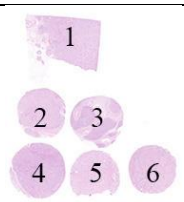
Assessment Run B35 2023
HER2 IHC

Purpose
Evaluation of the analytical accuracy of HER2 IHC tests performed by the NordiQC participants for demonstration and establishment of the HER2 protein overexpression level in breast carcinomas. The HER2 IHC assays PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) were used as reference standard methods, and accuracy was evaluated in six 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 used by the participants, 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 and recommended.

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****	**FISH: HER2 gene copy no.****	**FISH HER2 gene amplification status**
Breast carcinoma, no. 1	2+	4.1-4.54	>4	Amplified
Breast carcinoma, no. 2	1-2+	1.19	1-2	Unamplified
Breast carcinoma, no. 3	0-1+	1.39	1-3	Unamplified
Breast carcinoma, no. 4	3+	>6	>6	Amplified
Breast carcinoma, no. 5	1-2+	0.97-1.14	1-2	Unamplified
Breast carcinoma, no. 6	0	0.76	1-2	Unamplified

* HER2 immunohistochemical score (see table below) as achieved by using three CE-IVD approved HER2 IHC assays, HercepTest™ (SK001 and GE001, Dako/Agilent) and PATHWAY® (790-2991, Ventana/Roche), in the NordiQC reference laboratory.
** HER2 gene/chromosome 17 ratio achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision) in NordiQC reference laboratory.

Considering the emerging field of HER2-low, an additional breast carcinoma core was added to the TMA block circulated for this assessment to gain preliminary data on IHC test reproducibility for this entity. However, as stated above, the aim of this assessment was still to evaluate the demonstration of HER2 protein overexpression level according to the existing guidelines and the assessment marks given only related and based on this primary purpose.

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 carcinomas no. 3 and 6.
- Staining corresponding to score 0, 1+ or 2+ in carcinomas no. 2 and 5.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 1.
- Staining corresponding to score 3+ in carcinomas no. 4.
- No or only weak cytoplasmic reaction that did not interfere with the interpretation.

Staining was assessed as **good**, if (1) the HER2 gene amplified tumor no. 4 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by FISH/BRISH according to the ASCO 2018 guidelines) and the other breast carcinomas showed a reaction pattern as described above ***or*** (2) a less distinct and/or reduced number of neoplastic cells were demonstrated in the HER2 2+ gene amplified tumor no. 1 compared to the NordiQC reference standards determined by HercepTest™ and PATHWAY® ***or*** (3) a 2+ reaction was seen in the HER2 gene unamplified 0/1+ tumors no. 3 and 6.

Staining was assessed as **borderline,** if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive counterstaining or impaired morphology hampering the interpretation.

Staining was assessed as **poor** in case of a false negative staining (e.g., the IHC 3+ tumors or the 2+ tumor with HER2 gene amplification showing a 0 or 1+ reaction) ***or*** a false positive staining (e.g. the IHC 0, 1+ and 2+ tumors without HER2 gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B35	432
Number of laboratories returning slides	403 (93%)

Results

At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

In total 403 laboratories participated in this assessment and 90% achieved a sufficient mark (optimal or good).

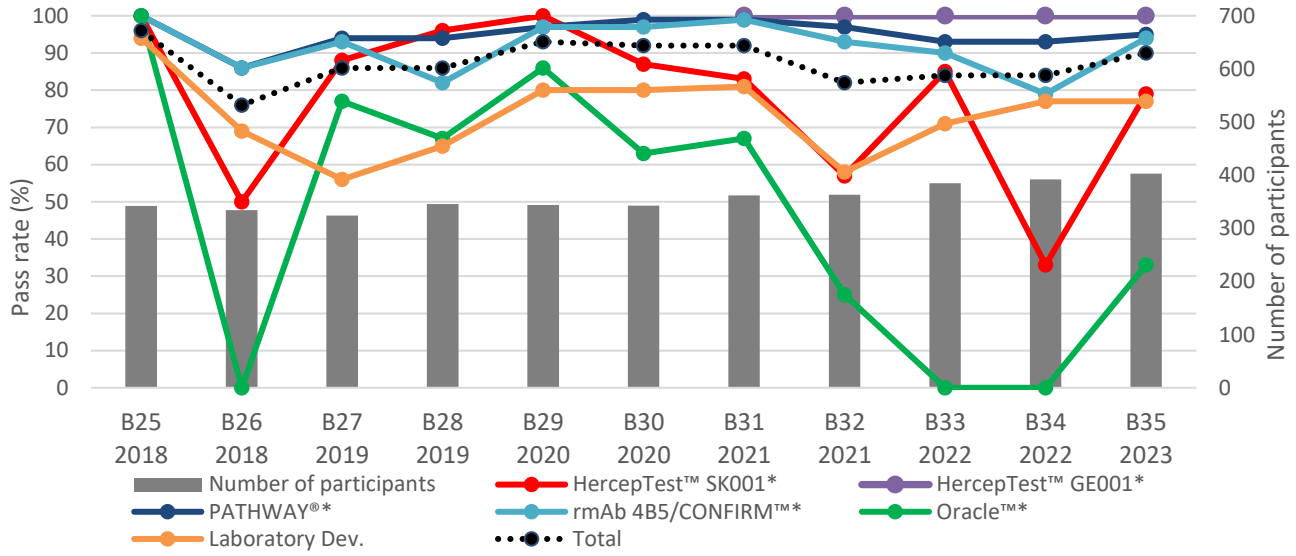
Conclusions

The overall pass rate improved compared to the latest three runs B32 to B34 and is nearly at the level seen in the three previous assessment runs B29-B31.
In this assessment, the **HercepTest™ GE001**, Dako/Agilent, for the Omnis platform provided the highest pass rate of 100% and 72% optimal results when using the vendor recommended protocol settings (VRPS). Although compared to the previous run B34, the pass rate for the "classical" **HercepTest™ SK001**, Dako/Agilent, for the Autostainer Link 48 platform improved from 33% to 79%, the proportion of optimal results decreased to 14%.
The two widely used and established FDA-/CE-IVD approved HER2 IHC assays from Ventana/Roche, **PATHWAY® 790-2991** and **VENTANA HER2/4B5 790-4493**, respectively, gave an overall pass rate of 95% and 94% when used by VRPS.
Laboratory developed tests (LDT's) based on RTU Abs without predictive claim or based on concentrated Abs gave a pass rate of 77%, 35% optimal.
As seen in the latest assessments, the CDx assay **Oracle™**, Leica Biosystems was less successful showing a pass rate of 29%, no optimal results.

Assessment marks for IHC HER2 CDx assays and HER2 LDTs are summarized in Table 1 (see page 3).

The historical pass rates of the NordiQC HER2 IHC assessments are illustrated in Graph 1 below.

Graph 1. **Pass rates of the HER2 IHC assessments in the NordiQC breast cancer module 2018-2023**



* pass rates using vendor recommended protocol settings

Table 1. **Assessment marks for IHC assays and antibodies run B35, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[4]**	22	Ventana/Roche	14	7	1	-	95%	64%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	137	Ventana/Roche	110	21	6	-	96%	80%
VENTANA HER2 rmab clone **4B5, 790-4493, (VRPS)[4]**	33	Ventana/Roche	24	7	2	-	94%	73%
VENTANA HER2 rmab clone **4B5, 790-4493, (LMPS)[5]**	77	Ventana/Roche	68	8	-	1	99%	88%
HercepTest™, pAb, **SK001, (VRPS)[4]**	14	Dako/Agilent	2	9	1	2	79%	14%
HercepTest™, pAb, **SK001, (LMPS)[5]**	5	Dako/Agilent	1	2	1	1	60%	20%
HercepTest™, rmAb **DG44, GE001, (VRPS)[4]**	25	Dako/Agilent	18	7	-	-	100%	72%
HercepTest™, rmAb **DG44, GE001, (LPMS)[5]**	2	Dako/Agilent	-	1	-	1	-	-
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	3	Leica Biosystems	-	1	1	1	-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	4	Leica Biosystems	-	1	1	2	-	-
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CB11**	1	Zytomed	-	-	1	-	-	-
rmAb clone **BP6020**	1	Biolynx	-	1	-	-	-	-
rmAb clone **EP3**	1 1 1	Biocare Cell Marque Epitomics	3	-	-	-	-	-
rmAb clone **QR3**	1	Quartett	-	1	-	-	-	-
rmAb clone **SP3**	3 1 5 3 2 2 1 1	Cell Marque Abcam Zytomed Thermo Fisher Scientific/Epredia Invitrogen Master Diagnostica DCS Unknown	2	8	3	5	56%	11%
pAb, **A0485**	47	Dako/Agilent	20	21	5	1	87%	43%
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Ab clone **246G0D3, PA216**	1	Abcarta/Abcepta	1	-	-	-	-	-
mAb clone **CB11, PA0983**	1	Leica Biosystems	-	-	-	1	-	-
Ab clone **MXR011, RMA-1022**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **EP3, AN726**	1	BioGenex	-	1	-	-	-	-
rmAb clone **EP3 ARMPD049R**	1	Diagnostic BioSystems	-	-	-	1	-	-
rmAb clone **EP3, 4362-C010a**	1	Sakura Finetek	-	1	-	-	-	-
rmAb clone **EP3, 8388-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	-	-	1	-	-	-
rmAb clone **SP3, 237R-17**	2	Cell Marque	-	1	-	1	-	-
Total	403		265	98	23	17		
Proportion			66%	24%	6%	4%	90%	

1) Suff.; Proportion of sufficient stains (optimal or good).
2) OR; Proportion of optimal results.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.
4) VRPS; Vendor Recommended Protocol Settings – RTU system used in compliance to protocol settings and package insert.
5) LMPS; Laboratory Modified Protocol settings - RTU system used by modified protocol settings focusing on retrieval conditions, Ab incubation time, detection system and IHC platform.

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): In total, 124 of 159 (78%) 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 30-64 min.) on BenchMark XT, GX or Ultra, 12-48 min. incubation of the primary Ab and UltraView as detection kit. Using these protocol settings, 129 of 134 (96%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 92 of 110 (84%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 36-64 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView as detection system. Using these protocol settings, 82 of 84 (98%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 3 of 19 (16%) 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, 30 min. incubation of the primary Ab and SK001 as detection system. Using these protocol settings, 10 of 13 (77%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 18 of 27 (67%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97°C for 30 min., 10 min. incubation of the primary Ab and GE001/GV800 as detection system. Using these protocol settings, 26 of 26 (100%) 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 according 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, Ultra PATHWAY® rmAb 4B5, **790-2991**	21/22 (95%)	14/22 (64%)	116/121 (96%)	99/121 (82%)
Ventana BenchMark GX, XT, Ultra VENTANA 4B5, **790-4493**	31/33 (94%)	24/33 (73%)	74/74 (100%)	66/74 (89%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	11/14 (79%)	2/14 (14%)	2/4	1/4
Dako Omnis HercepTest™ rmAb DG44, **GE001**	25/25 (100%)	18/25 (72%)	1/2	0/2
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	1/3	0/3	1/4	0/4

* Protocol settings recommended by vendor – Retrieval method & conditions, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Concentrated antibodies for laboratory developed (LD) assays
pAb **A0485**: 20 of 47 (43%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) low pH (Dako/Agilent) (14/25*), TRS High pH (Dako/Agilent) (3/9), CC1 (Ventana/Roche) (1/5) or Bond Epitope Retrieval Solution 2 (BERS2) pH 9 (Leica Biosystems) (2/4). The Ab was typically diluted in the range of 1:150-1000 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 32 of 34 (94%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP3**: 3 of 3 (100%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Tris/EDTA pH 9 (1/1), CC1 (Ventana/Roche) (1/1) or BERS2 pH 9 (Leica Biosystems) (1/1). The Ab was diluted in the range of 1:70-200 depending on the level of the total technical sensitivity of the protocol employed.

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

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

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS High pH	TRS 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**	1/5** (20%)	4/9 (44%)	2/5 (40%)	10/16 (63%)	1/5 (20%)	-	2/4	0/4

* 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 B35 for HER2, an increased pass rate of 90% was obtained compared to the levels seen in the latest three runs B32 (82%), B33 (84%) and B34 (84%) and is nearly at the level seen in the assessment runs B29-B31 (see Graph 1).

The insufficient results were primarily characterized by reduced proportion of positive cells, a too weak or false negative staining reaction being observed in 68% (27 of 40 results). The vast majority of laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinoma tissue core no. 4, with high level gene amplification, whereas too weak or false negative staining results were particularly and most critically observed as a 0/1+ IHC staining reaction in the HER2 gene amplified breast carcinoma, tissue core no. 1. This tumor was categorized as IHC 2+ in the NordiQC reference laboratory using the CE-IVD HER2 IHC assays: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (HER2/chr17 ratio >4) by FISH.
In the remaining insufficient results, these were characterized by e.g. a poor signal-to-noise ratio, impaired morphology and/or excessive cytoplasmic staining reaction compromising the read-out and scoring of the specific HER2 membranous reaction.

75% of the participants (n=302) used one of the CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY® (Ventana/Roche), VENTANA HER2 (4B5) (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica Biosystems) on the specified stainer with predictive claim for HER2 status in breast cancer. 5% (n=20) of the participants used one of the approved assays on another platform than specified by the vendor, while the remaining 20% (n=81) used a laboratory developed test (LDT) based on a concentrated primary Ab or a RTU format without a predictive claim. This segmentation has been relatively consistent in the last assessment runs.

The two Ventana/Roche assays, PATHWAY® 790-2991 and VENTANA HER2 (4B5) 790-4493, were most widely used and in total used by 67% of all participants (n=269). When applying the assays on the intended platform, Ventana BenchMark, an overall pass rate (irrespective of protocol settings) of 97% (242 of 250) was observed and 81% (203 of 250) of the results evaluated as optimal.
Similar to previous assessments, it was noticed that the majority of laboratories (78%; 195/250) used the two assays by modified protocol settings as shown in Tables 1 and 2. For both the PATHWAY® HER2 IHC assay 790-2991 and the VENTANA HER2 (4B5) assay 790-4493, the pass rate and proportion of optimal results were superior using the assay by laboratory modified settings compared to the recommended protocol settings (see Tables 1 and 2).

Comparable to runs B32 - B34, it was observed that 12% of the participants used OptiView or UltraView with amplification for the Ventana/Roche PATHWAY® HER2 IHC assay 790-2991 and VENTANA HER2 (4B5) 790-4493, substituting iView or UltraView as recommended by Ventana/Roche. As in the previous assessment, this modification was again found very successful providing a pass rate of 97% (28/29) which is superior to the vendor recommended protocol settings. However, this observation must be carefully evaluated as in previous assessment runs e.g. run B28, this modification frequently induced an insufficient result characterized by a false positive 3+ HER2 reaction in a 2+ HER2 gene unamplified breast carcinoma. In addition potentially also might increase the number of HER2 2+ cases on a daily basis hereby extending the number of cases reflexed to ISH for final HER2 status. This underlines that modifications of CDx assays should be meticulously validated by the end-users on a large cohort of breast carcinomas (e.g. n=100). This has been addressed by ASCO/CAP in both the 2013 guidelines for HER2 testing and the 2020 guidelines for ER/PR testing and in particular in detail in the publication by Torlakovic et al; "*Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine Part 3: Technical Validation of Immunohistochemistry", AIMM 2017;25:151–159.*

The recently launched Dako/Agilent HercepTest™ CDx assay GE001 for Dako Omnis based on the rmAb clone DG44 was the most widely used "non-Ventana" CDx assay and used by 7% (n=27) of all participants. As seen in Tables 1 and 2, the majority of all laboratories used the assay by vendor

recommended protocol settings and when used as "plug-and-play" a pass rate of 100% was obtained. However, the proportion of optimal results has decreased to 72% compared to 91% in run B34, which was mainly caused by an increased proportion and intensity of positive cells being visualized in the unamplified breast carcinomas, tissue cores 2, 3 and 5 compared to the levels characterized by NordiQC reference IHC methods and vast majority of participants.

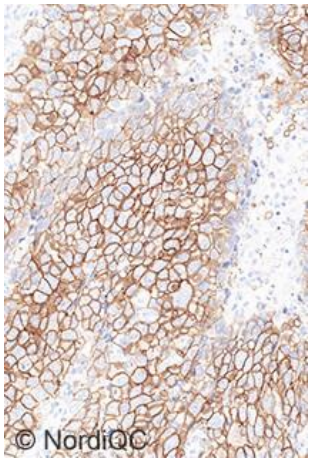
The "classic" Dako/Agilent HercepTest™ CDx assay SK001 for Dako Autostainer Link 48 provided a pass rate of 79% and 14% optimal results when used in concordance with the recommended protocol settings from Dako/Agilent. This was an improvement in the pass rate compared to the level seen in run B34 for this assay, however the proportion of optimal results has decreased. At present no plausible causes for the fluctuations of pass rates for SK001 as shown in Graph 1 can be identified and data must be interpreted with caution due to relatively few data points.

In this HER2 IHC assessment, 20% of the participants used LDTs based on concentrated Ab formats or generic RTU Abs without intended use or predictive claim for HER2 demonstration in breast carcinoma to guide decision with treatment with Herceptin or similar drugs. The proportion of laboratories using LDTs and CE-IVD approved HER2 IHC assays seems to be very consistent. In the three latest assessment runs B32 to B34, 21-22% of the participants used LDTs.

Overall, the LDTs in run B35 provided a pass rate of 79% (56 of 71), 35% (25 of 71) being optimal. During the last 4 HER2 IHC assessment runs B32 - B35 with focus on HER2 overexpression LDTs have showed a consistent improvement in pass rates from 58% to 79%.
The pAb A0485 from Dako/Agilent is still the most widely applied Ab within a LDT being used by 12% (47 of 403) of the participants and gave an overall pass rate of 87% and 43% optimal results.

Scoring consensus B35
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 80% (324 of 403) of the participants returning slides.
For 275 of the 324 (85%) 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 scoring guidelines.
Among laboratories with sufficient staining, 87% (256 of 293) of the scoring read-outs were in agreement with the NordiQC assessors. Disagreement was primarily related to the scoring of the HER2 status in the breast carcinoma, tissue core no. 3, which was characterized as 0/1+ both by the NordiQC reference standard methods and by the vast majority of all participants, but a minor proportion of participants scored this as 2+.
Among participants with insufficient staining results, 61% (19 of 31) scored their HER2 IHC results in consensus with the NordiQC assessor group. For this group the disagreement mainly was related to the scoring of the breast carcinoma, tissue core no. 1. The results submitted to NordiQC was scored as 2+ by NordiQC assessor team and as 0 or 1+ by the participant. The NordiQC assessment was primarily based on strict adherence to the ASCO/CAP guidelines but also to the level expected and characterized by the NordiQC HER2 IHC reference standard methods.

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 – excessive background reaction, same protocol



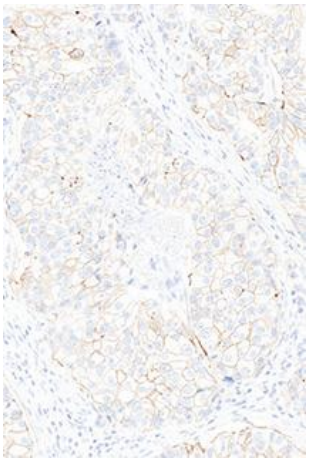
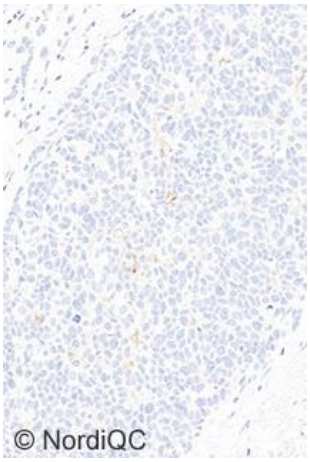


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.1-4.54.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



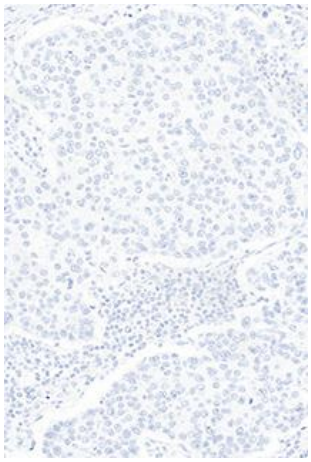
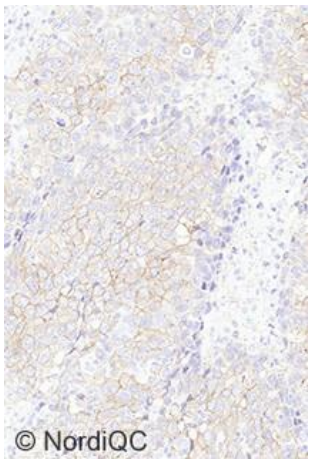


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.39.
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 6 with a HER2 / chr17 ratio of 0.76.
No staining reaction is seen corresponding to 0.



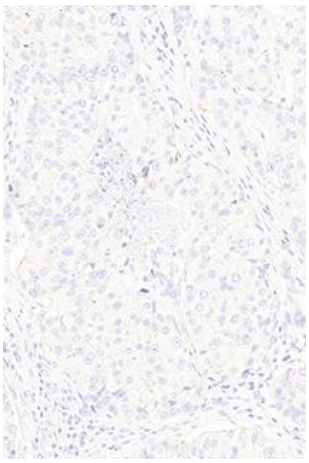
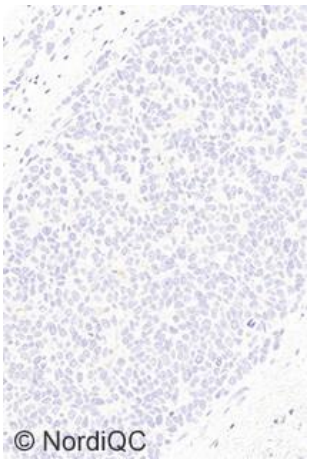


Fig. 2a.
Left: **Insufficient staining result** for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >6.
>10% of the neoplastic cells show a moderate complete membranous staining reaction corresponding to 2+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.1-4.54.
>10% of the neoplastic cells show a weak, but incomplete membranous staining reaction corresponding to 1+ (the core was scored as 0 by the participant and 1+ by NordiQC).



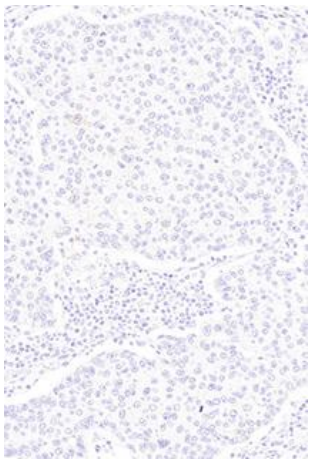
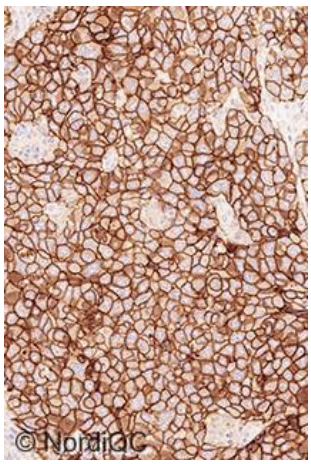


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.39.
<10% of the neoplastic cells show a weak partial membranous staining reaction corresponding to 0.
Right: Staining result for HER2 of the breast carcinoma no. 6 with a HER2 / chr17 ratio of 0.76.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.



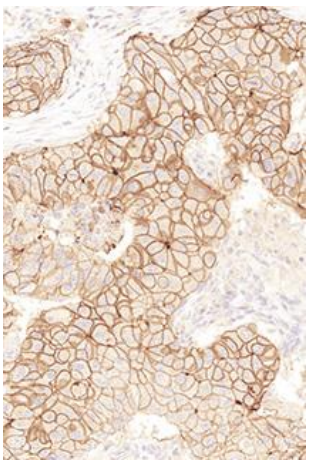
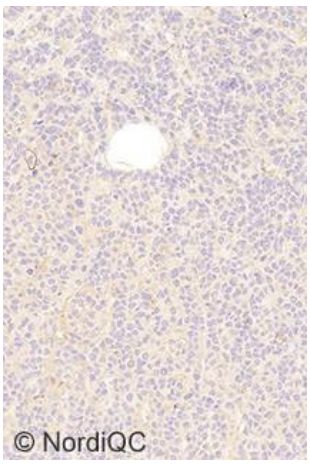


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >6.
>10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.1-4.54.
>10% of the neoplastic cells show a moderate to strong and complete membranous staining reaction corresponding to 3+. An excessive cytoplasmic staining reaction is seen, but the scoring is not compromised.



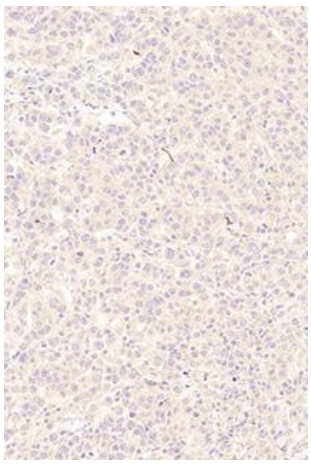


Fig. 3b.
Left: **Insufficient staining result** for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.39.
<10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+, however the diffuse cytoplasmic staining is interfering interpretation.
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 6 with a HER2 / chr17 ratio of 0.76.
A diffuse cytoplasmic staining reaction is observed hampering the read-out of the specific HER2 expression.

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