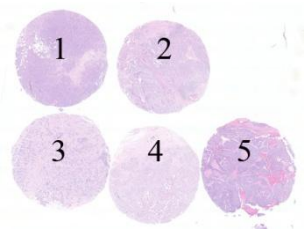
HER-2 IHC
Assessment Run B16 2013

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 carcinoma	2+	1.4 – 1.7 (u)
2.Breast carcinoma	2+	2.3 – 2.9 (a)
3.Breast carcinoma	0 or1+	1.1 – 1.4 (u)
4.Breast carcinoma	3+	> 6 (a)
5.Breast carcinoma	0 or 1+	1.0 – 1.3 (u)

* HER-2 immunohistochemical score (see table below) as achieved by using the three FDA/CE-IVD approved kits and antibodies, Oracle™ (Leica), HercepTest™ (Dako), and PATHWAY® (Ventana)
** 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 used 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** were:

- Staining corresponding to score 0 or 1+ in carcinomas no. 3 and 5.
- Staining corresponding to score 1+ or 2+ in carcinoma no 1.
- Staining corresponding to score 2+ or 3+ in carcinoma no 2.
- Staining corresponding to score 3+ in carcinoma no 4.
- No or only weak cytoplasmic reaction that did not affect interpretation of the true membranous HER-2 reaction.

A staining was assessed as good, if the HER-2 gene amplified tumour no. 4 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by ISH according to the ASCO/CAP guidelines and the Nordic national guidelines) 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+ tumours and the 2+ tumour without gene amplification showing a 3+ reaction).

Results

285 laboratories participated in this assessment. 91 % achieved a sufficient mark. Results for the HER-2 systems and antibodies (Abs) used and assessment marks are summarized in table 1.

Table 1. **Assessment marks for the IHC systems antibodies**

FDA approved HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY®, rmAb clone **4B5, 790-2991**	97	Ventana	86	4	0	7	93 %	95 %
CONFIRM™, rmAb clone **4B5, 790-4493**	38	Ventana	36	0	0	2	95 %	97 %
CONFIRM™, rmAb clone **4B5, 800-2996**	14	Ventana	14	0	0	0	100 %	100 %
HercepTest™ **SK001**	30	Dako	26	2	0	2	93 %	100 %
HercepTest™ **K5207**	11	Dako	9	1	0	1	91 %	100 %
HercepTest™ **K5204**	7	Dako	5	2	0	0	100 %	100 %
Oracle™ mAb clone **CB11, TA9145**	8	Leica	3	4	0	1	88 %	86 %
Concentrated antibodies for in-house HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff.OPS[2]
mAb clone **CB11**	3 1 1	Leica/Novocastra BioGenex Monosan	0	3	1	1	60 %	-
rmAb clone **EP1045Y**	1	Epitomics	0	0	0	1	-	-
rmAb clone **SP3**	13 1 1 1	Thermo/NeoMarkers Cell Marque Spring Bioscience Zytomed	8	7	1	0	94 %	100 %
pAb clone **A0485**	56	Dako	38	9	0	9	84 %	87 %
RTU Antibodies for in-house HER-2 systems	n							
rmAb clone **SP3, 237R**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	1	0	0	0	-	-
Total	285		227	32	2	24	-	-
Proportion	-		80 %	11 %	1 %	8 %	91 %	-

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** (790-2991, Ventana): 86 of 97 (89 %) stains 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 – 32 min. incubation of the primary Ab and UltraView or iView as detection kit. Using these protocol settings 88 of 93 (95 %) laboratories produced a sufficient staining.

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 36 of 38 (95 %) stains 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 UltraView or iView as detection kit.
Using these protocol settings 36 of 37 (97 %) laboratories produced a sufficient staining.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 14 of 14 (100 %) stains were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1(mild and standard) in the BenchMark XT, GX or Ultra, 16 – 32 min. incubation of the primary Ab and UltraView or iView as the detection kit. Using these protocol settings 14 of 14 (100 %) laboratories produced a sufficient staining.

HercepTest™ SK001 (Dako): 26 of 30 (87 %) stains were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest epitope retrieval solution at 97 - 98°C for 40 min. in a water bath or PT Link, 30 min. incubation of the primary Ab and EnVision as detection kit. Using these protocol settings 18 of 18 (100 %) laboratories produced a sufficient staining.

HercepTest™ K5207 (Dako): 9 of 11 (82 %) stains were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest epitope retrieval solution at 97 - 98°C for 40 min. in a water bath, 30 min. incubation of the primary Ab and EnVision as detection kit. Using these protocol settings 9 of 9 (100 %) laboratories produced a sufficient staining.

HercepTest™ K5204 (Dako): 5 of 7 (71 %) stains were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest epitope retrieval solution at 95 - 98°C for 40 min. in a water bath or PT Link, 30 min. incubation of the primary Ab and EnVision as detection kit. Using these protocol settings 5 of 5 (100 %) laboratories produced a sufficient staining.

Oracle™ (Leica) mAb clone CB11: 3 of 8 (38 %) stains were assessed as optimal. Protocols with optimal results were based on HIER in Bond Epitope Retrieval Solution (BERS1) for 20-25 min., 30 min. incubation of the primary Ab and Oracle polymer as detection kit. Using these protocol settings 6 of 7 (86 %) laboratories produced a sufficient staining.

Concentrated antibodies for in-house systems
rmAb **SP3**: 8 of 16 (50 %) stains were assessed as optimal. Protocols with optimal results were based on HIER using either Target Retrieval Solution (TRS) pH 9 (Dako) (1/1)*, CC1 (BenchMark, Ventana) (3/4), BERS2 (Bond, Leica) (1/3) or Citrate pH 6 (3/3). 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 11 of 11 (100 %) laboratories produced a sufficient staining.
* (number of optimal results/number of laboratories using this buffer)

pAb **A0485**: 38 of 56 (68 %) stains were assessed as optimal. Protocols with optimal results were based on HIER using either TRS pH 6.1 (Dako) (18/23), TRS low pH 6.1, 3-in-1 (Dako) (4/5), TRS pH 9 (Dako) (4/4), TRS pH 9 (3-in-1) (Dako) (2/4), CC1 (BenchMark, Ventana) (1/5), BERS1 (Bond, Leica) (4/5), BERS2 (Bond, Leica) (1/2), Tris-EDTA/EGTA pH 9 (1/2) or Citrate pH 6 (3/5). 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 45 of 52 (87 %) laboratories produced a sufficient staining.

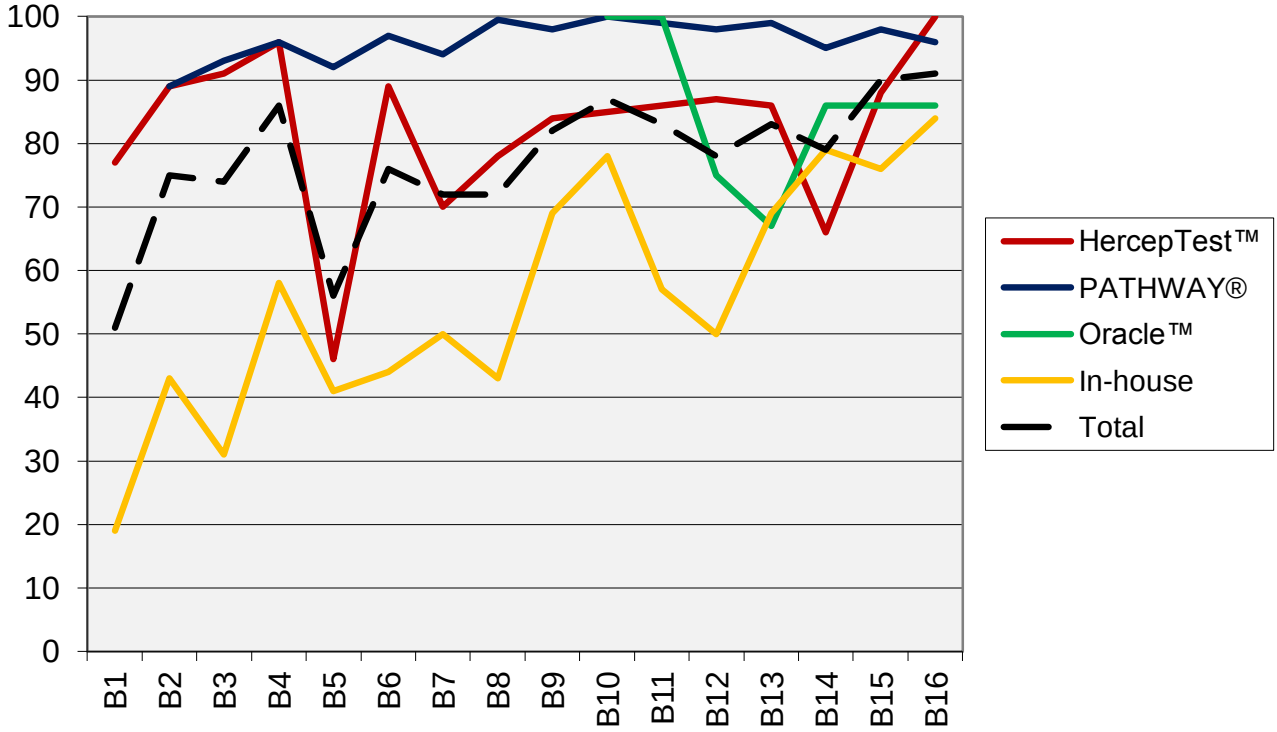
Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, the prominent feature of an insufficient HER-2 staining was a too weak or false negative staining reaction, which particularly – and most critically – was observed as a 0/1+ IHC reaction in the HER-2 gene amplified breast carcinoma no. 2. 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 – 2.9) by ISH. A false negative staining reaction of the breast carcinoma no. 2 was seen in 77 % of the insufficient results (20 of 26 laboratories), whereas a false positive staining characterized by a 3+ staining in the HER-2 non-amplified tumour no. 1, was seen in 15 % of the insufficient results (4 of 26). In the remaining insufficient results, a poor signal-to-noise ratio was seen, complicating the interpretation.
False negative results were seen both in in-house validated assays and FDA-/CE-IVD approved systems, while poor signal-to-noise ratios and false positive results only were seen in in-house validated assays. The weak and false negative results were for the in-house systems 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 in-house validated IHC assays, see fig 1.
The FDA-/CE-IVD approved HER-2 IHC systems PATHWAY® & CONFIRM™ (Ventana, rmAb clone 4B5) have consistently provided a high pass rate, superior to especially in-house HER-2 assays. Thus, the average pass rates in 16 consecutive NordiQC assessments for HER-2 IHC has been 96 % for PATHWAY®/CONFIRM™ compared to an average pass rate of 56 % of the in-house HER-2 assays.
In this assessment HercepTest™ (Dako) showed a pass rate of 100 % provided that protocol settings were applied as recommended by Dako (!)

The overall pass rate of 91 % obtained in this assessment is significantly higher compared to the pass rate of 51 % seen in the 1' run (B1) of HER-2 IHC in the NordiQC breast module. As seen in Fig. 1 especially the pass rate for in-house validated assays have been improved consistently from the B1 to B16. In run B1 the pass rate for this group was 19 % and in the present run B16 a pass rate of 84 % was seen.

The proportion of laboratories using in-house validated IHC assays is relatively consistent. In this run, 28 % of the participants (n=80) used in-house validated assays compared to 28 - 31 % in the last 5 assessments.

Figure. 1 Pass rate in the 16 HER-2 IHC assessments performed in the NordiQC breast module.



Scoring consensus
83% of the participating laboratories (236 of 285) submitted as requested their own scores (0, 1+, 2+, 3+) of the stained sections. Of these, 90% were in total agreement with the scores given by the NordiQC assessor group. A sufficient staining combined with interpretation in agreement with the NordiQC assessors was seen in 95 % (207 out of 217), which was an increase from 87 % obtained in the previous run B15.
An insufficient staining combined with interpretation in agreement with the NordiQC assessor group was seen in 37 % (7 out of 19) of the laboratories, which virtually was identical to the level seen in run B15. The discrepancy was typically related to the interpretation of the staining reaction in the tissue core no. 2, which by the laboratories was classified as 2+, while interpreted as 1+ by the NordiQC assessors in the stains submitted.

Conclusion
The FDA-/CE-IVD approved HER-2 IHC systems PATHWAY® & CONFIRM™ rmAb clone 4B5 (Ventana), HercepTest™ (Dako) and Oracle™ (Leica) were in this assessment the most reliable methods for semi-quantitative IHC determination of HER-2 protein expression. The overall pass rate increased to 91%, the highest observed in 16 NordiQC HER-2 runs performed. 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
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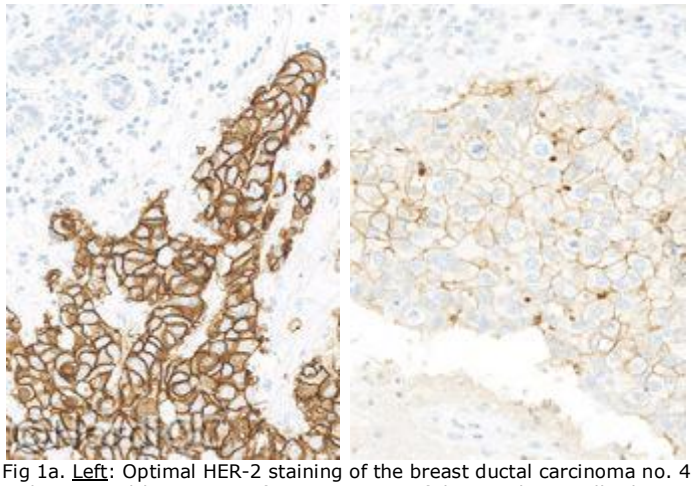

Fig 1a. Left: Optimal HER-2 staining of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of > 6.0.> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 2.3 – 2.9. > 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+.

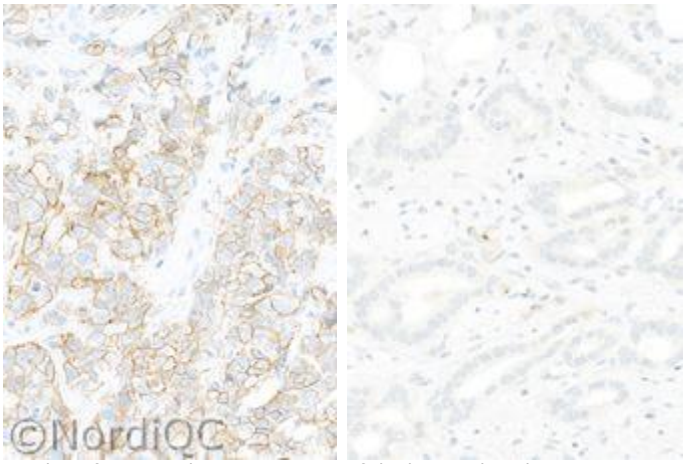

Fig 1b. Left: Optimal HER-2 staining of the breast ductal carcinoma no. 1 with a HER-2/chr17 ratio of 1.4 – 1.7. > 10 % of the neoplastic cells show a weak to moderate and complete membranous staining corresponding to 2+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio of 1.1 – 1.4. < 10 % of the neoplastic cells show a membranous staining corresponding to 0.

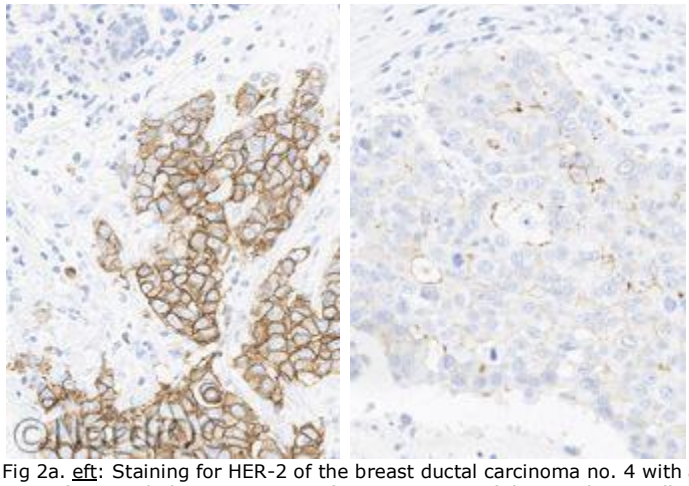

Fig 2a. eft: Staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of > 6.0.> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient and false negative staining for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 2.3 – 2.9.> 10 % of the neoplastic cells show a faint perceptible membranous staining corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be referred to ISH.

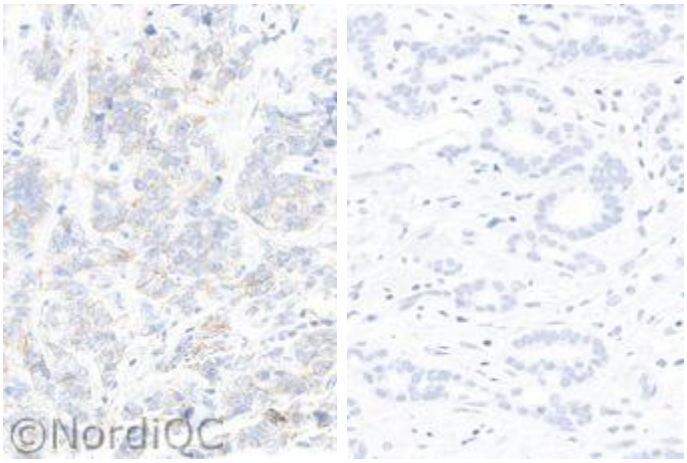

Fig 2b. Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of 1.4 – 1.7. > 10 % of the neoplastic cells show a faint perceptible membranous staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4. < 10 % of the neoplastic cells show a membranous staining corresponding to 0.

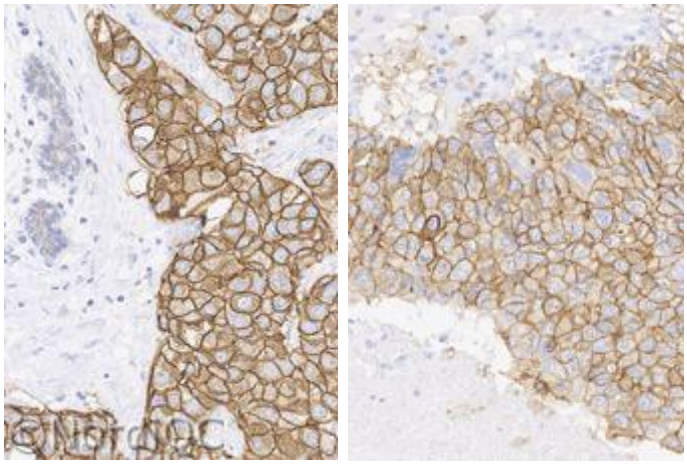

Fig 3a. Left: HER-2 staining of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of > 6.0. >30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 2.3 – 2.9. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Also compare with Figs. 3b, same protocol.

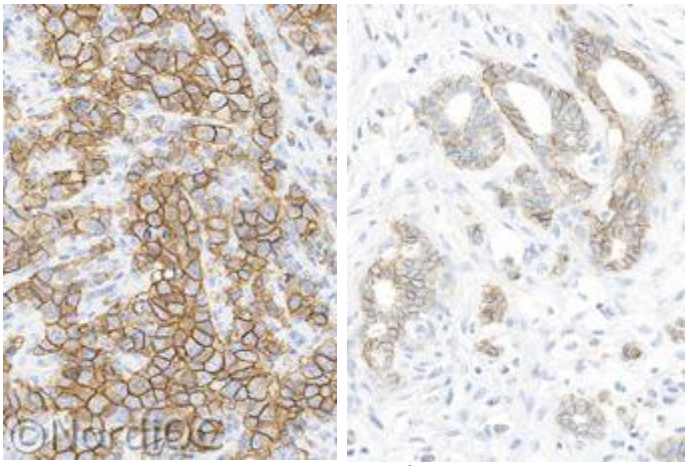

Fig 3b. Left: Insufficient and false positive ´HER-2 staining of the breast ductal carcinoma no. 1 with a HER-2/chr17 ratio of 1.4 – 1.7. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio of 1.1 – 1.4. > 10 % of the neoplastic cells show a weak to moderate incomplete membranous staining corresponding to 1+.

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