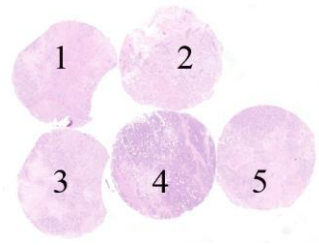
Assessment Run B15 2013
HER-2 IHC

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

	IHC	FISH
	HER-2, Score* (0, 1+, 2+, 3+)	**HER-2 gene/chr 17 ratio****
1.Breast Ductal Carcinoma	0-1+	1.1 – 1.3 (u)
2.Breast Ductal Carcinoma	0-1+	1.1 – 1.4 (u)
3.Breast Ductal Carcinoma	1-2+	1.3 – 1.6 (u)
4.Breast Ductal Carcinoma	2-3+	2.4 – 2.9 (a)
5.Breast Ductal Carcinoma	3+	> 6 (a)



* HER-2 immunohistochemical score (see table below) as achieved by using the FDA approved kit, PATHWAY®, Ventana, in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 Ratio achieved by using HER-2 FISH pharmDX™ Kit, Dako, and HER2 FISH, ZytoVision
a: amplified, u: unamplified

All tissues 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 were

- A clear and unequivocal staining marked as score 0 or 1+ in the carcinomas no. 1 and 2.
- A clear and unequivocal staining marked as score 1+ or 2+ in the carcinoma no 3.
- A clear and unequivocal staining marked as score 2+ or 3+ in the carcinoma no 4.
- A clear and unequivocal staining marked as score 3+ in the 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. 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
272 laboratories participated in this assessment. 90 % achieved a sufficient mark. Table 1 summarizes marks, HER-2 systems and used antibodies (Abs).

Table 1. **IHC systems/antibodies used and assessment marks for HER-2 run B15**

FDA-/CE-IVD approved HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	87	Ventana	83	4	0	0	100 %	100 %
CONFIRM™, rmAb clone **4B5, 800-2996**	28	Ventana	26	1	0	1	96 %	96 %
CONFIRM™, rmAb clone **4B5, 790-4493**	25	Ventana	23	2	0	0	100 %	100 %
HercepTest™ **SK001**	32	Dako	12	14	1	5	81 %	83 %
HercepTest™ **K5207**	8	Dako	5	3	0	0	100 %	100 %
HercepTest™ **K5204**	7	Dako	2	4	0	1	86 %	100 %
Oracle™ mAb clone **CB11, TA9145**	10	Leica	2	7	0	1	90 %	86 %
Antibodies for in-house HER-2 systems, conc. Ab	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11**	5 1 1	Leica/Novocastra BioGenex Monosan	2	5	0	0	100 %	100 %
mAb clone **e-2-4001+3B5**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb clone **SP3**	15 1 1 1	Thermo/NeoMarkers Cell Marque Diagnostic BioSystems Zytomed	8	3	2	5	61 %	77 %
pAb clone **A0485**	46	Dako	21	14	4	7	76 %	79 %
Antibodies for in-house HER-2 systems, RTU Ab	n							
rmAb clone **EP1045Y PME241**	1	Biocare	1	0	0	0	-	-
rmAb clone **SP3, 237R**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	1	0	0	0	-	-
Total	272		187	58	7	20	-	-
Proportion			69 %	21 %	3 %	7 %	90 %	-

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): 83 of 87 (95 %) protocols were assessed as optimal. Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1), short, mild or standard in the BenchMark XT or Ultra, 8 – 60 min incubation of the primary Ab and either iView, UltraView or OptiView as detection kit. Using these protocol settings 86 of 86 (100 %) laboratories produced a sufficient staining.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 26 of 28 (93 %) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 short, mild or standard in the BenchMark XT or Ultra, 16–50 min incubation of the primary Aband either iView or UltraView as detection kit. Using these protocol settings 27 of 28 (96 %) laboratories produced a sufficient staining.

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 23 of 25 (92 %) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1, mild or standard in the BenchMark XT

or Ultra, 4–32 min incubation of the primary Ab and either iView or UltraView as detection kit. Using these protocol settings 24 of 24 (100 %) laboratories produced a sufficient staining.

HercepTest™ SK001 (Dako): 12 of 32 (38 %) 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 19 of 23 (83 %) laboratories produced a sufficient staining.

HercepTest™ K5207 (Dako): 5 of 8 (63 %) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 95 - 98°C for 40 min in a water bath and 30 min incubation of the primary Ab. Using these protocol settings 7 of 7 (100 %) laboratories produced a sufficient staining.

HercepTest™ K5204 (Dako): 2 of 7 (29 %) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 98°C for 40 min in a water bath or PT Link and 30-60 min incubation of the primary Ab. Using these protocol settings 2 of 2 (100 %) laboratories produced a sufficient staining.

Oracle™ (Leica) mAb clone CB11: 2 of 10 (20 %) protocols were assessed as optimal. Protocols with optimal results were based on HIER in Bond Epitope Retrieval Solution (BERS1) for 25 min and 30 min incubation of the primary Ab. Using these protocol settings 6 of 7 (86 %) laboratories produced a sufficient staining.

Antibodies for in-house systems – concentrated antibodies

mAb **CB11**: 2 of 7 protocols (29 %) were assessed as optimal. Protocols with optimal results were based on HIER using Cell Conditioning 1 (Ventana) (1/2)* or BERS1 (Leica) (1/2). The mAb CB11 was diluted in the range of 1:100-1:350 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 of 3 (100 %) laboratories produced a sufficient staining.

* (number of optimal results/number of laboratories using this buffer)

rmAb **SP3**: 8 of 18 (44 %) protocols were assessed as optimal. Protocols with optimal results were based on HIER using either Target Retrieval Solution (TRS) pH 9 (Dako) (1/1), CC1 (Ventana) (2/2), BERS2 (Leica) (2/4), Tris-EDTA/EGTA pH 9 (1/5) or Citrate pH 6 (2/4). 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 10 of 13 (77 %) laboratories produced a sufficient staining.

pAb **A0485**: 21 of 46 (46 %) protocols were assessed as optimal. All protocols with optimal results were based on HIER using either TRS low pH 6.1 (Dako) (13/26), TRS pH 9 (Dako) (3/4), TRS pH 9 (3-in-1) (Dako) (1/5), CC1 (Ventana) (1/2), BERS2 (Leica) (1/1), Tris-EDTA/EGTA pH 9 (1/3) or EDTA/EGTA pH 8 (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 33 of 42 (79 %) laboratories produced a sufficient staining.

Antibodies for in-house HER-2 systems – Ready-To-Use antibodies

rmAb clone **EP1045Y** (product. no. PME342, Biocare): The protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 (Biocare) in a Pressure Cooker, 45 min incubation of the primary Ab and MACH4 (4U534) as detection system.

Comments

In this assessment and in concordance to the previous NordiQC assessments for 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. 4. This tumour was shown to be IHC 2+ in the NordiQC reference laboratories using the HER-2 IHC assay PATHWAY®(Ventana) and showed a low level of HER-2 gene amplification (ratio 2.4 – 2.9) by ISH. A false negative staining reaction of the breast carcinoma no. 4 was seen in 67 % of the insufficient results (18 of 27 laboratories), whereas a poor signal-to-noise ratio, typically caused by an excessive cytoplasmic staining reaction hampering the interpretationn, was seen in 30 % of the insufficient results (8 of 27) and in one case a false positive staining characterized by a 3+ staining in the 3 HER-2 non-amplified tumours, no. 1, 2 and 3 was seen.

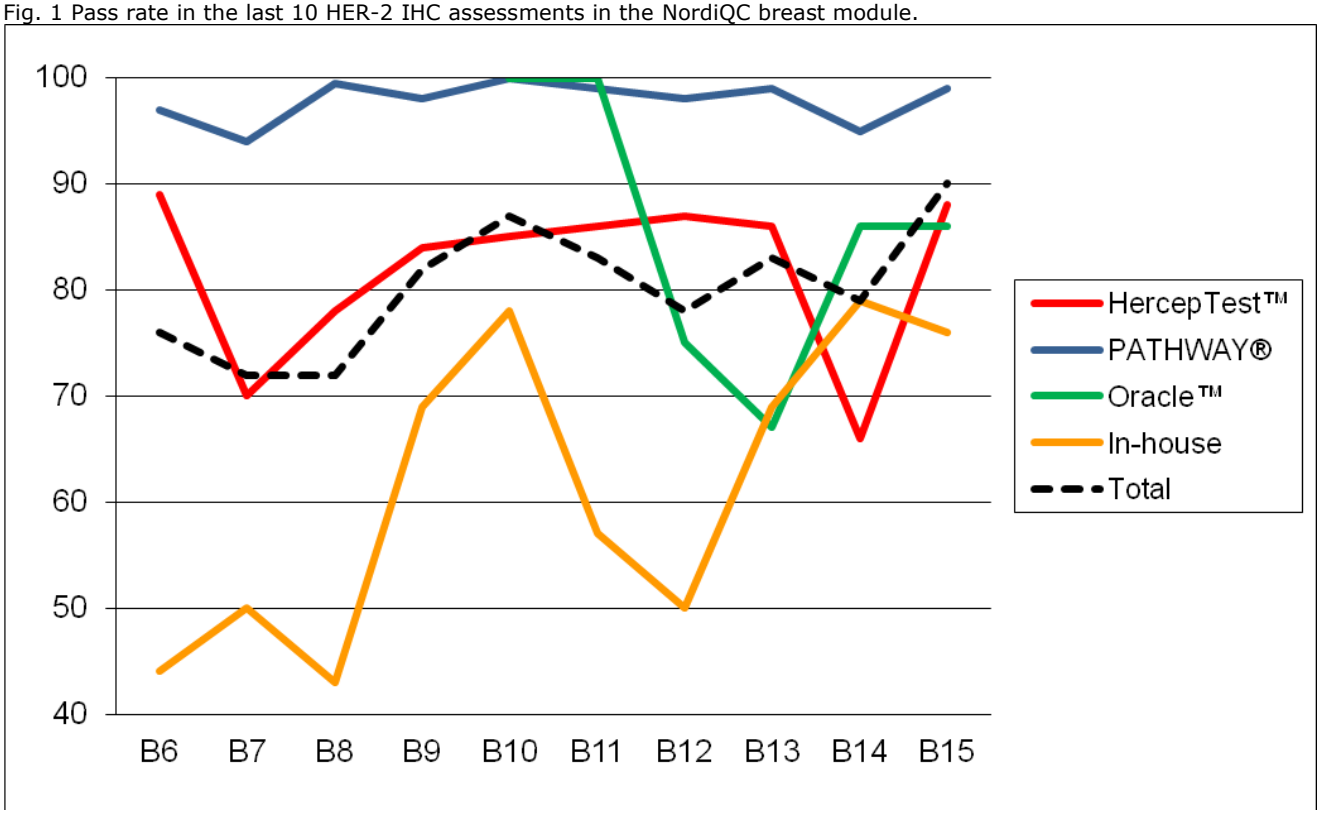
The false negative results and results with a poor signal-to-noise ratio were seen for both the in-house validated assays and the FDA-/CE-IVD approved systems, while the false positive result was seen by an in-house validated assay. The weak and false negative results were for the in-house systems typically caused by a too low sensitivity of the protocol applied e.g. a too low concentration of the primary Ab

and/or insufficient HIER. For the FDA-/CE-IVD approved systems no single plausible cause for insufficient and false negative staining reaction could be identified from the submitted protocols.
In the 3+, HER-2 gene amplified tumour (core no. 5), a relative high proportion of protocols based on HercepTest™(Dako) and Oracle™(Leica) gave a moderate to strong cytoplasmic staining reaction and a reduced proportion of cells with a strong continuous membrane reaction. This pattern was also observed for the pAb A0485, Dako and the mAb clone CB11, Leica applied within an in-house validated assay.

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 figure 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. The average pass rate in the 15 consecutive NordiQC assessment for HER-2 IHC has thus been 96 % for PATHWAY®/CONFIRM™ compared to an average pass rate of 54 % of the in-house HER-2 assays.

The use of in-house validated IHC assays decreases only slightly. In this run in-house validated assays were used by 28 % of the participants (n=75) compared to 29 - 31 % of the participants in the last 4 assessments.

Fig. 1 Pass rate in the last 10 HER-2 IHC assessments in the NordiQC breast module.



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

Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) on the stained sections. For 199 out of the 242 laboratories (82 %) 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 87 % (190 of 219), which was an increase from 84 % obtained in the previous run B14. An insufficient staining combined with an interpretation in concordance with the NordiQC assessor group was seen in 39 % (9 of 23) of the laboratories, compared to 51 % in the previous run.

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 the semi-quantitative IHC determination of the HER-2 protein expression.

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.

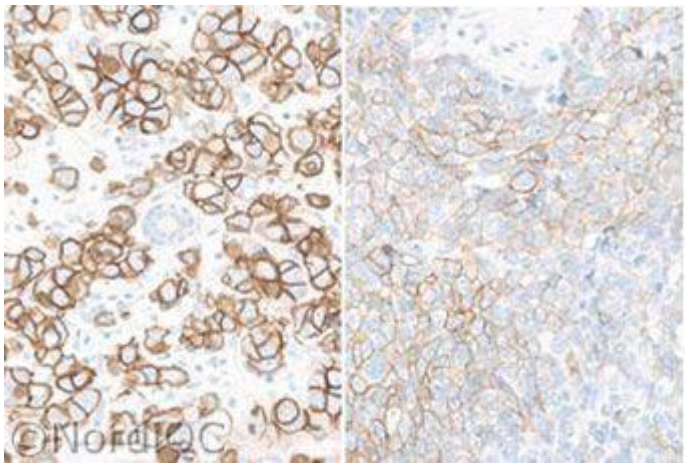


Fig 1a.
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of 2.4 – 2.9.
> 10 % of the neoplastic cells show a moderate complete membranous staining corresponding to 2+.

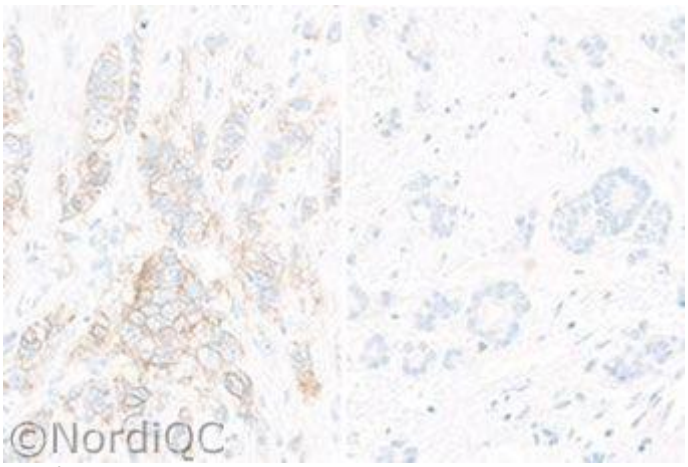


Fig 1b.
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of of 1.3 – 1.6.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4.
< 10 % of the neoplastic cells show a membranous staining corresponding to 0.

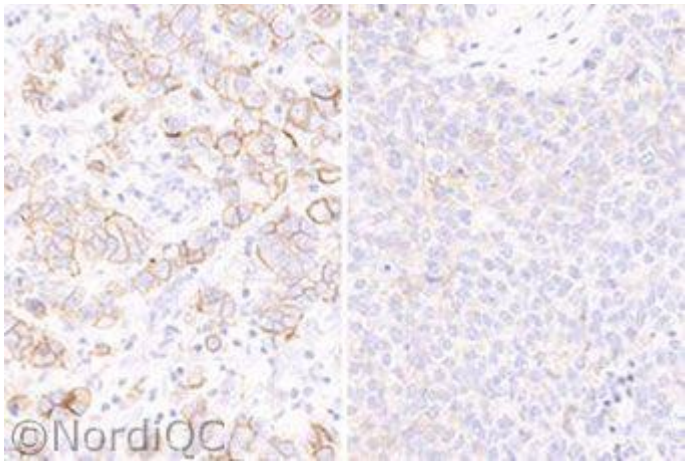


Fig 2a.
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10 % of the neoplastic cells show a weak to moderate and complete membranous staining corresponding to 2+.

Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 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.

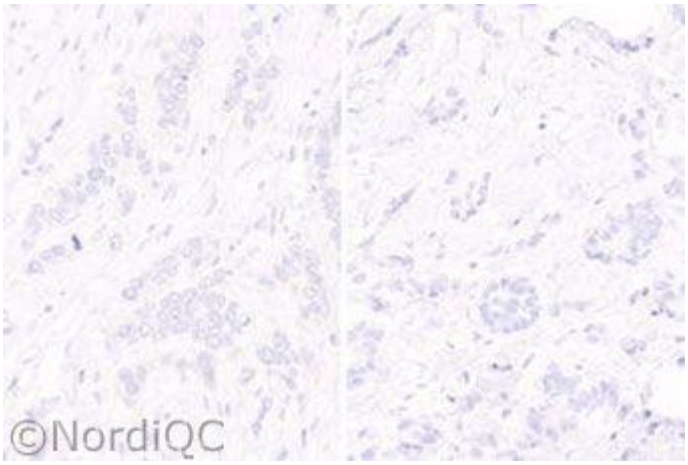


Fig 2b.
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.3 – 1.6.
< 10 % of the neoplastic cells show a membranous staining corresponding to 0.

Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4.
< 10 % of the neoplastic cells show a membranous staining corresponding to 0.

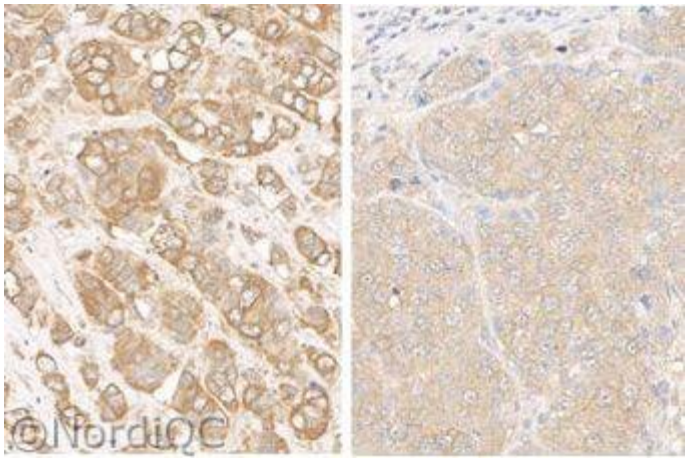


Fig 3a.
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0.
It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.

Right: Staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of 2.4 – 2.9.
It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.

Also compare with Figs. 3b., left & right, same protocol

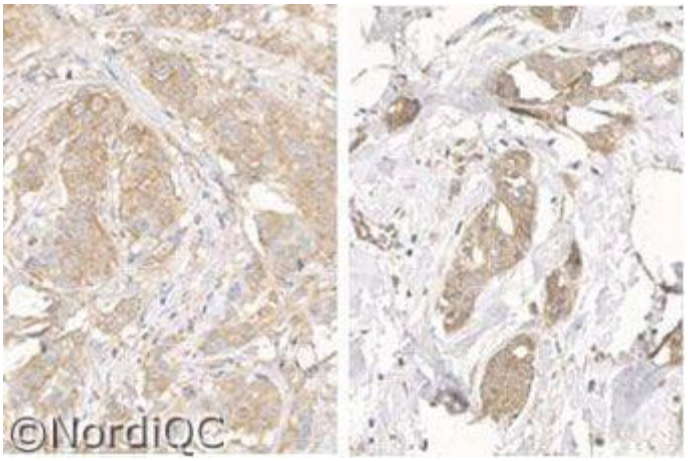


Fig 3b.
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.3 – 1.6.
It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.

Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4.
It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.

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