Assessment Run B28 2019

HER2 IHC

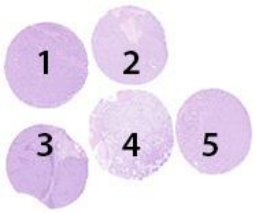
Purpose

Evaluation of the analytical accuracy of HER2 IHC tests performed by the NordiQC participants for the demonstration and establishment of the HER2 protein expression level in breast carcinomas. PATHWAY® (Ventana) and HercepTest™ (Dako) were used as reference standard methods, and accuracy was evaluated in five breast carcinomas with the dynamic and critical relevant expression levels of HER2. The obtained score in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, internal validation and extended quality control, e.g. regularly measuring the HER2 results, is necessary.

Material

The slide to be stained for HER2 comprised the following 5 materials:

	IHC: HER2 Score* (0, 1+, 2+, 3+)	**FISH: HER2 gene/chr 17 ratio****
1. Breast carcinoma, no. 1	3+	> 6.0 (clusters) (amplified)
2. Breast carcinoma, no. 2	0-1+	1.3 – 1.5 (unamplified
3. Breast carcinoma, no. 3	2+	2.9 – 3.4 (amplified)
4. Breast carcinoma, no. 4	2+	1.5 – 1.8 (unamplified)
5. Breast carcinoma, no. 5	3+	> 6.0 (clusters) (amplified)



* HER2 immunohistochemical score (see table below) as achieved by using the two FDA / CE-IVD approved HER2 IHC assays, HercepTest™ (Dako) and PATHWAY® (Ventana), in NordiQC reference laboratories.
** HER2 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 2018 ASCO/CAP guidelines:

Score 0	No staining is observed **or** membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells.
Score 1+	Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells.
Score 2+	Weak to moderate complete membrane staining observed in >10% of tumor cells.
Score 3+	Circumferential membrane staining that is complete, intense, and in >10% of tumor cells*.

**Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.*

Criteria for assessing a HER2 staining as **optimal** were:

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

Staining was assessed as **good**, if (1) the HER2 gene amplified tumours no. 1 and 5 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) a less distinct and/or reduced number of neoplastic cells were demonstrated in the the HER2 2+ gene amplified tumour no. 3 compared to the NordiQC reference standards determined by HercepTest™ and PATHWAY®.

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.

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

Participation

Number of laboratories registered for HER2, run B28	354
Number of laboratories returning slides	346 (98%)

Results: 346 laboratories participated in this assessment and 86% achieved a sufficient mark (optimal or good). Assessment marks for IHC HER2 assays and HER2 antibodies are summarized in Table 1.

Table 1. Assessment marks for **IHC assays and antibodies run B28, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
PATHWAY® rmAb clone **4B5, 790-2991**	157	Ventana/Roche	132	17	1	7	95%	97%
PATHWAY® rmAb clone **4B5, 790-2991[4]**	2	Ventana/Roche	-	1	-	1	-	-
rmAb clone **4B5, 790-4493**	60	Ventana/Roche	45	4	2	9	82%	92%
rmAb clone **4B5, 790-4493[4]**	1	Ventana/Roche	1	-	-	-	-	-
HercepTest™ **SK001**	25	Dako/Agilent	21	3	-	1	96%	95%
HercepTest™ **SK001[4]**	4	Dako/Agilent	3	-	-	1	-	-
Oracle™ mAb clone **CB11, TA9145**	9	Leica	2	4	1	2	67%	-
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11**	5	Leica/Novocastra	-	1	1	3	20%	-
mAb clone **e2-4001**	1	ThermoFisher Scientific	1	-	-	-		
rmAb clone **BSR44**	2	Nordic Biosite	-	-	-	2	-	-
rmAb clone **EP3**	1 1 1 1	Cell Marque Diagnostic BioSystems Epitomics Zytomed	1	2	-	1	-	-
rmAb clone **SP3**	9 5 2 1 1 1 1	ThermoFisher Scientific Cell Marque Zytomed Spring Biosystems Invitrogen enquire Master Diagnostica	9	7	2	2	79%	88%
rmAb clone **RM228**	1	RevMAb Biosciences	1	-	-	-	-	-
pAb, **A0485**	48	Dako/Agilent	29	9	-	10	79%	80%
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **CB11, PA0571**	1	Leica	-	1	-	-	-	-
mAb clone **CB11, PA0983**	1	Leica	-	-	-	1	-	-
Ab clone **GR011, 8362-C010**	1	Sakura Finetek	1	-	-	-	-	-
Ab clone **MXR001, RMA-0701**	1	Maixin	1	-	-	-	-	-
rmAb clone **EP3, PME342**	1	Biocare	-	-	-	1	-	-
rmAb clone **EP3, AN726**	1	BioGenex	-	-	-	1	-	-
rmAb clone **SP3, 237R**	1	Cell Marque	1	-	-	-	-	-
Total	346		248	49	7	42	-	-
Proportion			72%	14%	2%	12%	86%	-

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.
4) RTU system used on a different platform than it was developed for.

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): 132 of 157 (84%) 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 16-64 min.) on BenchMark XT, GX or Ultra, 12-48 min. incubation of the primary Ab and iView or UltraView as detection kit. Using these protocol settings, 125 of 129 (97%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **4B5** (790-4493, Ventana/Roche): 45 of 60 (75%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in CC1 (efficient heating time 24-52 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView as detection system. Using these protocol settings, 45 of 49 (95%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): 21 of 25 (84%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 20-40 min. in a water bath or PT Link, 30 min. incubation of the primary Ab and SK001 Polymer as detection system. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result.

Table 2 summarizes the proportion of sufficient and optimal marks for the most commonly used IVD approved assays. The performance was evaluated both as "true" plug-and-play systems performed accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the specific IHC stainer device are included.

Table 2. **Comparison of pass rates for vendor recommended and laboratory modified protocols**

CDx assay	**Vendor recommended protocol settings***		**Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra PATHWAY® rmAb 4B5 **790-2991**	42/45 (93%)	40/45 (89%)	107/112 (96%)	92/112 (82%)
Ventana BenchMark XT, GX, Ultra rmAb 4B5, **790-4493**	6/7 (86%)	6/7 (86%)	43/53 (81%)	39/53 (74%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	20/21 (95%)	17/21 (81%)	4/4	4/4
Leica Bond MAX, III Oracle™ mAb CB11 **TA9145**	2/4	1/4	4/5	4/5

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.

Concentrated antibodies for laboratory developed (LD) assays
pAb, **A0485**: 29 of 48 (60%) protocols were assessed as optimal. Optimal protocols were based on HIER using either TRS low pH (Dako) (18/28*), TRS High pH (Dako) (8/12), CC1 (Ventana) (1/2), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (1/2), PT module buffer 1 (Thermo Scientific) (1/1). The pAb A0485 was typically diluted in the range of 1:150-1,000 with either a 2-layer detection system (23/42) or a 3-layer detection system (6/6). Using these protocol settings, 36 of 45 (80%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP3**: 9 of 20 (45%) protocols were assessed as optimal. Optimal protocols were based on HIER using TRS High pH (Dako) (3/4), BERS2 (Leica) (2/10), CC1 (Ventana) (3/4) or EDTA/EGTA pH 8 (1/1). The rmAb clone SP3 was diluted in the range of 1:50-100 with either a 2-layer detection system (6/13) or 3-layer detection system (3/3). Using these protocol settings, 15 of 17 (88%) laboratories produced a sufficient staining result.

Table 3 summarizes the overall proportion of optimal staining results when using the two most frequently used concentrated Abs on the most commonly used IHC stainer platforms.

Table 3. **Optimal results for HER2 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako Agilent Autostainer**		**Dako Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH High	TRS pH Low pH	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
pAb clone **A0485**	5/7** (71%)	8/12 (75%)	3/4	10/14 (71%)	2/2	-	2/10 (80%)	-
rmAb clone **SP3**	1/2	-	2/2	-	3/4	-	2/10 (80%)	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Comments
In this NordiQC assessment B28 for HER2, an overall pass rate of 86% was observed similar to the level obtained in most of the recent assessments. However, in contrast to previous HER2 IHC assessments, where false negative staining reactions typically have characterized the insufficient results, both false positive and false negative results were seen in this run. A false positive staining reaction was seen in 40% (20 of 50) of the insufficient results and was characterized by an unequivocal 3+ IHC reaction in the breast carcinoma, tissue core no 4. This tumour was categorized as IHC 2+ in the NordiQC reference laboratory using the two FDA/CE-IVD HER2 IHC assays: PATHWAY® (Ventana) and HercepTest™ (Dako) and by FISH evaluated to be HER2 negative showing a HER2 gene / chromosome 17 ratio in the range of 1.5-1.8.
34% (17 of 50) of the insufficient results were caused by a false negative staining reaction. This was revealed by a 0/1+ IHC reaction in the HER2 gene amplified breast carcinoma, tissue core no. 3 categorized as IHC 2+ in the NordiQC reference laboratory and showing HER2 gene amplification (ratio 2.9-3.4) by FISH.
The remaining insufficient results showed e.g. a poor signal-to-noise ratio, impaired morphology, excessive cytoplasmic staining or severe artefacts compromising the interpretation of the HER2 level.

75% of the participants (n=258) used FDA/CE-IVD approved companion diagnostic HER2 IHC assays as PATHWAY® (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica), while the remaining 25% of laboratories used a laboratory developed (LD) assay based on a concentrated primary Ab or a RTU format being optimized for the IHC system used by the laboratory.

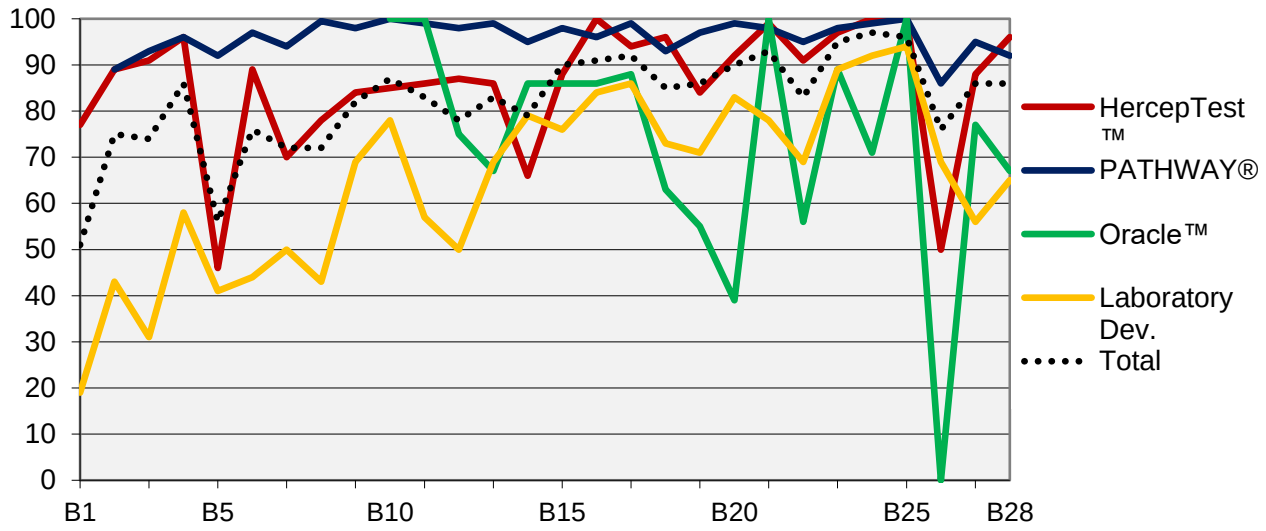
The Ventana/Roche PATHWAY® HER2 IHC assays 790-2991 and 790-4493 were used by the majority of participants. Despite these companion diagnostic (CDx) HER2 assays have been meticulously validated by the vendor and principally should be used strictly in concordance to the instructions and guidelines for the products, the vast majority of the laboratories modified the protocols for these HER2 IHC assays as shown in Table 2. In this assessment, only 24% (52 of 217) of the laboratories in fact used the two companion diagnostic assays accordingly to the package inserts, while 76% modified the protocols and hereby changing the CDx assay to a laboratory developed test (LDT).
For the laboratories using the two Ventana/Roche CDx assays as plug-and-play or by modified protocols, comparable pass rates of 92% and 91%, respectively were observed. However the proportion of optimal results was 89% and 79%, respectively and thus more accurate and concordant to the NordiQC reference standard results for these laboratories using the CDx assays as plug-and-play systems (see Table 2).
The most common modification observed was prolonged incubation of the primary Ab, change of efficient HIER time and/or using a more sensitive detection system than indicated in the package inserts e.g. UltraView with amplification kit or OptiView.
It was observed that an increased number of participants used OptiView for the HER2 IHC assays 790-2991 and 790-4493 substituting iView or UltraView. OptiView will typically amplify the analytical sensitivity of the IHC system 3-4 times compared to UltraView using otherwise identical protocol settings and consequently induce a potential risk of inaccurate HER2 IHC results. In total, 18 laboratories used OptiView and 7 obtained an insufficient result caused by a 3+ false positive staining reaction in the unamplified the breast carcinoma, tissue core no 4. This underlines that modifications of CDx assays must be meticulously validated by the end-users on a large cohort of breast carcinomas (n=100, ASCO/CAP 2013 guidelines) showing the diagnostic relevant and critical expression ranges of HER2.

The Dako/Agilent HercepTest™ CDx assay SK001 provided an overall pass rate of 96% (24 of 25). The vast majority of laboratories used the IHC assay in concordance with the recommended protocol settings from Dako/Agilent. 4 laboratories modified the protocol adjusting HIER and/or incubation times. No significant differences was observed for the laboratories using the IHC assay SK001 as plug-and-play versus laboratories modifying the protocols.

In this HER2 assessment, LDTs based on concentrated Ab formats or generic RTU Abs without intended use or claim for HER2 demonstration in breast carcinoma to guide decision with treatment with Herceptin or similar drugs provided a significant lower pass rate of 65% (54 of 83) compared to the FDA-/CE-IVD approved assays. rmAb SP3 and pAb A0485 from Dako were the most successful and widely used concentrates. If optimal protocol settings was applied, a pass rate of 88% and 80% was obtained.

In this assessment, the FDA-/CE-IVD approved HER2 IHC assays HercepTest™ and PATHWAY® /4B5 were most successful and provided a high pass rate superior to Oracle™ and LDTs as illustrated in Graph 1. The proportion of laboratories using the FDA-/CE-IVD approved HER2 IHC assays and LDTs is very consistent. In this run, 25% of the participants (n=88) used LDTs compared to 23-31% in the latest assessments.

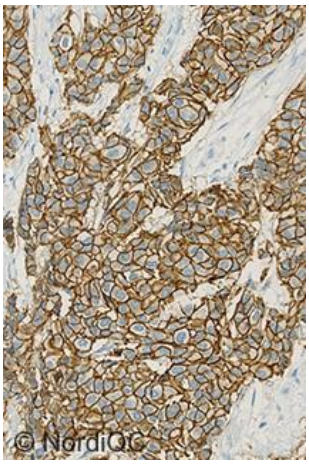
Graph 1. **Pass rates of 27 HER2 IHC assessments in the NordiQC breast cancer module**



Scoring consensus B28
Laboratories were requested to submit scores (0, 1+, 2+ or 3+) on the NordiQC homepage of their own HER2 stained slides. This was done by 84% (292 of 346) of the participants returning slides.
For 207 of the 292 (71%) responding participants, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group using the ASCO/CAP 2018 interpretation guidelines. This was similar to run B27 where 76% (211 of 276) of the scores were in consensus with the NordiQC assessor group. Among laboratories with sufficient staining, 74% (185 of 250) of the interpretations were in agreement with the NordiQC assessors. Among participants with insufficient staining, 52% were in consensus with the NordiQC assessor group (22 of 42).

Conclusion
The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/4B5** 790-2991/790-4493 (Ventana/Roche) and **HercepTest™** SK001 (Dako) were in this assessment the most accurate and successful assays for the semi-quantitative IHC determination of HER2 protein expression in breast carcinoma. Laboratory developed tests produced a lower pass-rate.
Inclusion of 2+ tumours with and without HER2 gene amplification in the control material for both EQA and internal quality control is essential to evaluate accuracy, precision and reproducibility of the IHC HER2 assays used by laboratories.

Figs 1a and 1b – optimal staining results, same protocol
Figs 2a and 2b – insufficient staining results - false positive, same protocol
Figs 3a and 3b – insufficient staining results – false negative, same protocol



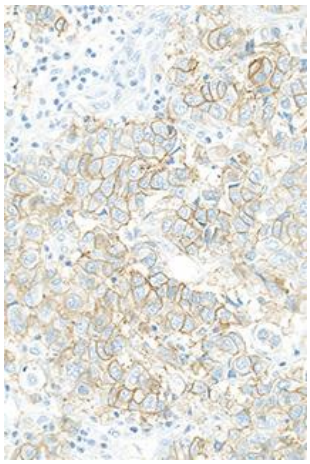
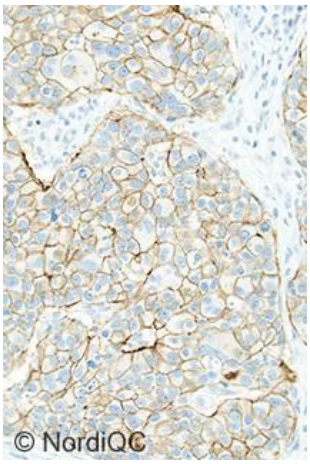


Fig 1a.
Left: Optimal staining result for HER2 of the breast ductal carcinoma no. 1 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast ductal carcinoma no. 3 with a ratio of HER2 / chr17 of 2.9-3.4.
> 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



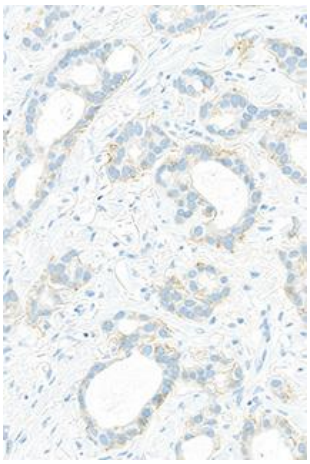
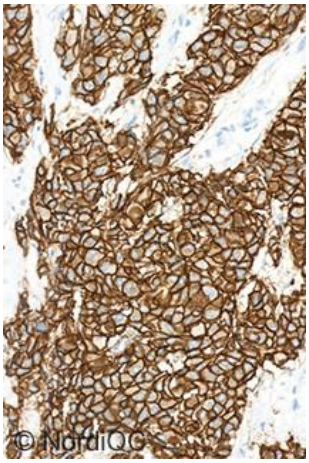


Fig 1b.
Left: Optimal staining result for HER2 of the breast ductal carcinoma no. 4 with a ratio of HER2 / chr17 of 1.5-1.8.
> 10% of the neoplastic cells show a weak-moderate membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER2 of the breast ductal carcinoma no. 2 with a HER2 / chr17 ratio of 1.3-1.5.
≥ 10% of the neoplastic cells show a faint, incomplete membranous staining reaction corresponding to 1+.



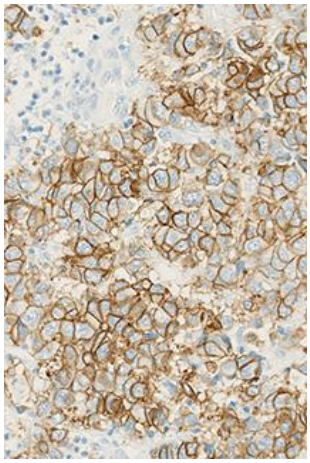
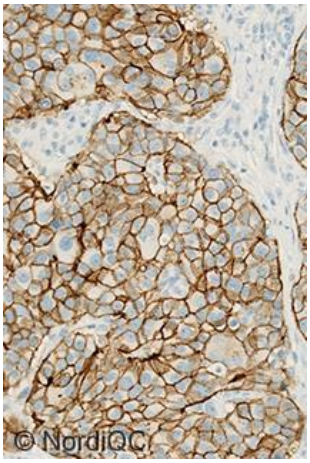


Fig 2a.
Left: Staining result for HER2 of the breast ductal carcinoma no. 1 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast ductal carcinoma no. 3 with a ratio of HER2 / chr17 of 2.9-3.4
> 10% of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+.



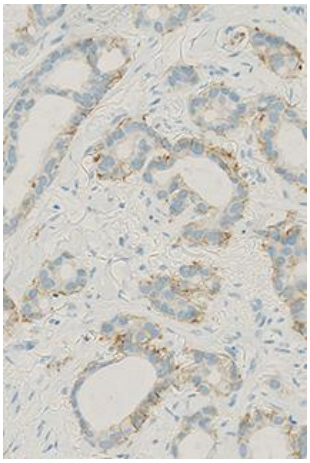
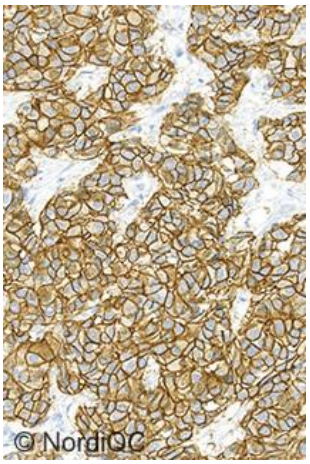


Fig 2b.
Left: Insufficient and false positive staining result for HER2 of the breast ductal carcinoma no. 4 with a ratio of HER2 / chr17 of 1.5-1.8.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+ (the core was scored as 3+ both by the participant and NordiQC).
Right: Staining result for HER2 of the breast ductal carcinoma no. 2 with a HER2 / chr17 ratio of 1.3-1.5.
≥ 10% of the neoplastic cells show a weak to moderate, incomplete membranous staining reaction corresponding to 1+.



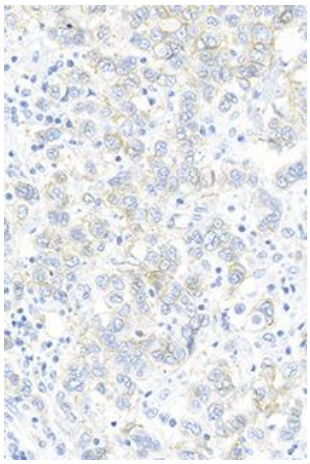
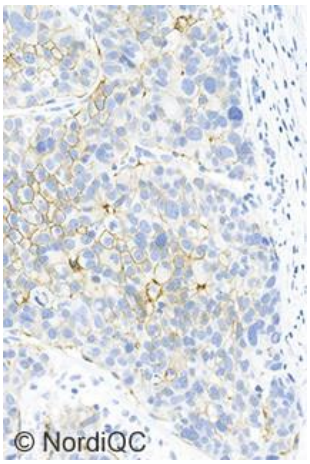


Fig 3a.
Left: Staining result for HER2 of the breast ductal carcinoma no. 1 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Insufficient and false negative staining result for HER2 of the breast ductal carcinoma no. 3 with a ratio of HER2 / chr17 of 2.9-3.4
≥ 10% of the neoplastic cells show a weak to moderate, incomplete membranous staining reaction corresponding to 1+.



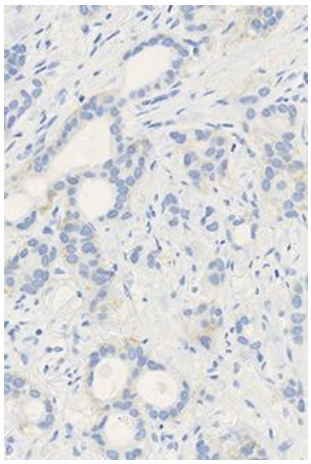


Fig 3b.
Left: Staining result for HER2 of the breast ductal carcinoma no. 4 with a ratio of HER2 / chr17 of 1.5-1.8.
≥ 10% of the neoplastic cells show a weak to moderate, incomplete membranous staining reaction corresponding to 1+.
Right: Staining result for HER2 of the breast ductal carcinoma no. 2 with a HER2 / chr17 ratio of 1.3-1.5.
Virtually all neoplastic cells are negative corresponding to 0.

SN/LE/RR 11.12.2019
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