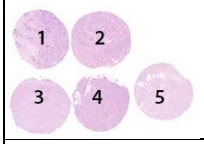
Assessment Run B33 2022

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 expression 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 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 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	0-1+	1.3	1-3	Unamplified
Breast carcinoma, no. 2	1-2+	1.4	1-3	Unamplified
Breast carcinoma, no. 3	2+	3.5	>6	Amplified
Breast carcinoma, no. 4	3+	>5 (clusters)	>6	Amplified
Breast carcinoma, no. 5	3+	>2 (clusters)	>6	Amplified

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

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. 1.
- Staining corresponding to score 0, 1+ or 2+ in carcinoma no. 2.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 3.
- Staining corresponding to score 3+ in carcinomas no. 4 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. 4 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 HER2 2+ gene amplified tumour no. 3 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+ tumour no. 1.

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+ tumours or the 2+ tumour with HER2 gene amplification showing a 0 or 1+ reaction) ***or*** a false positive staining (e.g. the IHC 2+ tumour without HER2 gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B33	415
Number of laboratories returning slides	386 (93%)

Results

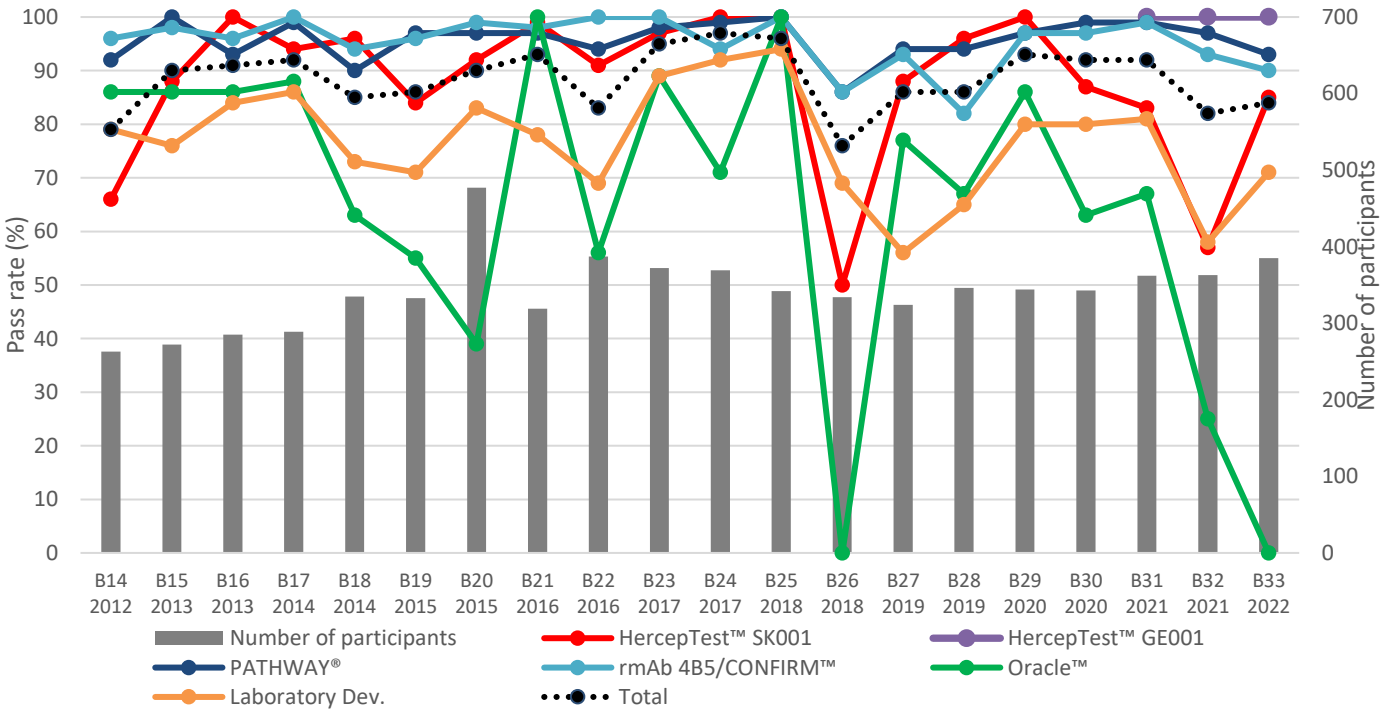
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

In total 386 laboratories participated in this assessment. One submitted a slide stained for HER2 on the PR slide and was excluded from the data analysis. Of the remaining 385 participants, 84% achieved a sufficient mark (optimal or good).

The overall pass rate was almost identical to the latest run B32 and slightly reduced compared to the level seen in the three previous assessment runs B29-B31.
In this and in concordance to recent NordiQC data, the two established FDA-/CE-IVD approved HER2 IHC assays from Ventana/Roche, PATHWAY® 790-2991 and HER2/4B5 790-4493 provided the highest proportion of optimal results, 79% and 76%, respectively with an overall pass rate of 93% and 90% when used by vendor recommended protocol settings (VRPS), see Tables 1 and 2, below.
The recently launched HercepTest™ GE001, Dako/Agilent, provided a high pass rate of 100% but with a reduced proportion of 35% optimal results using VRPS.
Laboratory developed tests (LDT's) based on RTU Abs without predictive claim or based on concentrated Abs were less successful giving a pass rate of 71%.

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

Graph 1. **Pass rates* of the HER2 IHC assessments in the NordiQC breast cancer module 2012-2022**



* pass rates using vendor recommended protocol settings

Table 1. **Assessment marks for IHC assays and antibodies run B33, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[4]**	29	Ventana/Roche	23	2	-	4	93%	79%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	127	Ventana/Roche	88	21	3	15	86%	69%
VENTANA HER2 (**4B5**), **790-4493, (VRPS)[4]**	21	Ventana/Roche	16	3	-	2	90%	76%
VENTANA HER2 (**4B5**), **790-4493, (LMPS)[5]**	80	Ventana/Roche	55	17	1	7	90%	69%
HercepTest™, pAb **SK001, (VRPS)[4]**	13	Dako/Agilent	7	4	1	1	85%	54%
HercepTest™, pAb **SK001, (LMPS)[5]**	3	Dako/Agilent	1	2	-	-	-	-
HercepTest™, rmAb **DG44 GE001, (VRPS)[4]**	17	Dako/Agilent	6	11	-	-	100%	35%
HercepTest™, rmAb **DG44 GE001, (LPMS)[5]**	4	Dako/Agilent	-	3	-	1	-	-
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	1	Leica Biosystems	-	-	-	1	-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	7	Leica Biosystems	1	3	2	1	43%	14%
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **10A7**	1	Leica Biosystems	-	-	-	1	-	-
rmAb clone **BP6020**	1	Bailing Biotechnology	-	1	-	-	-	-
rmAb clone **EP3**	1 1 1	Cell Marque Epitomics Zytomed	2	-	-	1	-	-
rmAb clone **QR3**	1	Quartett	-	1	-	-		
rmAb clone **SP3**	6 5 2 1 3	Cell Marque Thermo Fisher Scientific Zytomed DCS Unknown	1	5	3	8	35%	6%
rmAb clone **ZR218**	1	Zeta Corporation	-	-	-	1		
pAb, **A0485**	50	Dako/Agilent	21	22	-	7	86%	42%
pAb **IHC012**	1	GenomeMe	-	1	-	-	-	-
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Ab clone **MXR001, Kit-0043**	2	Fuzhou Maixin	1	1	-	-	-	-
rmAb clone **EP3 AN726**	1	BioGenex	-	-	-	1		
rmAb clone **SP3, MAD-000308QD**	2	Master Diagnostica	1	1	-	-	-	-
rmAb clone **SP3, 237R-17**	3	Cell Marque	-	1	1	1	-	-
Total	385		223	99	11	52		
Proportion			58%	26%	3%	13%	84%	

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, 111 of 156 (71%) 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 UltraView or OptiView as detection kit. Using these protocol settings, 118 of 136 (87%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 71 of 101 (70%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 30-64 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView as detection system. Using these protocol settings, 72 of 82 (88%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 8 of 16 (50%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 40-45 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, 14 of 16 (88%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 6 of 21 (29%) 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 Polymer as detection system. Using these protocol settings, 17 of 17 (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 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**	25/29 (93%)	23/29 (79%)	105/121 (87%)	86/121 (71%)
Ventana BenchMark XT, GX, Ultra VENTANA 4B5, **790-4493**	19/21 (90%)	16/21 (65%)	69/77 (90%)	53/77 (69%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	11/13 (85%)	7/13 (54%)	3/3	1/3
Dako Omnis HercepTest™ rmAb DG44, **GE001**	17/17 (100%)	6/17 (35%)	3/4	0/4
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	0/1	0/1	4/7 (43%)	1/7 (14%)

* 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**: 21 of 50 (42%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) low pH (Dako/Agilent) (12/28*), TRS High pH (Dako/Agilent) (7/14), CC1 (Ventana/Roche) (1/4) or Novocastra low pH 6 (1/1). The Ab was typically diluted in the range of 1:100-1.000 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 39 of 46 (85%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

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 GX / 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**	3/7** (43%)	4/9 (44%)	4/7 (57%)	8/19 (42%)	1/4	-	0/3	-

* 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 B33 for HER2, a pass rate of 84% was obtained and almost identical to the latest run B32 (82%) but slightly reduced compared to the level seen in the three previous assessment runs B29-B31 (see Graph 1).

The insufficient results were primarily characterized by a too weak or false negative staining reaction being observed in 84% (53 of 66 results). Virtually all laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinomas, tissue cores no. 4 and 5, 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. 3. This tumour was categorized as IHC 2+ in the NordiQC reference laboratories using the two FDA/CE-IVD HER2 IHC assays: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (ratio 3.5) by FISH.
In 5% of the insufficient results a false positive staining result was observed being characterized by a 3+ IHC staining result in the HER2 non-amplified breast carcinoma, tissue core no. 2, expected to be 1-2+.
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 interpretation of the specific HER2 membranous reaction.

76% of the participants (n=293) used one of the FDA/CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY®, 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. 2% (n=9) of the participants used one of approved assays on another platform than specified by the vendor, while the remaining 22% (n=83) 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 Ventana/Roche PATHWAY® HER2 IHC assay 790-2991 and VENTANA HER2 (4B5) 790-4493 were used by 67% of all participants (n=257). When applying the assays on the intended platform, BenchMark, an overall pass rate of 88% was observed and 72% of the results evaluated as optimal. The overall pass rate was slightly reduced compared to the level of 93% observed in the latest run B32.
Similar to previous assessments, it was noticed that the majority of laboratories (80%, 198 of 248) used the two assays by modified protocol settings as shown in Table 1 and 2. For the PATHWAY® HER2 IHC assay 790-2991, the pass rate and proportion of optimal results was superior, when applied in concordance to the instructions and guidelines provided by the vendor, whereas for the VENTANA HER2 (4B5) assay 790-4493, the pass rate and proportion of optimal results were fully comparable using the assay as "plug-and-play" and strictly compliant to the recommended protocol settings or using modified protocols (see Tables 1 and 2).

Comparable to B32, it was observed that 10% 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. In previous runs as B28, this modification frequently induced an insufficient result characterized by a false positive 3+ HER2 reaction in a 2+ HER2 gene unamplified breast carcinoma and/or potentially also 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 used by 5% (n=21) of all participants and provided an overall pass rate of 95%, 29% optimal results. As seen in Table 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. Similar to the results in run B32, the proportion of optimal results was reduced compared to the level seen for the Ventana/Roche CDx assays based on rmAb clone 4B5. The relatively low level of optimal results was mainly caused by an increased cytoplasmic staining reaction complicating the interpretation of the coexisting membranous reaction especially in the breast carcinoma, tissue no. 3, expected to be "HER2 positive" being identified as IHC 2+ and showed HER2 gene amplification.
In addition, the breast carcinoma, tissue core no. 1, expected to be 0/1+ occasionally showed a 2+ IHC reaction and hereby an additional ISH test being required and accordingly to the assessment criteria consequently scored as "Good" providing an otherwise optimal end expected result in all other tissues.

The "classic" Dako/Agilent HercepTest™ CDx assay SK001 for Dako Autostainer Link 48 provided a pass rate of 85% and 54% optimal results when used in concordance with the recommended protocol settings from Dako/Agilent. This was a significant improvement compared to the level seen in run B32 for this assay. 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.

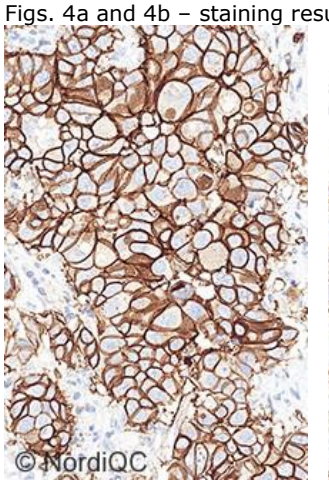
In this HER2 IHC assessment, 22% of the participants used LDTs based on concentrated Ab formats or generic RTU Abs without intended use or predictive claim for HER2 demonstration in breast carcinoma to guide decision with treatment with Herceptin or similar drugs. Overall, the LDTs provided a pass rate of 71% (59 of 83) and 31% optimal (26 of 83).
The pAb A0485 from Dako/Agilent was the most widely applied Ab within a LDT being used by 13% (n=50 of 385) of the participants and gave an overall pass rate of 86% and 42% optimal results.

The proportion of laboratories using the FDA-/CE-IVD approved HER2 IHC assays and LDTs is very consistent. In the two latest assessment runs B32 and B33, 22% of the participants used LDTs compared to 23-31% in previous assessments.

Scoring consensus B33
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 83% (320 of 385) of the participants returning slides.
For 265 of the 320 (83%) 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. This was virtually identical to the level of 82% seen in run B32 and slightly improved to the level of 75% and 77% observed in runs B30 and B31, respectively.
Among laboratories with sufficient staining, 86% (234 of 272) 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 carcinomas, tissue cores no. 1 and 3. Tissue core no. 1 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+. The results in tissue core no. 3 were also by a minority of participants obtaining a sufficient mark, scored as 1+, whereas the submitted result scored as 2+ by the NordiQC assessor group.
Among participants with insufficient staining results, 65% scored their HER2 IHC results in consensus with the NordiQC assessor group (31 of 48). For this group the disagreement solely was related to the scoring of the breast carcinoma, tissue core no. 3. The results submitted to NordiQC was scored as 1+ by NordiQC assessor team and as 2+ 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 two HER2 IHC reference standard methods.

Conclusion
The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/VENTANA HER2 (4B5)** 790-2991/790-4493 from Ventana/Roche and **HercepTest™**, GE001 Dako/Agilent were in this assessment the most successful assays for the semi-quantitative IHC determination of HER2 protein expression in breast carcinoma. In this context it was observed that the two Ventana/Roche CDx assays gave the highest proportion of optimal results.
Laboratory developed assays based on concentrated formats and RTU formats without a predictive claim provided a lower pass rate and a reduced proportion of optimal results. Inclusion of 2+ tumours with and without HER2 gene amplification in the control material for both EQA and internal quality control seems to be essential to evaluate accuracy, precision and reproducibility for HER2 IHC testing.

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
Figs. 4a and 4b – staining results evaluated as good – excessive cytoplasmic staining reaction, same protocol



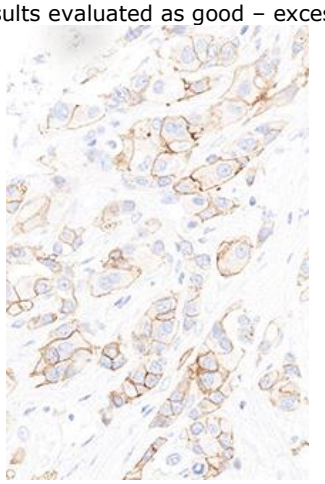
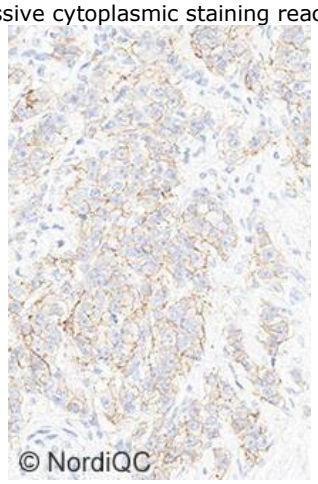


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >5.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 3.5.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.



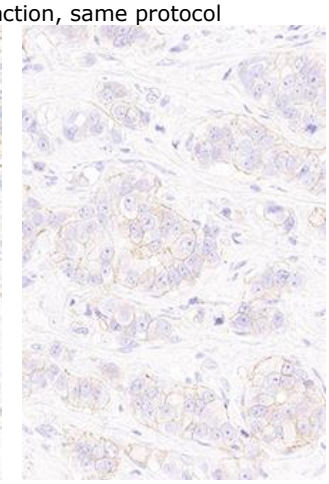
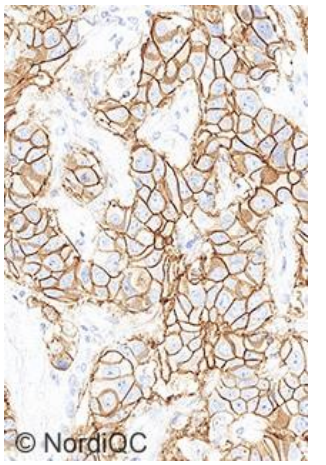


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.4.
>10% of the neoplastic cells show a weak complete membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 1 with a HER2 / chr17 ratio of 1.3.
>10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 1+.



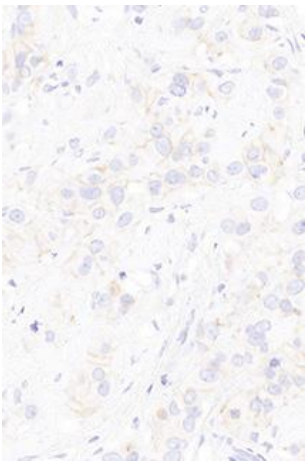
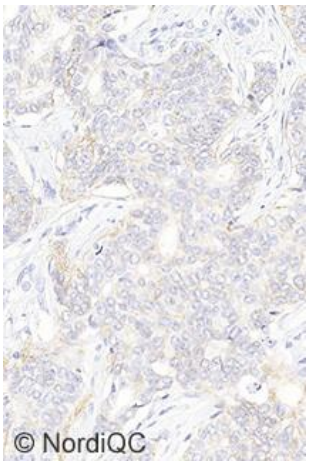


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >5.
>10% of the neoplastic cells show a strong complete membranous staining reaction corresponding to 3+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 3.5
>10% of the neoplastic cells show a weak to moderate, but incomplete membranous staining reaction corresponding to 1+ (the core was scored as 1+ both by the participant and NordiQC).



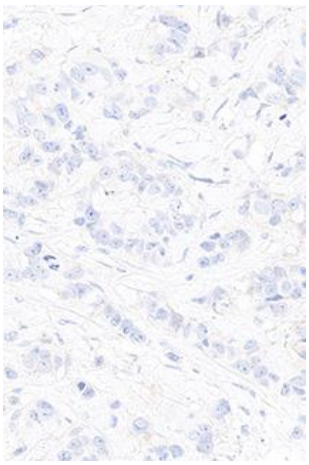
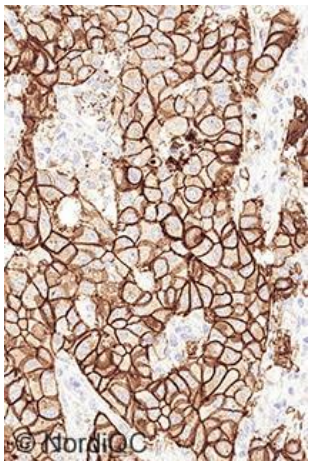


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.4.
<10% of the neoplastic cells show a weak partial membranous staining reaction corresponding to 0.
Right: Staining result for HER2 of the breast carcinoma no. 1 with a HER2 / chr17 ratio of 1.3.
No staining reaction is seen corresponding to 0.



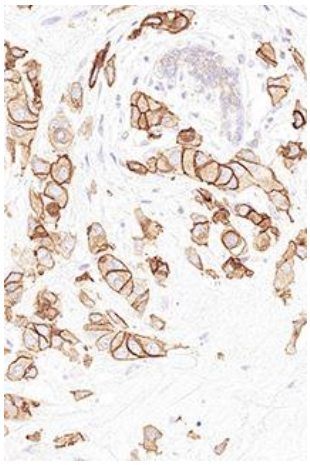
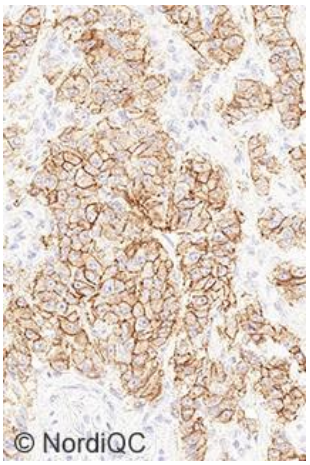


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >5.
>10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 3.5. >10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+. An excessive cytoplasmic staining reaction is seen, but the scoring not compromised.



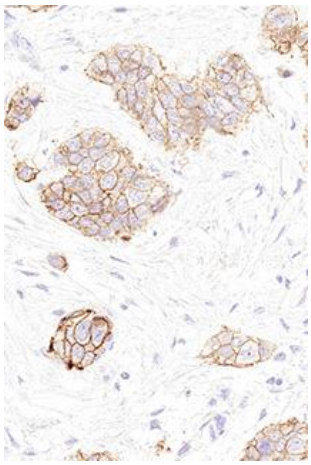
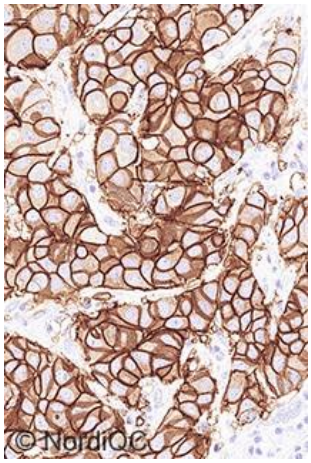


Fig. 3b.
Left: **Insufficient and false positive staining result** for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.4.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+ (the core was scored as 3+ both by the participant and NordiQC).
Right: Insufficient staining result for HER2 of the breast carcinoma no. 1 with a HER2 / chr17 ratio of 1.3.
Overall a 2+ result is observed and not as expected 0-1+. In areas focally a 3+ reaction was seen.



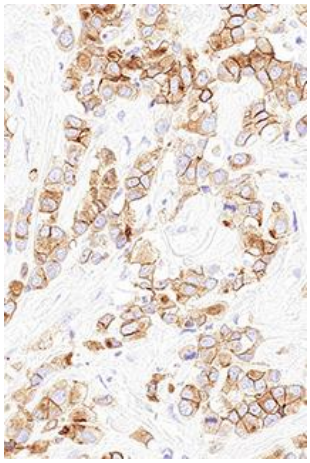
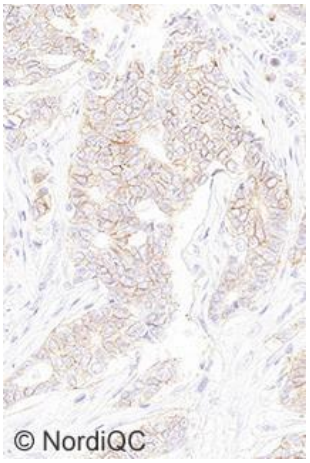


Fig. 4a.
Left: Staining result for HER2 of the breast carcinoma no. 4 with a ratio of HER2 / chr17 of >5.
>10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: **Staining result evaluated as good** for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 3.5. The excessive cytoplasmic staining reaction and reduced membranous staining reaction comprises the read-out. Compare with Fig. 1a, right.
The tumour was scored as 2+ both by the participant and NordiQC.



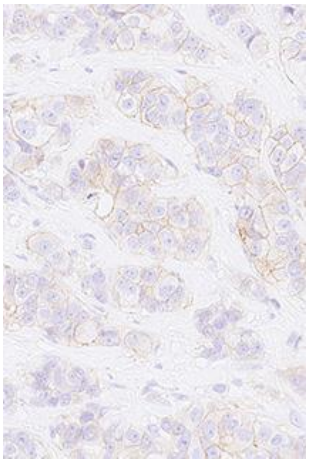


Fig. 4b.
Left: Staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.4.
>10% of the neoplastic cells show a weak complete membranous staining reaction corresponding to 2+.
Right: Staining result for HER2 of the breast carcinoma no. 1 with a HER2 / chr17 ratio of 1.3.
>10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 1+.

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