Assessment Run B21 2016

HER-2 IHC

Material

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

	IHC: HER-2 Score* (0, 1+, 2+, 3+)	FISH: HER-2 gene/chr 17 ratio**
1.Breast carcinoma	3+	> 6.0 (clusters) (amplified)
2.Breast carcinoma	2+	2.4 – 2.9 (amplified)
3.Breast carcinoma	1-2+	1.2 – 1.7 (unamplified)
4.Breast carcinoma	0-1+	1.1 – 1.4 (unamplified)
5.Breast carcinoma	0-1+	0.9 – 1.2 (unamplified)

* HER-2 immunohistochemical score (see table below) as achieved by using the three FDA approved kits and antibodies, HercepTest™ Dako, Oracle™ Leica and PATHWAY® Ventana, in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)

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

IHC scoring system according to the 2013 ASCO/CAP guidelines

Score 0	No staining is observed or incomplete membrane staining is observed in ≤ 10% of the tumour cells.
Score 1+	A faint perceptible and incomplete membrane staining is observed in more than 10% of the tumour cells.
Score 2+	A weak to moderate circumferential incomplete membrane staining is observed in more than 10% of the tumour cells or an intense circumferential complete membranous staining in ≤ 10% of the tumour cells.
Score 3+	An intense circumferential complete membrane staining is observed in more than 10% of the tumour cells.

NordiQC criteria for assessing a HER-2 staining result as **optimal** were:

- Staining reaction corresponding to score 0 or 1+ in carcinomas no. 4 and 5.
- Staining reaction corresponding to score 0, 1+ or 2+ in carcinoma no. 3.
- Staining reaction corresponding to score 2+ or 3+ in carcinoma no. 2.
- Staining reaction corresponding to score 3+ in carcinoma no. 1.
- No or only weak cytoplasmic reaction that did not interfere with the interpretation.

The staining reaction was assessed as **good**, if (1) the HER-2 gene amplified tumour no. 1 showed a 2+ reaction and the other breast carcinomas showed reaction pattern as described above (equivocal 2+ IHC staining should always be analyzed by ISH according to the ASCO/CAP guidelines) ***or*** (2) the HER-2 gene non-amplified tumour no. 4 and/or 5 showed a 2+ reaction and the other breast carcinomas showed the expected reaction pattern.

The staining reaction 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.

The staining reaction was assessed as **poor** in case of a false negative staining (e.g., the 3+ tumour or the 2+ tumour with gene amplification showed a 0 or 1+ reaction) or a false positive staining (e.g., the 0/1+ tumors and the 2+ tumour without gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B21	339
Number of laboratories returning slides	319 (94%)

Results: 319 laboratories participated in this assessment and 93% achieved a sufficient mark. Assessment marks for IHC HER-2 assays and HER-2 antibodies are summarized in table 1.

Table 1. **Assessment marks for IHC assays run B21, HER-2 IHC**

FDA approved HER-2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	119	Ventana/Roche	110	6	1	2	97%	97%
CONFIRM™, rmAb clone **4B5, 790-4493**	64	Ventana/Roche	62	1	0	1	98%	100%
CONFIRM™, rmAb clone **4B5, 800-2996**	2	Ventana/Roche	2	0	0	0	-	-
HercepTest™ **SK001**	42	Dako/Agilent	39	1	0	2	95%	98%
HercepTest™ **K5207**	8	Dako/Agilent	7	1	0	0	100%	100%
HercepTest™ **K5204**	5	Dako/Agilent	2	3	0	0	100%	100%
Oracle™ mAb clone **CB11, TA9145**	6	Leica	4	2	0	0	100%	100%
Antibodies[3] for laboratory developed HER-2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **10A7**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **CB11**	6 1 1	Leica/Novocastra Biogenex Klinipath	3	2	2	1	62%	100%
rmAb clone **EP1045Y**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb clone **EP3**	1 1 1	Biocare Bio SB PathnSitu	2	1	0	0	-	-
rmAb clone **SP3**	17 2 2 1 1 1	Thermo/NeoMarkers Thermo/Pierce Zytomed Cell Marque Immunologic Spring Bioscience	13	7	1	3	83%	85%
pAb clone **A0485**	33	Dako	20	6	3	4	79%	82%
Unknown	1	Unknown	0	0	0	1	-	-
Antibodies for laboratory developed HER-2 assays, RTU	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostics	0	0	0	1	-	-
Ab clone **MXR001, RMA-0701**	1	Maixin	1	0	0	0	-	-
Total	319		265	32	7	15	-	-
Proportion			83%	10%	2%	5%	93%	-

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.

Detailed Analysis
FDA/CE IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana): 110 of 119 (92%) protocols were assessed as optimal. Protocols with optimal results were typically based on heat induced epitope retrieval (HIER) in Cell Conditioning 1 (CC1) (efficient heating time 20-64 min.) in BenchMark XT, GX or Ultra, 12 – 40 min. incubation of the primary Ab and Iview, UltraView or OptiView as detection kit. Using these protocol settings, 115 of 118 (98%) laboratories produced a sufficient staining result (optimal or good).
2 protocols were performed on a BOND MAX/BOND III (Leica), both provided an optimal result.

CONFIRM™ rmAb clone **4B5** (790-4493, Ventana): 62 of 64 (97%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 20-64 min.) in BenchMark XT, GX or Ultra, 12 – 44 min. incubation of the primary Ab and Iview, UltraView or OptiView as detection kit. Using these protocol settings, 63 of 63 (100%) laboratories produced a sufficient staining result.

CONFIRM™ rmAb clone **4B5** (800-2996, Ventana): 2 of 2 protocols were assessed as optimal. Both protocols were based on HIER in CC1 (efficient heating time 36 min.) in the BenchMark Ultra, 16 or 28 min. incubation of the primary Ab and UltraView as detection kit.

HercepTest™ pAb (SK001, Dako): 39 of 42 (93%) protocols 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 and 20-30 min. incubation of the primary Ab. Using these protocol settings, 40 of 41 (98%) laboratories produced a sufficient staining result.

HercepTest™ pAb (K5207, Dako): 7 of 8 (88%) protocols were assessed as optimal. Protocols with 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 30 min. incubation of the primary Ab. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result.
Two protocols were performed on a BOND MAX/BOND III (Leica) - both provided optimal results.

HercepTest™ pAb (K5204, Dako): 2 of 5 (40%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97°C for 40 min. in a water bath or PT Link and 30 min. incubation of the primary Ab. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

Oracle™ mAb clone **CB11** (TA9145, Leica): 4 of 6 (67%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in Bond Epitope Retrieval Solution 1 (BERS1) or BERS2 for 20-25 min and 30 min incubation of the primary Ab. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result.

Concentrated antibodies for laboratory developed (LD) assays

mAb **CB11**: 3 of 8 (38%) protocols were assessed as optimal. Optimal protocols were based on HIER using Target Retrieval Solution (TRS) pH 9 (Dako) (1/1)*, Bond Epitope Retrieval Solution 1 pH 6 (BERS1, Leica) (4/4) or PT Module Buffer 1, pH 6 (Thermo) (1/1). The mAb clone CB11 was diluted in the range of 1:300-600 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb **EP3**: 2 of 3 (67%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS pH 9 (3-in-1) (Dako) (1/2) or Tris-EDTA/EGTA pH 9 (1/1). The rmAb clone SP3 was diluted in the range of 1:100-400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb **SP3**: 13 of 24 (54%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS pH 9 (3-in-1) (Dako) (3/4), Cell Conditioning 1 (CC1) (BenchMark, Ventana) (2/6), Bond Epitope Retrieval Solution 2 pH 9 (BERS2, Leica) (3/5), BERS1 pH 6 (Leica) (1/1), Tris-EDTA/EGTA pH 9 (2/2) or Citrate pH 6 (2/4). The rmAb clone SP3 was typically diluted in the range of 1:50-200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 17 of 20 (85%) laboratories produced a sufficient staining result.

pAb **A0485**: 20 of 33 (61%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH 6.1 (Dako) (15/19), TRS pH 9 (3-in-1) (Dako) (1/2), TRS pH 9 (Dako) (1/5), BERS1 pH 6 (Bond, Leica) (2/2) or BERS2 (Bond, Leica) (1/1). The pAb A0485 was typically diluted in the range of 1:200-800 depending on the total sensitivity of the protocol employed. Using these protocol settings, 23 of 28 (82%) laboratories produced a sufficient staining result.

Comments
In this assessment and in concordance with the previous NordiQC assessments of HER-2 IHC, insufficient HER-2 staining results were characterized by too weak or false negative staining reactions. This was particularly and most critically observed as 0/1+ IHC reaction in the low level HER-2 gene amplified breast carcinoma core no. 2. This tumour was categorized as 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 2.4 – 2.9) by ISH. False negative staining reaction of the breast carcinoma no. 2 was seen in 64% of the insufficient results (14 of 22). The remaining insufficient results were typically characterized by a poor signal-to-noise ratio, impaired morphology, excessive counterstaining complicating the interpretation or false positive 3+ IHC staining in the HER-2 non-amplified tumours.
False negative results were seen both in laboratory developed (LD) and FDA-/CE-IVD approved assays, while false positive results only were seen in LD assays.
False negative results were for the LD assays typically caused by a too low sensitivity of the protocol applied (e.g. too low concentration of the primary Ab, too short incubation time of the primary Ab and/or insufficient HIER). For the FDA-/CE-IVD approved systems, used according to the official package inserts for the respective systems, no single cause for insufficient and false negative staining reactions could be identified from the protocols submitted. However, it was observed that an extended off-label use compared to previous assessment was applied for the approved systems and e.g. both the Ventana PATHWAY® /CONFIRM™ and Dako HercepTest™ were applied on the Bond IHC platform and as such not within the intended use and consequently must be considered as a LD assay. 4 of 5 protocols based on PATHWAY® /CONFIRM™ or HercepTest™ and performed on another IHC platform as intentional provided a sufficient result while one a false positive result.
The Ventana PATHWAY® /CONFIRM™ HER2 IHC assay was also increasingly used off-label by the participants applying OptiView as detection system instead of UltraView or iView as recommended by Ventana. In this assessment no impact on the analytical sensitivity and specificity was revealed. In contrast, internal studies previously performed in the NordiQC reference laboratory indicated a less precise and robust HER2 IHC assay if UltraView was substituted by OptiView PATHWAY® /CONFIRM™. OptiView will typically amplify the sensitivity of the IHC system 3-4x compared to level seen for UltraView, if all other parameters are maintained. Consequently if OptiView is applied, the HER2 IHC assay must be adjusted at other parameters e.g incubation time of the primary Ab, HIER settings etc to provide the level validated by Ventana, which as mentioned can cause a less precise and robust assay.

In this assessment, all three FDA-/CE-IVD approved HER-2 IHC assays PATHWAY® /CONFIRM™, HercepTest™ and Oracle™ from Ventana, Dako and Leica respectively, provided a higher pass-rate superior to LD assays as illustrated in Fig. 1. PATHWAY®/CONFIRM™ have provided a consistent high pass rate throughout all HER-2 IHC runs in NordiQC. In this assessment the FDA/CE-IVD approved system Oracle™, Leica showed a noticeable increase in the proportion of sufficient results compared to the latest three NordiQC HER-2 IHC assessments. As only a relatively small number of participants used the Oracle™ system, no conclusions for the divergent pass rates can be drawn.

The proportion of laboratories using LD assays is relatively consistent, though slightly decreasing. In this run, 23% of the participants (n=73) used LD assays compared to 25 - 31% in the last 10 assessments. Despite an overall improvement of the pass rate for LD HER-2 assays from run B1 to B21 has been achieved, the pass rate and proportion of optimal results still is inferior to the FDA/CE-IVD approved systems as e.g. PATHWAY® /CONFIRM™ and HercepTest™. In general, the three FDA-/CE-IVD approved HER-2 assays provided a proportion of optimal results of 92% (226 of 246), whereas only 53% of LD HER-2 assays were assessed as optimal (39 of 73). As shown in Fig. 2, LD HER-2 assays both provided a reduced proportion of sufficient results but also a shift from optimal to good, typically caused by 2+ staining reaction in one or both of the HER-2 non-amplified tumours (no. 2 and 5) expected to show a 0/1+ staining reaction. The staining reaction of 2+ in these tumours would not directly lead to a wrong diagnosis but require an additional ISH test due to the less precise IHC result.

The overall pass rate of 93% obtained in this assessment is very satisfactory and is comparable to the pass rates seen in the last 5 runs indicating a stable level has been reached. A significant improvement compared to the pass rate of 51% seen in run B1, 2006 has thus been obtained.

Figure 1. **Pass rates of 20 HER-2 IHC assessments in the NordiQC breast cancer module**

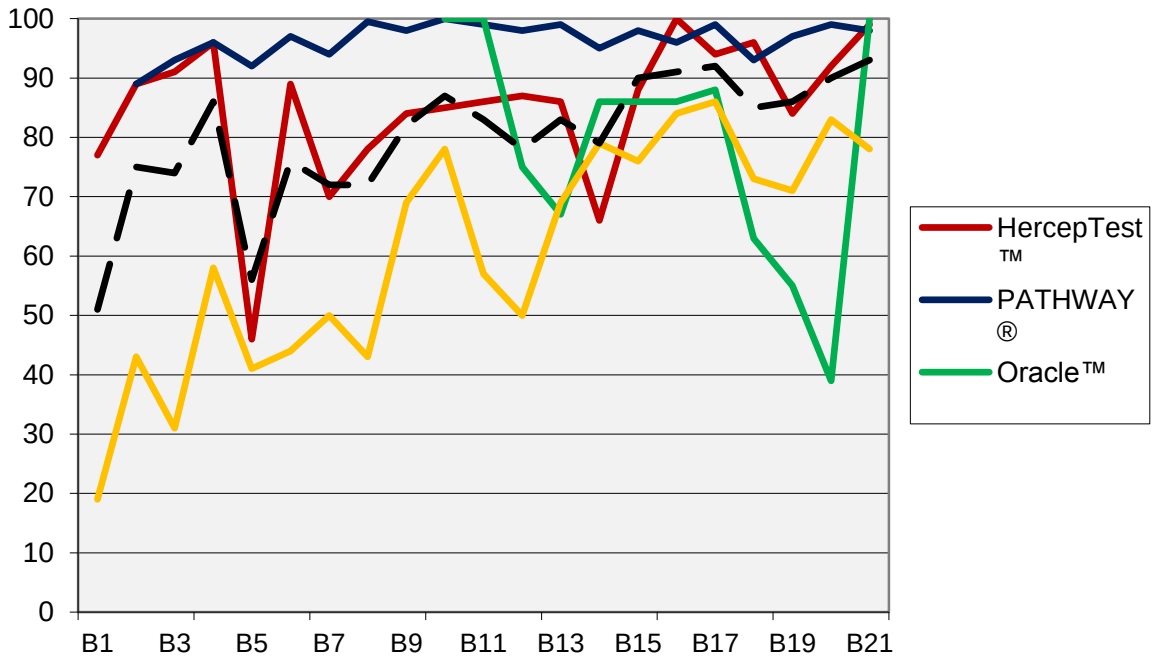
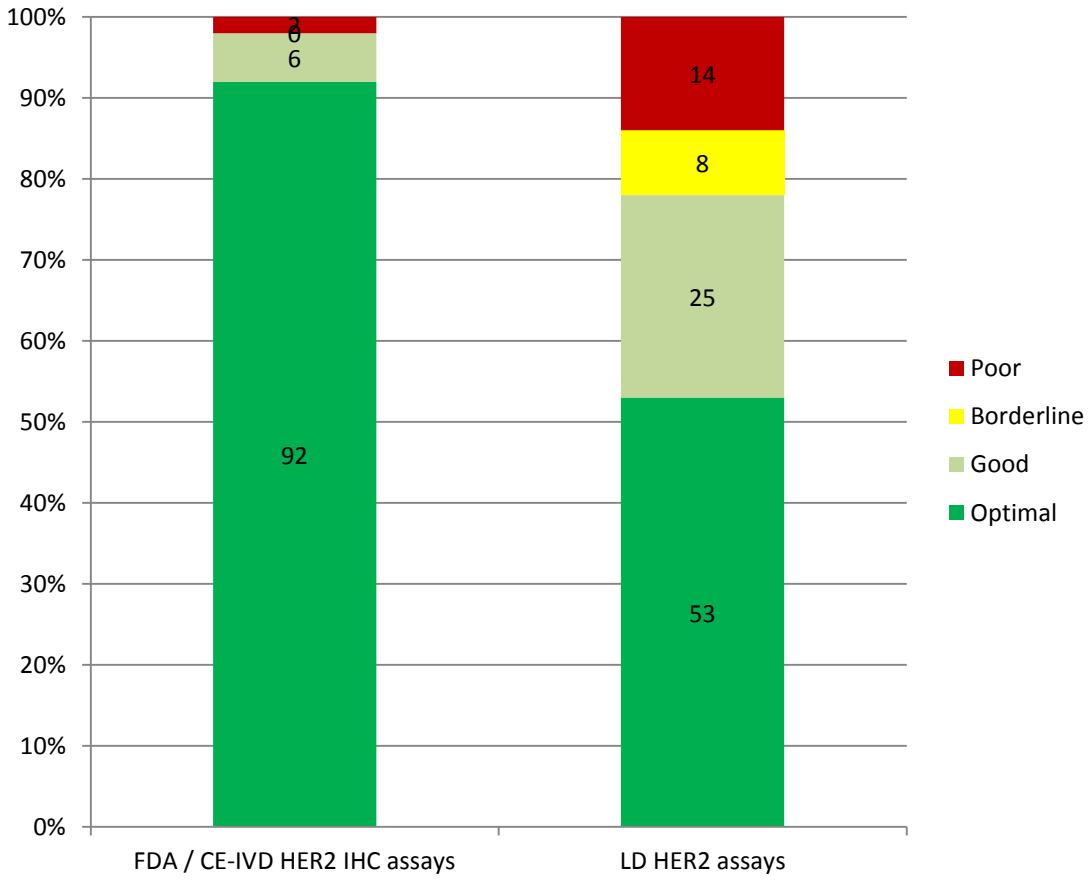


Figure 2. **Proportion of assessment marks using FDA-/CD-IVD and LD assays**



Scoring consensus
The laboratories were requested to submit their own scores (0, 1+, 2+, 3+) of their stained sections, which was effectuated by 84% (268 of 319) of participants. For 247 of the 268 participants (92%) responding, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group using the ASCO/CAP 2013 interpretation guidelines. This was an increase compared to the level of 81% observed in run B20. Sufficient staining and interpretation in agreement with the NordiQC assessors were seen in 97% (239 of 246), an increase from 84% in run B20. Insufficient staining and interpretation in concordance with the NordiQC assessor group was seen in 35% (7 of 20) of the participants. Typically the tumour no. 2 was interpreted as 2+ by the laboratory, but 0-1+ by the NordiQC assessor group. This was to some extent extraordinary as the ASCO/CAP 2013 HER-2 IHC guidelines have lowered the staining threshold for 2+ tumours and thus a higher level of consensus was expected.

Conclusion
The FDA-/CE-IVD approved HER-2 IHC assays **PATHWAY®/CONFIRM™** rmAb clone 4B5 (Ventana) **HercepTest™** (Dako) and **Oracle**™ (Leica) were in this assessment the most precise assays for the semi-quantitative IHC determination of HER-2 protein expression. Laboratory developed assays produced a lower pass-rate and were less precise for the HER-2 status requiring an additional ISH test for final evaluation.
Inclusion of 2+ tumours with and without HER-2 gene amplification in control material is essential to evaluate precision and performance stability of the IHC HER-2 assays used by laboratories.

HER2 – B21

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

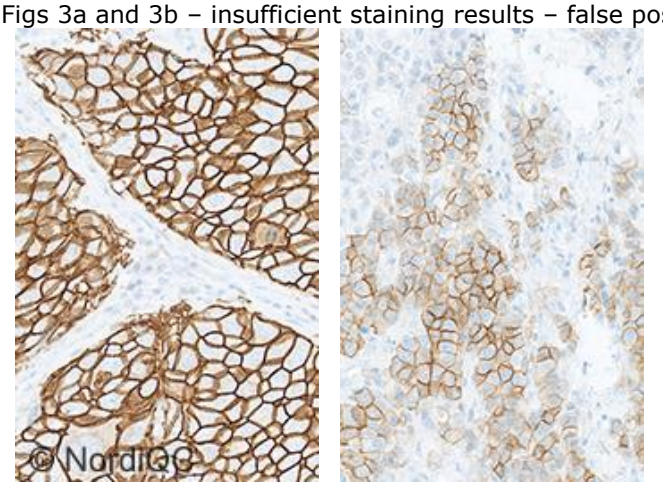


Fig. 1a
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 2.4 – 2.9.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

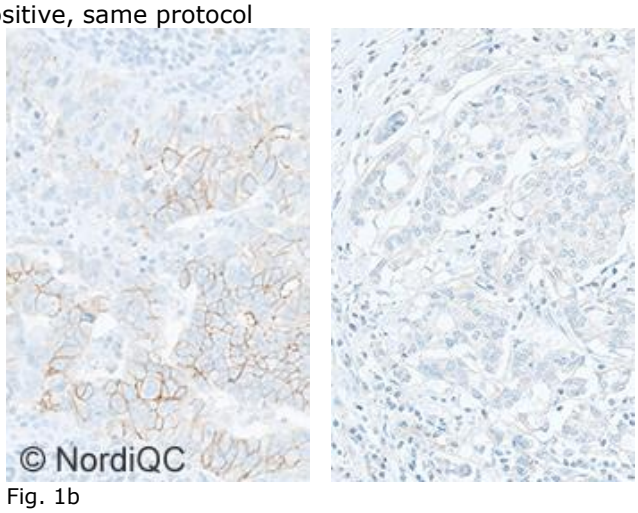
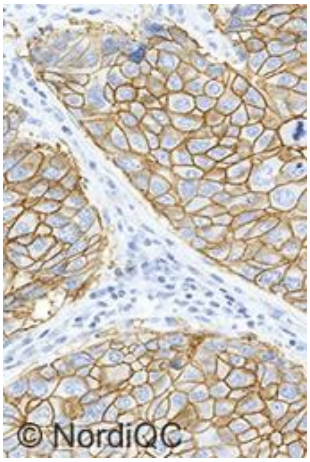


Fig. 1b
Left: Optimal staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.2 – 1.7.
> 10% of the neoplastic cells show a weak to moderate membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2 / Chromosome 17 ratio of 1.2– 1.5.
> 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 1+.



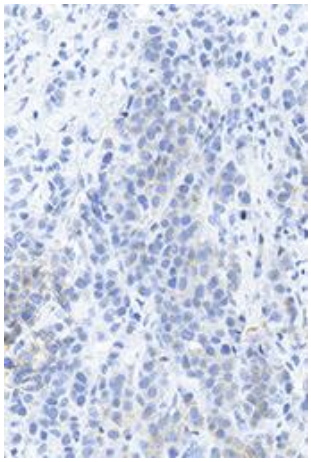
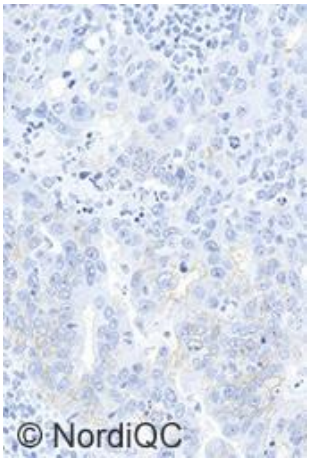


Fig. 2a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Insufficient and false negative staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 2.4 – 2.9.
> 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.



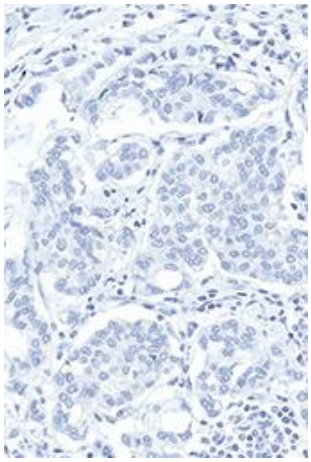
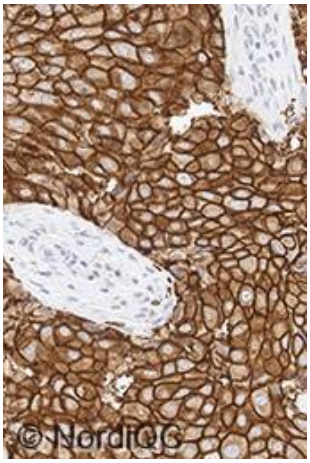


Fig. 2b
Left: Staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.2 – 1.7.
> 10% of the neoplastic cells show a faint perceptible membranous staining reaction corresponding to 1+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4.
< 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 0.



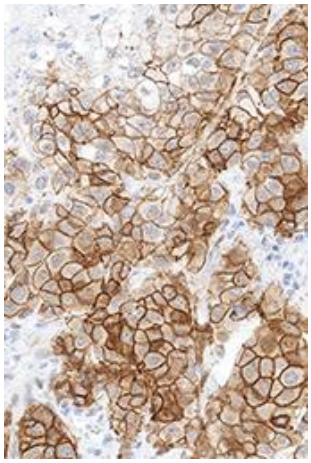
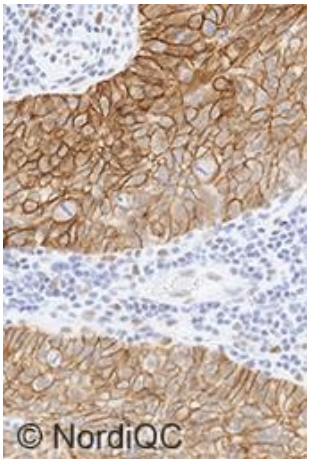


Fig. 3a
Left: Staining result for HER-2 of the breast ductal carcinoma no. 1 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 2 with a ratio of HER-2 / Chromosome 17 of 2.4 – 2.9.
> 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.



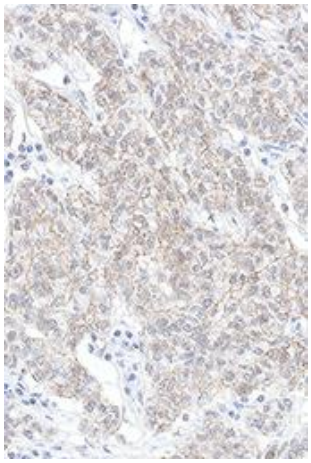


Fig. 3b
Left: Insufficient and false positive staining result for HER-2 of the breast ductal carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.2 – 1.7.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER-2 of the breast ductal carcinoma no. 4 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.4.
> 10% of the neoplastic cells show a faint membranous staining reaction corresponding to 2+.

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