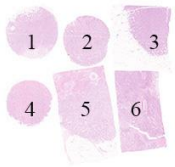
Assessment Run B38 2024
HER2 IHC

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.

Considering the emerging field of HER2-low, four relevant breast carcinoma (BC) samples for this category (HER2 0-2+, unamplified) were included in the TMA block circulated for this assessment. As stated above, the main aim of this assessment was to evaluate the classical demonstration of HER2 protein overexpression level according to the existing guidelines and the successful and unsuccessful results were mainly based on this primary purpose. However, with perspective on HER2-low classification, an otherwise optimal IHC assay for HER2 overexpression was downgraded to good, when any HER2-low positive or negative BC samples changed category compared to the expected result as listed in the table below.

Material
The slide to be stained for HER2 comprised the following 6 materials:

	**IHC: HER2 Score* (0, 1+, 2+, 3+)**	**FISH: HER2 gene/chr17 ratio****	**FISH: HER2 gene copy no.****	**FISH HER2 gene amplification status**
Breast carcinoma, no. 1	1-2+	1.93	2.8	Unamplified
Breast carcinoma, no. 2#	1-2+	1.04	1.4	Unamplified
Breast carcinoma, no. 3	1-2+	1.24	1.3	Unamplified
Breast carcinoma, no. 4	0	0.81	1.3	Unamplified
Breast carcinoma, no. 5	3+	7.43	8.6	Amplified
Breast carcinoma, no. 6	2+	2.89	4.3	Amplified

* HER2 immunohistochemical score (see table below) as achieved by using two CE-IVD approved HER2 IHC assays, HercepTest™ (GE001, Dako/Agilent) and Ventana HER2 4B5 (790-4493, 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.
# Breast carcinoma no. 2 was excluded from assessment, due to extensive heterogenous HER2 expression in the reference slides.

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

KEY POINTS FOR HER2 IHC ASSAYS
- Companion diagnostic IHC assays were more successful than laboratory developed assays.
- The **HercepTest™ GE001 assay**, Dako/Agilent, for Omnis provided the highest pass rate and proportion of optimal results.
- The recently launched Ventana/Roche **RxDx 4B5 HER2 assay 790-7167** was most reproducible among the Ventana/Roche HER2 assays.
- IHC assays showed high analytical concordance for HER2 overexpression and only moderate concordance for HER2 Low.

IHC scoring system according to the 2023 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 1+ or 2+ in carcinomas no. 1 and 3.
- Staining corresponding to score 0 in carcinoma no. 4.
- Staining corresponding to score 3+ in carcinoma no. 5.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 6.
- 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. 6 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by FISH/BRISH according to the ASCO/CAP 2023 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. 6 compared to the NordiQC reference standards determined by HercepTest™ and PATHWAY® ***or*** (3) a 1+ reaction was seen in the HER2 gene unamplified 0 tumor no. 4 ***or*** (4) a 0 reaction was seen in the HER2 unamplified tumors no. 1 and/or 3.

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+ tumor 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 B38	446
Number of laboratories returning slides	418 (94%)

Results
At the time of the assessment, 94% 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.

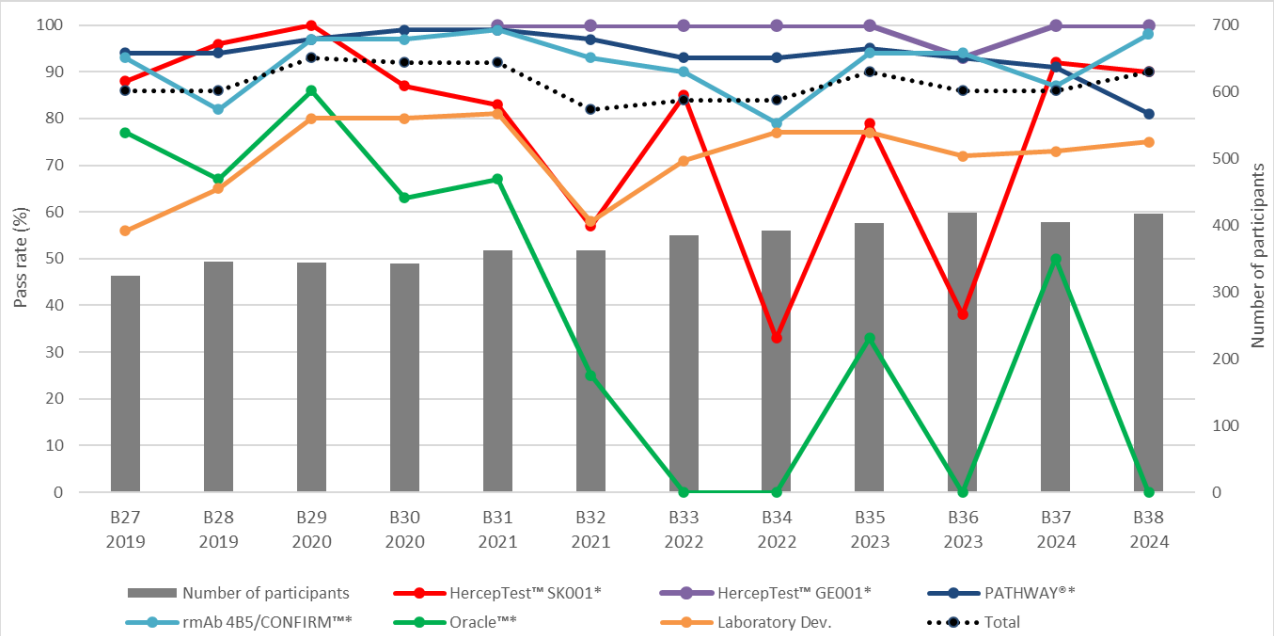
In total, 418 laboratories participated in this assessment and 90% achieved a sufficient mark (optimal or good).

Conclusions
In this assessment, the **HercepTest™, GE001**, Dako/Agilent, for the Omnis platform was most successful providing an overall pass rate of 100% and 82% optimal results when using the vendor recommended protocol settings (VRPS).
The newly launched FDA-/CE-IVD approved assay **Ventana RxDx HER2 4B5**, **790-7167** (Ventana/Roche) was also very successful giving a pass rate of 100%, 71% optimal when applied by VRPS. For unexplained reasons, the widely used and established FDA-/CE-IVD approved HER2 IHC assay **PATHWAY® 790-2991** (Ventana/Roche) gave an inferior overall pass rate of 81%, 58% optimal when using VRPS.
Laboratory developed tests (LDT's) based on RTU Abs without predictive claim or based on concentrated Abs gave a pass rate of 75%, 42% optimal.
Many assays were successful for HER2 classical overexpression but showed a decreased agreement and concordance for HER2 Low compared to the level expected and defined by the NordiQC reference methods.

Assessment marks for HER2 IHC CDx assays and HER2 LDTs (conc. Ab and RTU) are summarized in Tables 1a-1d (see pages 3-5).

The historical pass rates of the NordiQC HER2 IHC assessments are illustrated in Graph 1 (see page 3).

Graph 1. **Pass rates of the HER2 IHC assessments in the NordiQC breast cancer module 2019-2024**



* pass rates using vendor recommended protocol settings

Table 1a. **Assessment marks for HER2 IHC assays and antibodies run B38**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
IVD approved HER2 assays	351	244	85	1	21	94%	70%
Concentrated antibodies	58	22	20	1	15	72%	38%
Ready-To-Use antibodies	9	6	2	1	0	89%	67%
Total	418	272	107	3	36		
Proportion		65%	25%	1%	9%	90%	

1) Suff.: Proportion of sufficient stains (optimal or good).
2) OR: Proportion of optimal results.

Table 1b. **Assessment marks for IVD approved HER2 IHC CDx assays**

IVD approved HER2 CDx assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[3]**	31	Ventana/Roche	18	7	1	5	81%	58%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)**[4]	99	Ventana/Roche	73	22	0	4	96%	74%
VENTANA HER2 rmAb clone **4B5, 790-4493, (VRPS)[3]**	46	Ventana/Roche	27	18	0	1	98%	59%
VENTANA HER2 rmAb clone **4B5, 790-4493, (LMPS)**[4]	97	Ventana/Roche	75	17	0	5	95%	77%
VENTANA RxDx HER2 rmab clone **4B5, 790-7167, (VRPS)[3]**	14	Ventana/Roche	10	4	0	0	100%	71%
VENTANA RxDx HER2 rmab clone **4B5, 790-7167, (LMPS)**[4]	9	Ventana/Roche	5	4	0	0	100%	56%
HercepTest™, pAb, **SK001, (VRPS)[3]**	10	Dako/Agilent	5	4	0	1	90%	50%
HercepTest™, pAb, **SK001, (LMPS)**[4]	2	Dako/Agilent	0	1	0	1	-	-
HercepTest™, rmAb **DG44, GE001, (VRPS)[3]**	33	Dako/Agilent	27	6	0	0	100%	82%
HercepTest™, rmAb **DG44, GE001, (LPMS)**[4]	5	Dako/Agilent	4	1	0	0	100%	80%
Oracle™ mAb clone **CB11**, **TA9145, (VRPS)[3]**	3	Leica Biosystems	0	0	0	3	-	-
Oracle™ mAb clone **CB11, TA9145, (LPMS)**[4]	2	Leica Biosystems	0	1	0	1	-	-
Total	351		244	85	1	21		
Proportion			70%	24%	0%	6%	94%	

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

Table 1c. **Assessment marks for laboratory developed HER2 assays, concentrated antibodies**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP3**	2	Biocare	3	1	0	2	67%	50%
	2	Cell Marque						
	1	Epitomics						
	1	Zytomed						
rmAb clone **SP3**	3	Thermo Fisher Scientific/Epredia	1	3	1	1	67%	17%
	1	Cell Marque						
	1	Master Diagnostica						
	1	Invitrogen						
rmAb clone **QR003**	2	Quartett	0	1	0	1	-	-
rmAb clone **BP6020, BX50015B**	1	Biolynx	1	0	0	0	-	-
pAb, **A0485**	43	Dako/Agilent	17	15	0	11	74%	40%
Total	58		22	20	1	15		
Proportion			38%	34%	2%	26%	72%	

1) Suff.: Proportion of sufficient stains (optimal or good).
2) OR: Proportion of optimal results.

Table 1d. **Assessment marks for laboratory developed HER2 assays, Ready-To-Use antibodies**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **246G0D3, PA216**	1	Abcarta/Abcepta	1	0	0	0	-	-
rmAb clone **MXR011, RMA-1022**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **EP3, 8388-C010**	1	Sakura Finetek	0	1	0	0	-	-
rmAb clone **EP3, AC-0014EU**	1	Epitomics	1	0	0	0	-	-
rmAb clone **SP3, MAD-000308QD**	3	Master Diagnostica	1	1	1	0	-	-
rmAb clone **SP3, 237R-17/18**	1	Cell Marque	1	0	0	0	-	-
Total	9		6	2	1	0		
Proportion			67%	22%	11%	0%	89%	

1) Suff.: Proportion of sufficient stains (optimal or good).
2) OR: Proportion of optimal results.

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): In total, 91 of 130 (70%) 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 GX, XT, Ultra or Ultra Plus, 12-32 min. incubation of the primary Ab and UltraView DAB as detection kit. Using these protocol settings, 91 of 100 (91%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 102 of 143 (71%) 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, GX, Ultra or Ultra plus, 12-32 min. incubation of the primary Ab and UltraView DAB as detection system. Using these protocol settings, 105 of 106 (99%) laboratories produced a sufficient staining result.

Ventana RxDx HER2 rmAb clone **4B5** (790-7167, Ventana/Roche): In total, 15 of 23 (65%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 30-36 min.) on BenchMark GX, Ultra or Ultra plus, 12-16 min. incubation of the primary Ab and UltraView DAB as detection system. Using these protocol settings, 14 of 14 (100%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 5 of 12 (42%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97-98°C for 40 min. in the PT Link, 30 min. incubation of the primary Ab and SK001 as detection system. Using these protocol settings, 9 of 10 (90%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 31 of 38 (82%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in Target Retrieval Solution, Low pH at 97°C for 30 min., 10 min. incubation of the primary Ab and GE001/GV800 as detection system. Using these protocol settings, 33 of 33 (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 platform 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, Ultra Plus PATHWAY® rmAb 4B5, **790-2991**	25/31 (81%)	18/31 (58%)	89/93 (96%)	70/93 (75%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA 4B5, **790-4493**	45/46 (98%)	27/46 (59%)	89/92 (97%)	73/92 (79%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA RxDx 4B5, **790-7167**	14/14 (100%)	10/14 (71%)	9/9 (100%)	5/9 (56%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	9/10 (90%)	5/10 (50%)	0/1	0/1
Dako Omnis HercepTest™ rmAb DG44, **GE001**	33/33 (100%)	27/33 (82%)	4/4	3/4
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	0/3	0/3	1/2	0/2

* 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**: 17 of 43 (40%) protocols were assessed as optimal. Optimal protocols were typically based on HIER using either TRS low pH (Dako/Agilent) (7/20*), TRS High pH (Dako/Agilent) (5/8), CC1 (Ventana/Roche) (1/4), Bond™ Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (2/5) or Bond™ Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/6). 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, 30 of 38 (79%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP3**: 3 of 6 (50%) protocols were assessed as optimal. All 3 optimal protocols were based on HIER using BERS2 (Leica Biosystems). 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 antibody	Dako/Agilent Autostainer[1]		Dako/Agilent Omnis		Ventana/Roche BenchMark[2]		Leica Biosystems Bond[3]	
	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**	3/3**	3/9 (33%)	2/5 (40%)	4/11 (36%)	1/4	-	2/6 (33%)	2/5 (40%)
rmAb clone **EP3**	-	-	-	-	-	-	3/3	-

* 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
1) Autostainer Link 48
2) BenchMark XT, Ultra
3) Bond MAX, III, Prime

Comments

In this NordiQC assessment run B38 for HER2 IHC an overall pass rate of 90% was seen and slightly superior to the average level of 88% obtained in the assessment runs from 2019-2024 (see Graph 1).

The insufficient results were primarily characterized by a reduced proportion of positive cells, a too weak or false negative staining reaction being observed in 90% (35/39) of slides receiving an assessment mark borderline or poor. The vast majority of laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinoma, tissue core no. 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. 6. This tumor was categorized as IHC 2+ in the NordiQC reference laboratory using the CE-IVD HER2 IHC assays: Ventana

HER2 4B5 (790-4493, Ventana/Roche) and HercepTest™ (GE001, Dako/Agilent) and showed HER2 gene amplification (HER2 gene/chr17 ratio of 2,89) by FISH.

The remaining insufficient results were characterized by either poor signal-to-noise or excessive cytoplasmic staining reaction compromising the read-out and scoring of the specific HER2 membranous reaction.

81% of the participants (338/418) 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 platform with predictive claim for HER2 status in breast cancer. 3% (13/418) of the participants used one of the approved assays on another platform than specified by the vendor, while the remaining 16% (67/418) used a laboratory developed test (LDT) based on a concentrated primary Ab or RTU format without a predictive claim.

The well-stablished Ventana/Roche assays **PATHWAY®**, **790-2991** and **VENTANA HER2 (4B5)**, **790-4493** and the recently launched **Ventana RxDx HER2 4B5**, **790-7167** were most widely applied and in total used by 71% of all participants (296/418). When applying these assays on the intended platforms, Ventana BenchMark, a cumulated overall pass rate of 92% (84/91) was observed when applied by vendor recommended protocol settings (VRPS), compared to 96% (187/194) when used by laboratory modified protocol settings (LPMS) (see Tables 1b and 2).

For unexplained reasons, the Ventana/Roche **PATHWAY®** HER2 IHC assay **790-2991**, gave an inferior performance compared to the two other Ventana/Roche 4B5 HER2 assays. When used by VRPS, the **PATHWAY®** HER2 IHC assay gave a pass rate of 81% compared to e.g. 100% for the **Ventana RxDx HER2 4B5** assay. The inferior performance was related to both an increased proportion of false negative results for the breast carcinoma tissue core no. 6 expected to be HER2 IHC 2+ and amplified, but more widely giving a 0 IHC reaction in one or more of the breast carcinomas expected to be HER2 Low (cores 1 and3; expected to be 1+ or 2+). For the latter observation a change in HER2 score from "HER2 Low positive" to "HER2 Low negative" in one or more of the included breast carcinomas, this performance was accepted as a sufficient result but downgraded to "Good" from "Optimal" provided that the expected results was obtained for the classical HER2 overexpression status in all samples.

Similar to runs B32 - B37, it was observed that 11% (33/296) of the participants used OptiView or UltraView with amplification for the Ventana/Roche assays **PATHWAY®** HER2 IHC assay **790-2991**, **VENTANA HER2 (4B5) 790-4493** and **Ventana RxDx HER2 4B5**, **790-7167** substituting UltraView as recommended by Ventana/Roche. In this assessment, this modification resulted in both a high pass rate of 100% (33/33) and also an increased level of optimal results at 79% (26/33). 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, it might potentially also increase the number of HER2 2+ cases on a daily basis and therefore extend the number of cases reflexed to ISH for final HER2 status, but especially in the diagnostic area of HER2 Low, the change might affect the proportion of HER2 Low cases and diagnostic accuracy for this entity. 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.*
As indicated above and with perspective on HER2-low IHC classification, any significant change to the validated VRPS such as exchanging a 2-step detection system with a 3-step detection system most likely will lead to different proportions of HER2 0 and HER2-low (1+ and 2+ unamplified). Currently, no secondary confirmatory method, such as FISH, is available to verify or validate the accuracy of IHC protocols for HER2-low classification. Therefore, it is strongly recommended to regularly assess the performance metrics of HER2 scores assigned to diagnostic breast carcinomas to ensure the expected levels are achieved.

The most 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 was used by 9% (n=38) of all participants. As seen in Tables 1b and 2, the vast majority of laboratories used the assay by vendor recommended protocol settings (VRPS) and when used as "plug-and-play" a pass rate of 100% (33/33) was achieved, as seen in most assessment runs B31-B38. (see Graph 1). The proportion of optimal results was 82% and as such the most successful and accurate IHC assay for both classical HER2 overexpression and HER2 Low in the breast carcinomas included in this assessment run.

The "classic" Dako/Agilent **HercepTest™** CDx assay **SK001** for Dako Autostainer Link 48 provided a pass rate of 90% (9/10) when used accordingly to VRPS and comparable to the level seen in the recent run B37. However, as shown in Graph 1, a fluctuation of the pass rates for SK001 with VRPS has been observed in previous assessments and most likely impacted by technical issues related to a semi-automated platform. Since run B29 and the introduction of the Dako/Agilent 2' gen **HercepTest™** for Omnis a consistently reduced number of SK001 based protocols have been submitted to the NordiQC breast module.

In this HER2 IHC assessment, 16% (67/418) 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 of treatment with Herceptin or similar drugs. The proportion of laboratories using LDTs has thus shown a slow, but consistent decrease in the NordiQC breast module for HER2 IHC with 16% being at the lowest level. Overall, the LDTs in run B38 provided a pass rate of 75% (50/67), 42% (28/67) being optimal.
The pAb **A0485** from Dako/Agilent is still the most widely applied Ab within a LDT being used by 10% (43/418) of the participants and gave an overall pass rate of 74% and 40% optimal results and similar to the performance seen in previous runs.

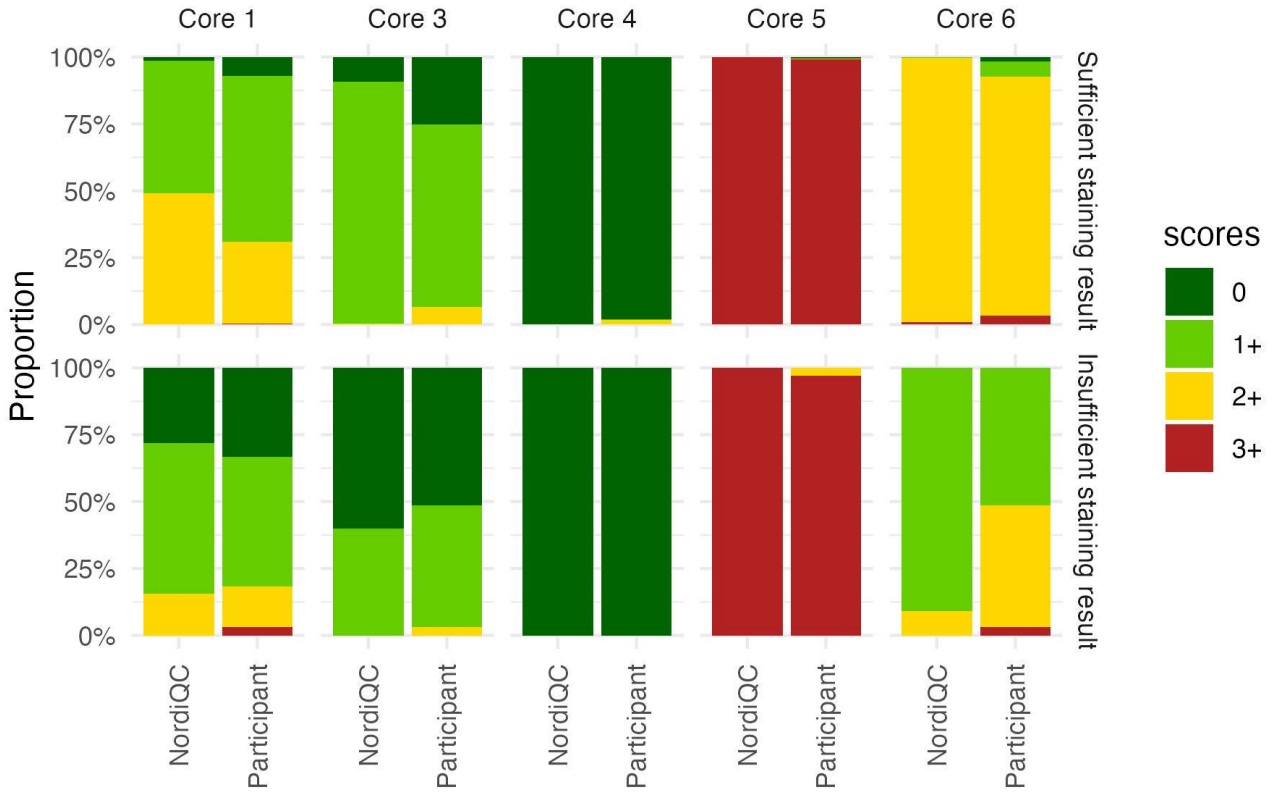
Scoring consensus B38
Participants were asked to submit self-evaluated scores (0, 1+, 2+, or 3+) for their HER2-stained slides on the NordiQC webpage. Of the 418 laboratories that returned slides, 82% (343/418) provided their self-assessments. In total, 43% (149/343) of these participants achieved full concordance with the NordiQC assessor group's scoring across all tissues in the multi-tissue sections, based on the ASCO/CAP 2023 scoring guidelines. This outcome is comparable to the previous assessment (B37), where a more precise scoring was carried out with heightened focus on HER2-low.

Table 4. **HER2 IHC scoring consensus results between scores submitted by participants and same tissue cores analyzed by the NordiQC assessor team**

		Participants				
NordiQC	**HER2**	**0**	**1+**	**2+**	**3+**	**Indeterminate**
	0	384	13	5	0	0
	1+	73	365	55	0	1
	2+	10	89	354	13	0
	3+	2	0	5	339	0
	Indeterminate	3	3	1	0	0

Among laboratories that produced sufficient staining results, 44% (135/310) of scoring read-outs were in complete agreement with the NordiQC assessors. Discrepancies primarily arose in the scoring of HER2 status for breast carcinoma tissue cores 1 and 3. These cores accounted for 78% (211/273) of all discordant scores, with participants frequently assigning lower scores than the NordiQC assessor team.

Graph 2. **Comparison of HER2 IHC Scores by Participants and NordiQC Assessors for Each Tissue Core**



Overall, a high level of consensus was observed between the HER2 IHC scores from participants and the NordiQC assessor team for tissue cores 1–5 (see Graph 2). For tissue core 6 (2+ with amplification), the majority of participants with sufficient staining results also scored it as 2+, in alignment with the NordiQC assessors. However, among participants with insufficient staining (typically due to weak reactions in core 6), 28% (18/66) still assigned a score of 2+.

A high degree of agreement was observed for tissue cores 1 and 3, which exhibited HER2-low patterns (1+/2+, unamplified). Notably, both NordiQC and participants with insufficient results more frequently scored tissue core 3 as 0 compared to participants with sufficient staining results.

Figs. 1a and 1b – **optimal staining results** for both HER2 overexpression and HER2 Low, same protocol

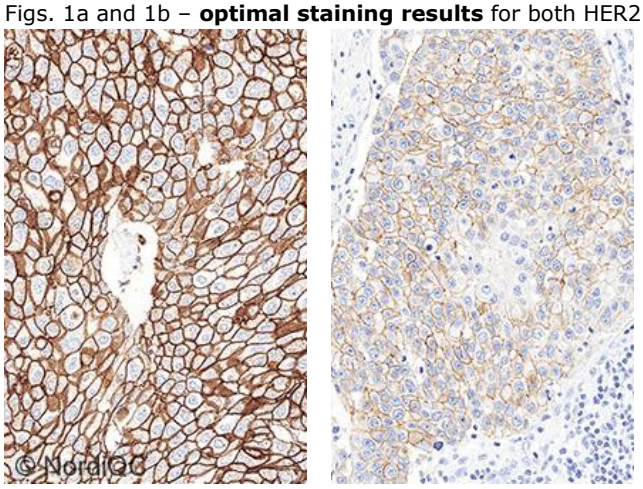


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of 2.89.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

Both tumours are categorized as HER2 positive - "classical overexpression".

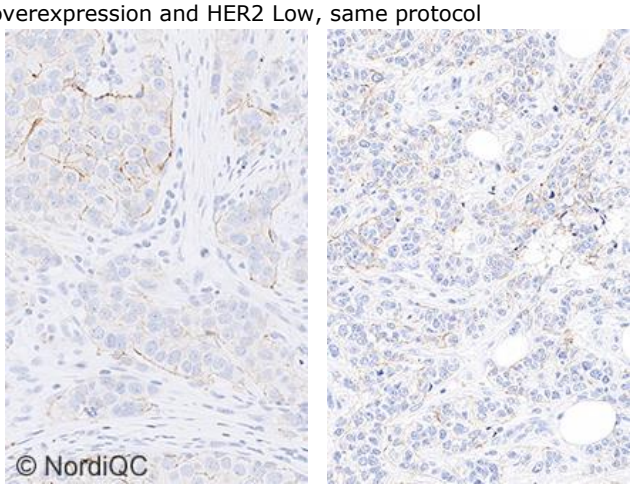


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 1, with a HER2/chr17 ratio of 1.93.
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+ (areas close to be 2+).
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 3, with a HER2/chr17 ratio of 1.24.
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+.

Both tumours are categorized as HER2 Low positive.

Figs. 2a and 2b – **insufficient staining results** - false negative for HER2 overexpression and HER2 Low, same protocol

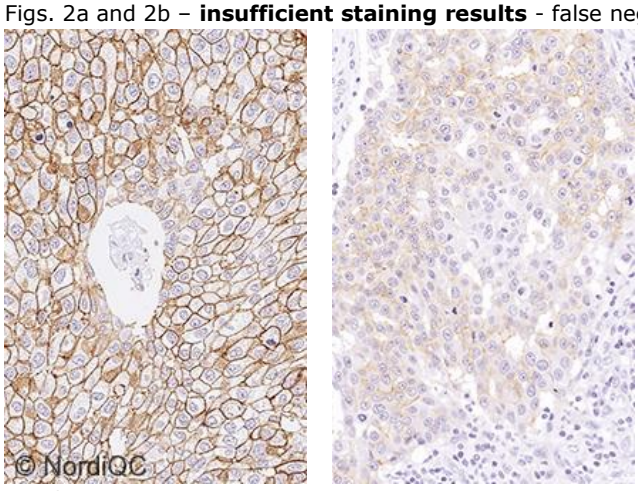


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a strong membranous staining reaction corresponding to 3+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of 2.89.
>10% of the neoplastic cells show a weak, but incomplete membranous staining reaction corresponding to 1+.
Both the participant and NordiQC scored the result as 1+.

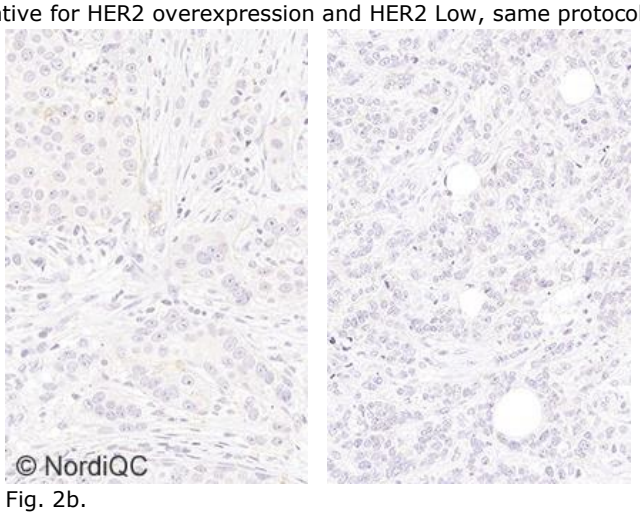


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 1, with a ratio HER2/chr17 of 1.93.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.
Both the participant and NordiQC scored the result as 0.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 3, with a HER2/chr17 ratio of 1.09.
No staining reaction is seen corresponding to 0.

Both tumours were categorized as HER 0 impacting HER2 Low classification as the expected 1+ status changed to 0.

Figs. 3a and 3b – **staining result assessed as Good** – expected result for HER2 overexpression, but failed HER2 Low.

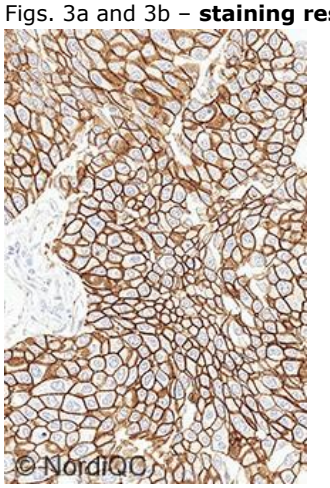


Fig. 3a.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of 2.89.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

Both tumours are categorized as HER2 positive - "classical overexpression".

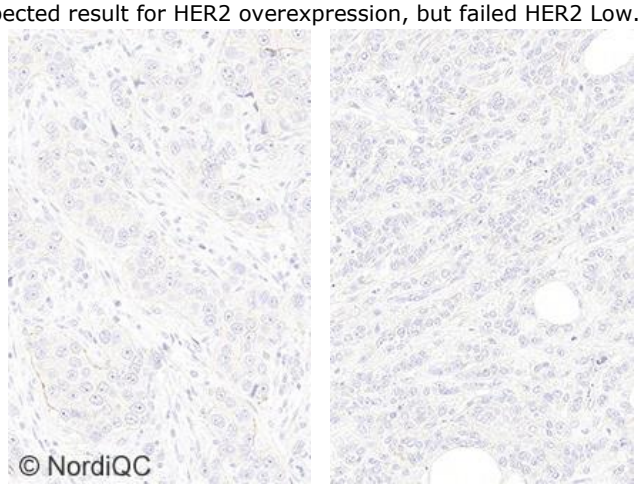


Fig. 3b.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 1, with a ratio HER2/chr17 of 1.93.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 3, with a HER2/chr17 ratio of 1.09.
No staining reaction is seen corresponding to 0.

Both the participant and NordiQC scored the result as 0 in the two tumours.

Both tumours were categorized as HER 0 impacting HER2 Low classification as the expected 1+ status changed to 0.

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