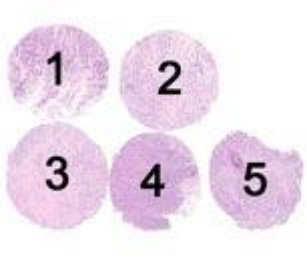
# Assessment Run B14 2012
# HER-2 IHC

## 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 – 1.4 |
| 2. Breast ductal carcinoma | 0-1+ | 1.2 – 1.5 |
| 3. Breast lobular carcinoma | 1-2+ | 1.3 – 1.7 |
| 4. Breast ductal carcinoma | 2-3+ | 2.5 – 2.8 |
| 5. Breast ductal carcinoma | 3+ | > 6.0 |




* HER-2 immunohistochemical score (guidelines 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 (HER-2/chr17) ratio as achieved by using HER-2 FISH pharmDX™ Kit, Dako.

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 were:

- 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

263 laboratories participated in this assessment. 79 % achieved a sufficient mark. Antibodies (Abs) used and marks are summarized in table 1.

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** | 86 | Ventana | 77 | 2 | 0 | 7 | 92 % | 93 % |
| CONFIRM™, rmAb clone **4B5, 790-4493** | 19 | Ventana | 17 | 0 | 0 | 2 | 89 % | 94 % |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CONFIRM™, rmAb clone **4B5, 800-2996** | 27 | Ventana | 27 | 0 | 0 | 0 | 100 % | 100 % |
| HercepTest™ **SK001** | 28 | Dako | 10 | 2 | 0 | 16 | 43 % | 60 % |
| HercepTest™ **K5204** | 9 | Dako | 3 | 0 | 1 | 5 | 33 % | 75 % |
| HercepTest™ **K5207** | 9 | Dako | 4 | 0 | 0 | 5 | 44 % | 80 % |
| Oracle™ mAb clone **CB11, TA9145** | 9 | Leica | 4 | 2 | 0 | 3 | 67 % | 86 % |
| **Abs for in-house HER-2 systems, concentrated Abs.** | | | | | | | | |
| mAb clone **CB11** | 5<br>1<br>1 | Leica/Novocastra<br>BioGenex<br>Monosan | 4 | 3 | 0 | 0 | 100 % | 100 % |
| mAb clone **e-2-4001+3B5** | 1 | Thermo/NeoMarkers | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **EP1045Y** | 1<br>1 | BioGenex<br>Epitomics | 0 | 0 | 0 | 2 | - | - |
| rmAb clone **EP3** | 1 | Epitomics | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **SP3** | 14<br>1 | Thermo/NeoMarkers<br>Zytomed | 10 | 1 | 1 | 3 | 73 % | 79 % |
| pAb clone **A0485** | 46 | Dako | 29 | 11 | 3 | 3 | 87 % | 87 % |
| **Abs for in-house HER-2 systems, RTU Abs.** | | | | | | | | |
| mAb clone **CB11, RTU-CB11** | 2 | Leica/Novocastra | 1 | 0 | 1 | 0 | - | - |
| rmAb clone **SP3, 237R-17** | 1 | Cell Marque | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **SP3, MAD-000308QD** | 1 | Master Diagnostica | 0 | 0 | 0 | 1 | - | - |
| **Total** | 263 | | 187 | 21 | 6 | 49 | - | - |
| **Proportion** | | | 71 % | 8 % | 2 % | 19 % | 79 % | - |

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): 77 of 86 (90 %) protocols were assessed as optimal. These protocols were typically based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1), mild or standard in BenchMark XT or ULTRA, 12-32 min incubation of the primary Ab and either iView, UltraView or OptiView used as detection kit. Using these protocol settings 78 out of 84 (93 %) laboratories produced a sufficient staining.

**CONFIRM**™ rmAb clone **4B5** (790-4493, Ventana): 17 of 19 (89 %) protocols were assessed as optimal. These protocols were typically based on HIER in CC1, mild or standard in BenchMark XT or ULTRA, 8-32 min incubation of the primary Ab and either iView, UltraView or OptiView used as detection kit. Using these protocol settings 17 out of 18 (94 %) laboratories produced a sufficient staining.

**CONFIRM**™ rmAb clone **4B5** (800-2996, Ventana): 27 of 27 (100 %) protocols were assessed as optimal. These protocols were typically based on HIER in CC1 mild in BenchMark XT or ULTRA, 4-40 min incubation of the primary Ab and either iView or UltraView used as detection kit.

**HercepTest**™ **SK001** (Dako): 10 of 28 (36 %) protocols were assessed as optimal. These protocols 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 30 min incubation of the primary Ab. Using these protocol settings 12 out of 20 (60 %) laboratories produced a sufficient staining.

**HercepTest**™ **K5204** (Dako): 3 of 9 (33 %) protocols were assessed as optimal. These protocols were typically based on HIER in HercepTest epitope retrieval solution at 99°C for 40 min in a water bath or PT Link and 30-40 min. incubation of the primary Ab. Using these protocol settings 3 out of 4 (75 %) laboratories produced a sufficient staining.

**HercepTest™ K5207** (Dako): 4 of 9 (44 %) protocols were assessed as optimal. These protocols were based on HIER in HercepTest epitope retrieval solution at 98 - 99°C for 40 min in a water bath and 30 min. incubation of the primary Ab. Using these protocol settings 4 out of 5 (80 %) laboratories produced a sufficient staining.

**Oracle™** (Leica) mAb clone **CB11**: 4 of 9 (44 %) protocols were assessed as optimal. These protocols were based on HIER in Bond Epitope Retrieval Solution 1 (BERS1) for 25 min. and 30 min. incubation of the primary Ab. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining.

**In-house systems - concentrated antibodies**
mAb **CB11**: 4 of 7 protocols (57 %) were assessed as optimal. These protocols were based on HIER using CC1 (Ventana) (2/3)* or Tris-EDTA/EGTA pH 9 (2/2). The mAb CB11 was diluted in the range of 1:90 - 1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 (100 %) laboratories produced a sufficient staining.
* (number of optimal results/number of laboratories using this buffer)

rmAb **SP3**: 10 of 15 (67 %) protocols were assessed as optimal. These protocols were based on HIER using either Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/1), TRS pH 9 (Dako) (1/1), CC1 (Ventana) (2/2), Bond Epitope Retrieval Solution 2 (Leica) (1/3), Tris-EDTA/EGTA pH 9 (2/4) or Citrate pH 6 (3/4). 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 11 of 14 (79 %) laboratories produced a sufficient staining.

pAb **A0485**: 29 of 46 (63 %) protocols were assessed as optimal. These protocols were based on HIER using either Target Retrieval Solution (TRS) low pH 6.1 (Dako) (14/21), TRS pH 9 (Dako) (4/6), TRS pH 9 (3-in-1) (Dako) (1/1), CC1 (Ventana) (3/3), BERS 1 (Leica) (1/1), BERS 2 (Leica) (1/1), Tris-EDTA/EGTA pH 9 (3/5) or Citrate pH 6 (2/8). The pAb A0485 was typically diluted in the range of 1:60 - 1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 40 of 46 (87 %) laboratories produced a sufficient staining.

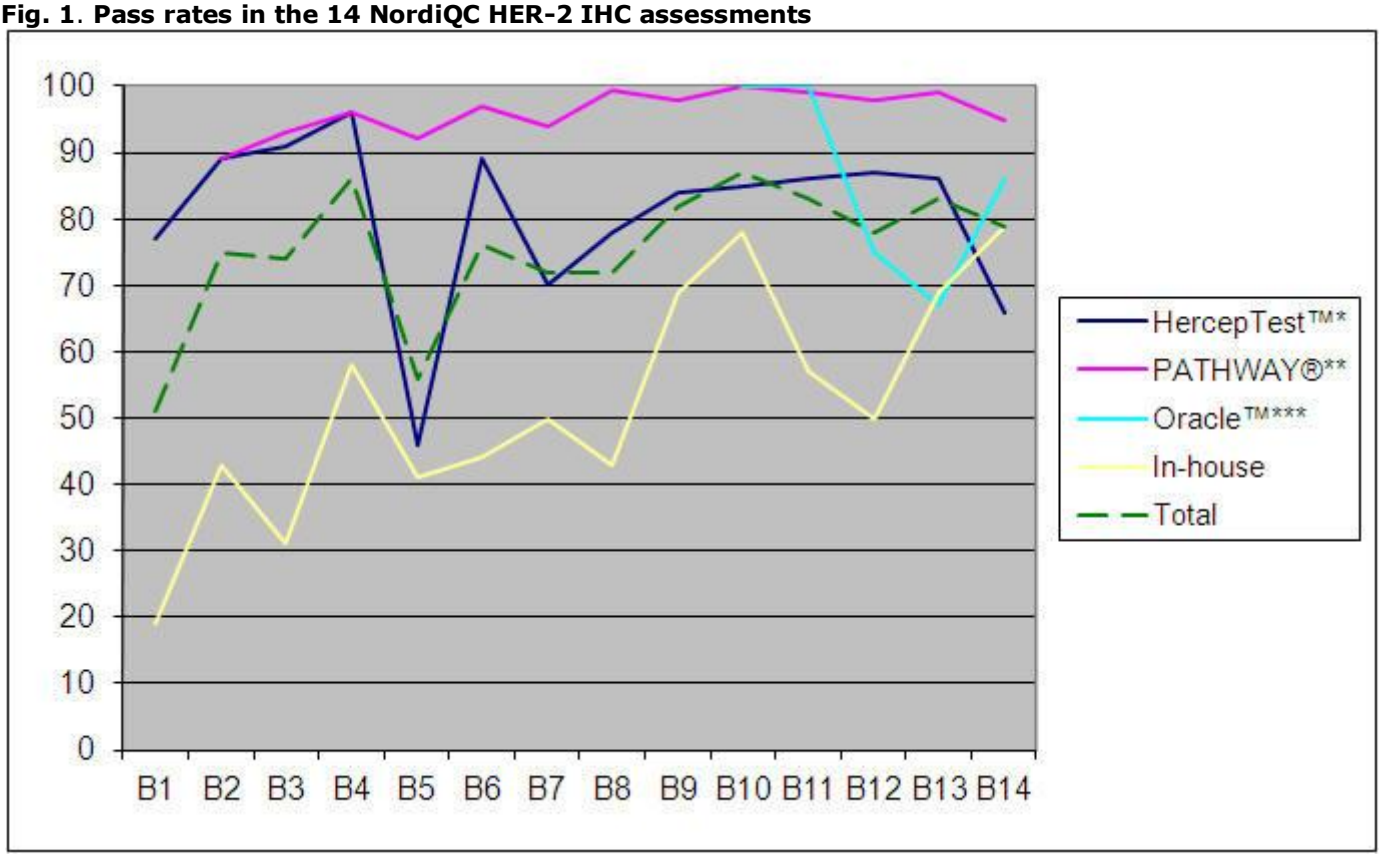
**Comments**
In concordance with the previous NordiQC assessments of HER-2 IHC, the prominent feature of an insufficient HER-2 staining was a too weak or false negative staining reaction, which most critically was observed as a 0/1+ IHC reaction in the HER-2 gene amplified breast carcinoma no. 4. This 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 – 2.8) by ISH. The weak or false negative staining reactions were seen in 89 % of the insufficient results (49 of 55 laboratories), whereas 9 % (5 of 55 laboratories) were characterized by a poor signal-to-noise ratio caused by an excessive cytoplasmic staining reaction hampering the interpretation. Only in 1 of 55 laboratories (2 %) a false positive staining was seen in the 3 HER-2 non-amplified tumours, no. 1, 2 & 3.
The false negative and poor signal-to-noise ratio results were seen for both in-house validated assays and the FDA-/CE-IVD approved systems, while the false positive result was seen in an in-house validated assay. Weak and false negative results were for the in-house systems typically caused by 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 originally was calibrated).
For the FDA-/CE-IVD approved systems 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 and/or insufficient HIER), but most frequently no reason for the insufficient result could be identified from the protocol settings submitted. This was seen for both the HercepTest™ (Dako) PATHWAY®/CONFIRM™ (Ventana) and Oracle™ (Leica). For the FDA-/CE-IVD approved system HercepTest™, SK001 Dako a significant decline in the pass rates and proportion of sufficient results was observed in this run. For the first time in the NordiQC HER-2 IHC assessments the pass rate of HercepTest™ (SK001, K5204 & K5207) was inferior to in-house IHC validated assays (66 % and 79 %, respectively), which is illustrated in Fig. 1.
At present no single parameter or plausible cause for the decline can be identified. The inferior performance was mainly seen for the HercepTest™ kit SK001, being used by the majority. Dako has been informed about the observation, and has willingly offered to support laboratories with insufficient results. Dako can be contacted on dakosupport@dako.com.

As illustrated in Fig. 1, the FDA-/CE-IVD approved HER-2 IHC systems PATHWAY® & CONFIRM™ (Ventana, rmAb clone 4B5) have consistently given a high and superior pass rate especially compared to the in-house HER-2 assays. Thus, the average pass rate in the 14 consecutive runs has been 96 % for PATHWAY®/CONFIRM™ compared to an average pass rate of 52 % of the in-house HER-2 assays.

In this run in-house validated assays used by 76 = 29 %* of the participants (compared to 32 % and 33 % in the two previous runs) had a pas rate of 79%, which is the same as the FDA approved kits grouped together. We find

no explanation the increased pass rate for in-house assays. However, fluctuating pass rates generally may be associated with less robust systems. The tissue cores used for the HER2 tests have all been carefully validated to possess the same patterns in terms of HER expression and gene status, but they are obtained from different patients.
* Unfortunately 19% was erroneously indicated in the fist version of this page.

**Fig. 1. Pass rates in the 14 NordiQC HER-2 IHC assessments**

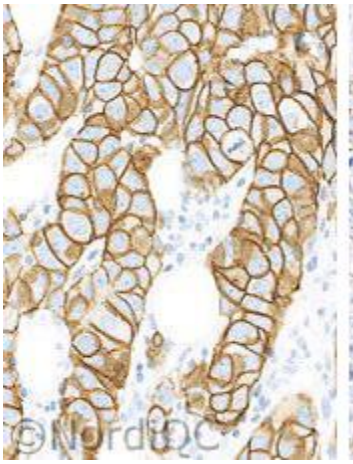



* HercepTest™ code no. K5204, K5206, K5207 & SK001, Dako
** PATHWAY® & CONFIRM™, rmAb clone 4B5, Ventana
*** Oracle™, Leica

**Scoring consensus**
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) on the stained sections. For 175 out of the 230 laboratories (76 %) responding, scores on all tissues in the multi-tissue sections given by the laboratories were in concordance with scores given by the NordiQC assessor group. A sufficient staining combined with an interpretation in concordance with the NordiQC assessors was seen in 84 % (148 out of 177), which was a slight increase from 79 % obtained in the previous run B13. An insufficient staining combined with an interpretation in concordance with the NordiQC assessor group was seen in 51 % (27 out of 53) of the laboratories, which was significantly higher than the previous assessment (18 %).

**Conclusion**
The FDA-/CE-IVD approved HER-2 IHC systems PATHWAY® &CONFIRM™ rmAb clone 4B5 (Ventana) were in this assessment the most reliable methods for the semi-quantitative IHC determination of the HER-2 protein expression. In this assessment HercepTest™, Dako gave an inferior performance compared to previous assessments.
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.
The scoring consensus between the laboratories and NordiQC was improved. This was seen both for the laboratories obtaining a sufficient and an insufficient staining result.

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



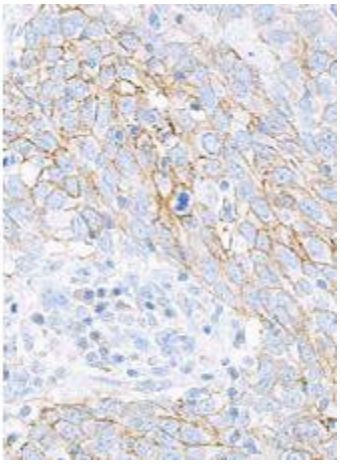
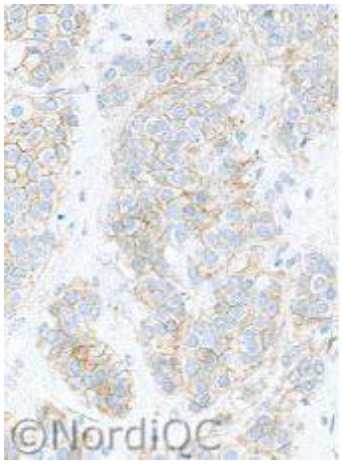


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of 2.5 – 2.8. > 10 % of the neoplastic cells show a weak to moderate complete membranous staining reaction corresponding to 2+.



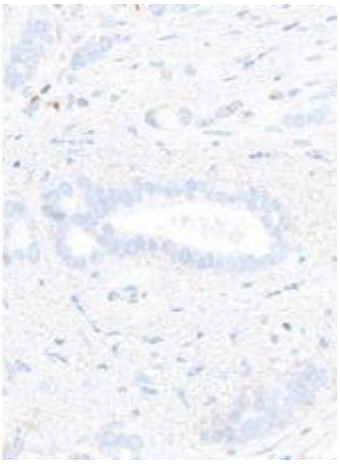
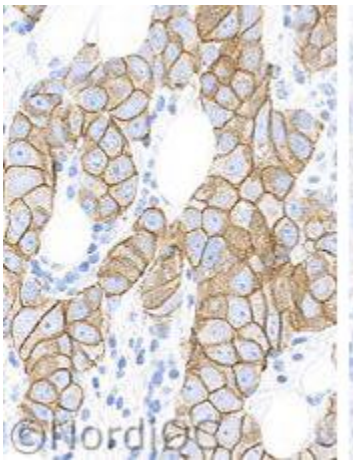


Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of of 1.3 – 1.7. > 10 % of the neoplastic cells show a weak complete membranous staining reaction corresponding to 2+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.2 – 1.5. A faint membranous staining reaction is seen in < 10 of the neoplastic cells corresponding to 0.



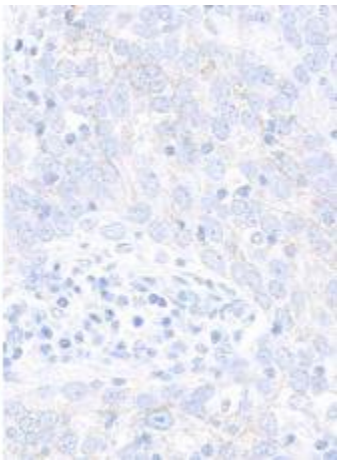
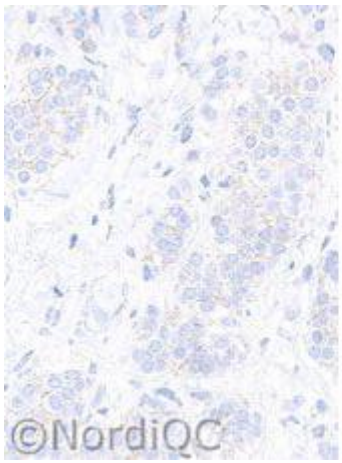


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of 2.5 – 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.



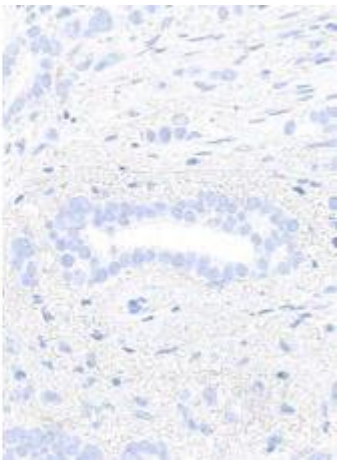


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of 1.3 – 1.7. A faint perceptible membranous staining reaction is seen in > 10 % of the neoplastic cells corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.2 – 1.5.
No staining reaction is seen corresponding to 0.

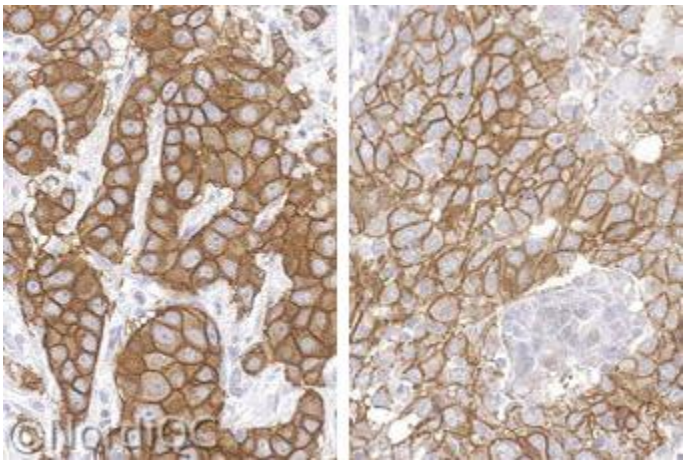


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a ratio of HER-2/chr17 of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+. An excessive cytoplasmic staining reaction is seen.
Right: Staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio of HER-2/chr17 of 2.5 – 2.8.
> 30 % of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.

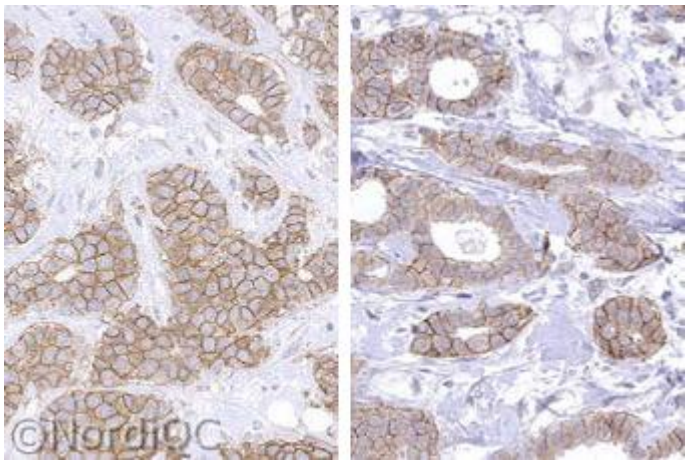


Fig. 3b
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2/chr17 of 1.3 – 1.7. > 30 % of the neoplastic cells show a moderate to strong membranous staining reaction corresponding to 3+.
Right: Insuffcient staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.2 – 1.5. > 10 % of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+. In the NordiQC reference laboratories this tumour was 1+.

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