Assessment Run B40 2025
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 Ventana HER2 4B5 (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**	**HER2-low status**
Breast carc. no. 1	1-2+	1.87	3.6	Unamplified	Positive
Breast carc. no. 2	2+	3.33	5.9	Amplified	
Breast carc. no. 3	3+	8.75	10.5	Amplified	
Breast carc. no. 4	1-2+	1.35	1.8	Unamplified	Positive
Breast carc. no. 5	1-2+	1.11	1.6	Unamplified	Positive
Breast carc. no. 6	0	1.19	1.6	Unamplified	Negative

* 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.

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.
- IHC assays showed high analytical concordance for HER2 overexpression and only moderate concordance for HER2-low.
- The **HercepTest™ GE001 assay**, Dako/Agilent, for Omnis provided the highest pass rate and proportion of optimal results.
- The most commonly used Ventana/Roche **4B5 based assays 790-2991, 790-4493** and **790-7167** gave an overall pass rate of 94% when used by recommended protocol settings.

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, 4 and 5.
- Staining corresponding to score 0 in carcinoma no. 6.
- Staining corresponding to score 3+ in carcinoma no. 3.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 2.
- 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. 3 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. 2 compared to the NordiQC reference standards determined by HercepTest™ and 4B5 ***or*** (3) a 1+ reaction was seen in the HER2 gene unamplified 0 tumor no. 6 ***or*** (4) a 0 reaction was seen in one or more of the HER2 unamplified tumors no. 1, 4 and/or 5.
The scoring criteria for good as described for paragraphs (1) and (2) were related to accuracy for HER2 classical overexpression while the criteria for (3) and (4) were related to accuracy for HER2 Low.

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). The scoring criteria primarily related to accuracy for HER2 classical overexpression.

Participation

Number of laboratories registered for HER2, run B39	440
Number of laboratories returning slides	410 (93%)

Results
At the time of the 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.

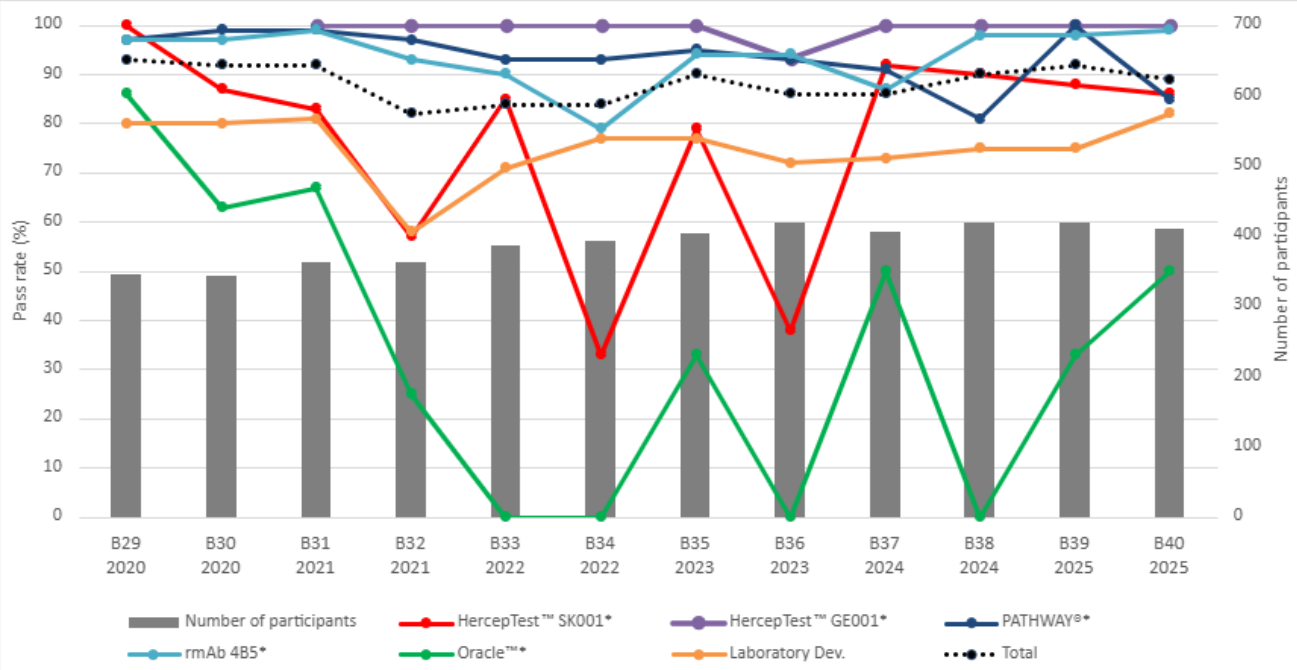
In total, 410 laboratories participated in this assessment and 89% achieved a sufficient mark (optimal or good).

Conclusions
In this assessment, the **HercepTest™, GE001**, Dako/Agilent, for the Omnis platform was most successful providing an overall pass rate of 100% and 76% optimal results when using the vendor recommended protocol settings (VRPS).
The most commonly used Ventana/Roche **4B5 based assays 790-2991**, **790-4493** and **790-7167** gave an overall pass rate of 94% and 61% optimal results 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 82%, 26% 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 2020-2025**



* pass rates using vendor recommended protocol settings

Table 1a. **Assessment marks for HER2 IHC assays and antibodies run B40**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
IVD approved HER2 assays	349	220	95	5	28	91%	63%
Concentrated antibodies	49	11	30	3	5	84%	24%
Ready-To-Use antibodies	12	4	5	1	2	75%	33%
Total	410	235	130	9	35		
Proportion		58%	32%	2%	8%	89%	

1) Suff.: Proportion of sufficient results (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]**	26	Ventana/Roche	15	7	1	3	85%	58%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[4]**	73	Ventana/Roche	41	17	2	13	79%	56%
VENTANA HER2 rmAb clone **4B5, 790-4493, (VRPS)[3]**	35	Ventana/Roche	24	11	-	-	100%	69%
VENTANA HER2 rmAb clone **4B5, 790-4493, (LMPS)[4]**	86	Ventana/Roche	62	19	-	5	94%	72%
VENTANA RxDx HER2 rmab clone **4B5, 790-7167, (VRPS)[3]**	41	Ventana/Roche	24	15	-	1	95%	59%
VENTANA RxDx HER2 rmab clone **4B5, 790-7167, (LMPS)[4]**	35	Ventana/Roche	18	14	1	3	91%	51%
HercepTest™, pAb, **SK001, (VRPS)[3]**	7	Dako/Agilent	4	2	-	1	86%	57%
HercepTest™, pAb, **SK001, (LMPS)[4]**	7	Dako/Agilent	7	-	-	-	100%	100%
HercepTest™, rmAb **DG44, GE001, (VRPS)[3]**	34	Dako/Agilent	26	8	-	-	100%	76%
Oracle™ mAb clone **CB11**, **TA9145, (VRPS)[3]**	4	Leica Biosystems	-	2	-	2	-	-
Oracle™ mAb clone **CB11, TA9145, (LPMS)[4]**	1	Leica Biosystems	-	-	1	-	-	-
Total	349		221	95	5	28		
Proportion			63%	27%	2%	8%	91%	

1) Suff.: Proportion of sufficient results (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	Cell Marque	1	2	-	1	-	-
	2	Biocare						
rmAb clone **SP3**	2	Master Diagnostica	1	1	-	-	-	-
	1	Thermo Fisher Scientific/Epredia	-	1	-	-	-	-
	1	Cell Marque	1	-	-	-	-	-
	1	Invitrogen	-	-	-	1	-	-
	1	Diagnostic Biosystems	-	-	1	-	-	-
rmAb clone **QR003**	2	Quartett	-	2	-	-	-	-
pAb, **A0485**	37	Dako/Agilent	8	24	2	3	86%	22%
Total	49		11	30	3	5		
Proportion			23%	61%	6%	10%	84%	

1) Suff.: Proportion of sufficient results (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 **MXR011, RMA-1022**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **EP3, 8388-C010**	2	Sakura Finetek	1	1	-	-	-	-
rmAb clone **EP3, GT224502**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **QR003, P-H001-30**	1	Quartett	-	-	-	1	-	-
rmAb clone **SP3, MAD-000308QD**	2	Master Diagnostica	1	1	-	-	-	-
rmAb clone **SP3, 237R**	1	Cell Marque	-	-	1	-	-	-
rmAb clone **SP3, RMPD008**	1	Diagnostic Biosystems	-	-	-	1	-	-
rmAb clone **SP3, NOA-RBG026**	1	Zytomed Systems	-	1	-	-	-	-
rmAb clone **BP6020, BX50015**	1	BioLynx Biotechnology	-	1	-	-	-	-
Ab clone **DY49224, 4911042**	1	Dakewe	-	1	-	-	-	-
Total	12		4	5	1	2		
Proportion			43%	36%	7%	14%	79%	

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

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): In total, 50 of 92 (61%) protocols applied on the BenchMark Ultra and Ultra Plus 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 Ultra or Ultra Plus, 12-48 min. incubation of the primary Ab and UltraView DAB or OptiView as detection kit. Using these protocol settings, 66 of 82 (81%) laboratories produced a sufficient staining result (optimal or good).
6 laboratories used product no. 790-2991 for staining on another platform. Data was not included in the description above

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 84 of 119 (71%) protocols applied on the BenchMark GX, XT, Ultra and Ultra Plus were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 30-64 min.), 12-32 min. incubation of the primary Ab and UltraView DAB or OptiView as detection system. Using these protocol settings, 100 of 103 (97%) laboratories produced a sufficient staining result.
3 laboratories used product no. 790-4493 for staining on another platform. Data was not included in the description above

Ventana RxDx HER2 rmAb clone **4B5** (790-7167, Ventana/Roche): In total, 42 of 76 (55%) protocols applied on the BenchMark Ultra and Ultra Plus were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 30-64 min.) 12-32 min. incubation of the primary Ab and UltraView DAB or OptiView as detection system. Using these protocol settings, 59 of 62 (95%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 5 of 8 (63%) protocols applied on Dako Autostainer Link 48 were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97-98°C for 30-40 min. in the PT Link, 30 min. incubation of the primary Ab and SK001 as detection system. Using these protocol settings, 7 of 8 (88%) laboratories produced a sufficient staining result.
6 laboratories used product no. SK001 for staining on another platform. Data was not included in the description above

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 26 of 34 (76%) protocols applied on Dako Omnis were assessed as optimal. Protocols with optimal results were based on HIER in Target Retrieval Solution (TRS), 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, 30 of 30 (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 Ultra, Ultra Plus PATHWAY® rmAb 4B5, **790-2991**	22/26 (85%)	15/26 (58%)	52/66 (79%)	35/66 (53%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA 4B5, **790-4493**	35/35 (100%)	24/35 (69%)	79/84 (94%)	60/84 (71%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA RxDx 4B5, **790-7167**	40/41 (95%)	24/41 (59%)	32/35 (91%)	18/35 (51%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	6/7 (86%)	4/7 (57%)	1/1	1/1
Dako Omnis HercepTest™ rmAb DG44, **GE001**	34/34 (100%)	26/34 (76%)	-	-
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	2/4	0/4	0/1	0/1

* 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**: 8 of 37 (22%) protocols were assessed as optimal. Optimal protocols were typically based on HIER using either TRS low pH (Dako/Agilent) (6/15*) or TRS High pH (Dako/Agilent) (2/7). The Ab was typically diluted in the range of 1:150-600 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 15 of 18 (83%) 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 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**	(1/2)	50% (3/6)	20% (1/5)	33% (3/9)	(0/3)	-	(0/4)	(0/7)

* 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 III, Prime

Comments

In this NordiQC assessment run B40 for HER2 IHC an overall pass rate of 89% was seen which is slightly inferior compared to the latest assessment run B39 with a pass rate of 92% (see Table 1a and 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 82% (36/44) 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. 3, with high level gene amplification, whereas too weak or false negative staining results were particularly and most critically observed as a 1+ IHC staining reaction in the HER2 gene amplified breast carcinoma, tissue core no. 2. 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 3.33) by FISH. The remaining insufficient results were characterized by either poor signal-to-noise, excessive counterstaining or excessive cytoplasmic staining reaction compromising the read-out and scoring of the specific HER2 membranous reaction.

The above-mentioned observations mainly related to HER2 status for classical overexpression. However, as HER2 IHC is moving into the identification of HER2-low in breast carcinoma, the results were also evaluated for this entity. As stated in the assessment criteria (see page 2), an IHC result changing the

HER2 score from "HER2-low positive" to "HER2-low negative" or opposite in one or more of the included breast carcinomas, was accepted as a sufficient result but downgraded to "Good" from "Optimal" provided that the expected results were obtained for the classical HER2 overexpression status in all samples.

In this assessment, 32% (n=130/410) of all results were scored as Good. In 76% of these, the "downgraded" assessment score was related to a change for HER2-low status from HER2-low positive to HER2-low negative. In 19%, a significantly increased number of positive cells was observed with increased need for HER2 ISH status for final treatment stratification. In the last 5% the downgrading was related to technical issues as e.g. excessive counterstaining or cytoplasmic reaction interfering with the interpretaion.

85% of the participants (348/410) used one of the CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY® (Ventana/Roche), VENTANA HER2 (4B5) (Ventana/Roche), Ventana RxDx HER2 (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica Biosystems) on the specified stainer platform with predictive claim for HER2 status in breast cancer. 3% (14/410) of the participants used one of the approved assays on another platform than specified by the vendor, while the remaining 15% (61/410) used a laboratory developed test (LDT) based on a concentrated primary Ab or RTU format without a predictive claim.

The well-established Ventana/Roche HER2 IHC 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 72% of all participants (296/410). When applying these assays on the intended platforms, Ventana BenchMark, a cumulated overall pass rate of 94% (96/102) was observed when applied by vendor recommended protocol settings (VRPS), compared to 88% (170/193) when used by laboratory modified protocol settings (LPMS) (see Tables 1b and 2). 9 laboratories used one of the three IHC assays on other IHC stainer platforms.

In this assessment run B40 all three HER2 IHC assays **PATHWAY®**, **790-2991, VENTANA HER2 (4B5)**, **790-4493** and **Ventana RxDx HER2 4B5**, **790-7167** gave slightly higher pass rates by VRPS compared to LMPS as shown in Tables 1b and 2. The **PATHWAY®**, **790-2991** assay with recommended protocol for HER2 gave a slightly inferior performance compared to the two other Ventana/Roche HER2 IHC assays. In total 98 participants used this assay and a pass rate of 86% (79/98) was observed. 14/19 laboratories obtaining an insufficient result characterized by a false negative staining reaction used protocol settings that typically provided sufficient results among other laboratories. Upon thorough review of the data, no shared lot numbers or other recurring errors were identified that could account for the issues with the **PATHWAY®**, **790-2991**. However, similar data and aberrant results have been observed in earlier runs with other of the Ventana assays e.g. in run B39, where the **Ventana RxDx HER2 4B5**, **790-7167** assay had a pass rate of 88%, which is now 95% in this run.
Virtually all the results evaluated as Good and downgraded from Optimal were related to a change in one of the HER2-low positive samples (tissue cores no 1,4 and/or 5) to a HER2-low negative status and caused by too low level of analytical sensitivity of the IHC assay performed.

The 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 8% (n=34) of all participants. As seen in Tables 1b and 2, all laboratories used the assay by vendor recommended protocol settings (VRPS) and when used as "plug-and-play" a pass rate of 100% (29/29) was achieved, as seen in most assessment runs B31-B39. (see Graph 1).

The Dako/Agilent **HercepTest™** CDx assay **SK001** for Dako Autostainer Link 48 provided a pass rate of 100% (7/7) optimal results when used accordingly to VRPS. In run B39 the level of optimal results was only 50%, and in general the pass-rate shown in Graph 1 displays a fluctuation for SK001 assay most likely impacted by technical issues related to the semi-automated platform. Since run B29 and the introduction of the Dako/Agilent 2nd generation **HercepTest™** for Omnis a consistently reduced number of SK001 based protocols have been submitted to the NordiQC breast module.

In this HER2 IHC assessment, 15% (62/410) 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 15% being at the lowest level so far. Overall, the LDTs in run B40 provided a pass rate of 82% (51/62), 26% (16/62) being optimal.
The pAb **A0485** from Dako/Agilent is still the most widely applied Ab within a LDT being used by 9% (37/410) of the participants and gave an overall pass rate of 86% and 22% optimal results and the performance as such very similar to the level seen in previous runs.

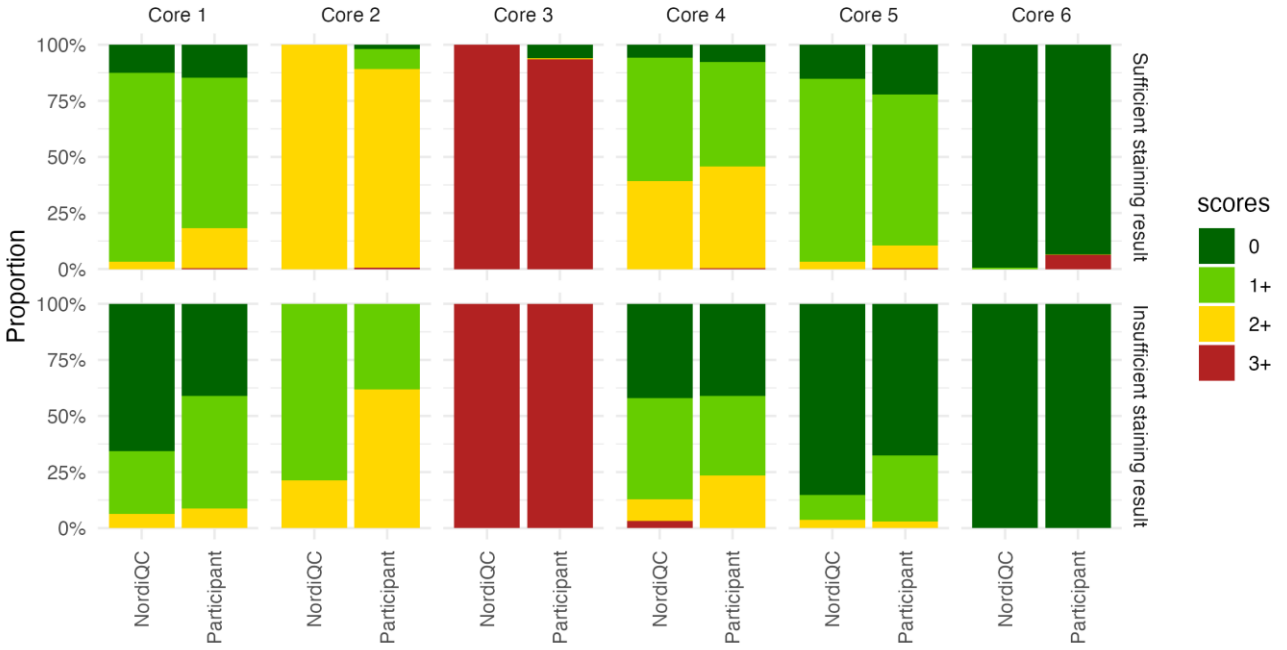
Scoring consensus B40
Participants were asked to submit self-evaluated scores (0, 1+, 2+, or 3+) for their HER2-stained slides on the NordiQC webpage. Of the 410 laboratories that returned slides, 81% (332/410) provided their own assessment scores. In total, 37% (115/332) of these participants achieved 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 similar to the previous assessments (B39 (37 %) and B38 (43%)). The cores with highest disagreement were core 1, 4 and 5 (agreement percentage 68%, 75% and 71%) indicating that the disagreement was especially related to the HER2-low scoring.

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	406	50	6	19	1
	1+	78	509	123	2	0
	2+	6	56	383	3	0
	3+	18	0	2	313	0
	Indeterminate	11	4	2	0	0

Among laboratories that produced sufficient staining results, 37% (108/298) of scoring read-outs were in complete agreement with the NordiQC assessors.

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 1–6 (see Graph 2). For tissue core 2 (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 2), a large proportion of participants still scored this as 2+.

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

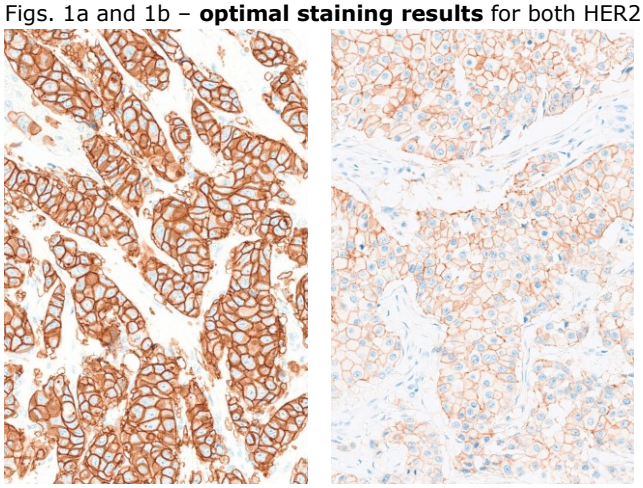


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 3, with a HER2/chr17 ratio of 8.75.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>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".

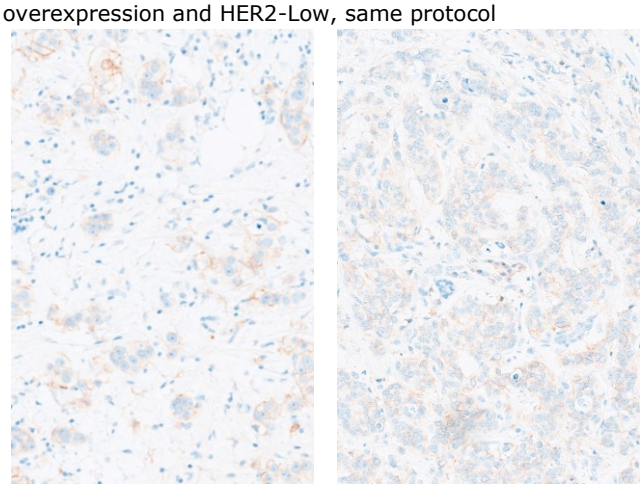


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 1, with a HER2/chr17 ratio of 1.87
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of 1.11.
>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

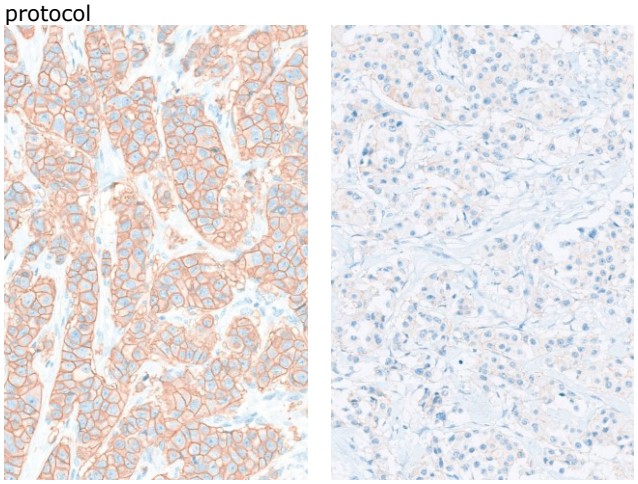


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 3, with a HER2/chr17 ratio of 8.75.
>10% of the neoplastic cells show a moderate membranous staining reaction corresponding to 3+. The tumour will be reflexed to ISH and hereby HER2 classical overexpression can be determined and no direct misclassification. Both the participant and NordiQC scored the result as 3+ ("low level").
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>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+.

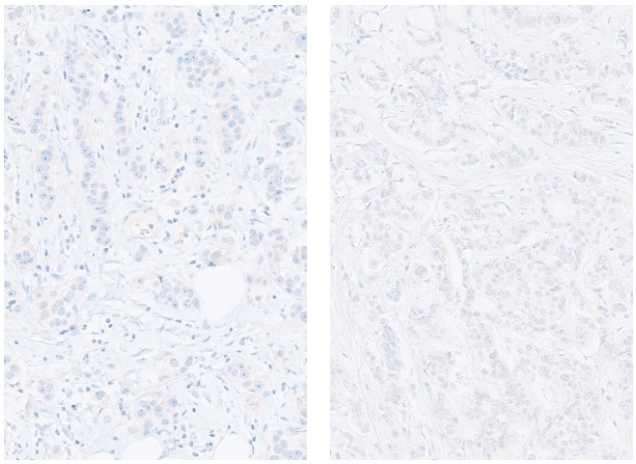


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 1, with a ratio HER2/chr17 of 1.87.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.
Both the participant and NordiQC scored the result as 0.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of 1.11.
No staining reaction is seen corresponding to 0.
Both the participant and NordiQC scored the result as 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 Optimal versus Good** – expected result for HER2 overexpression, but failed HER2 Low.
3a left and 3b left same protocol (assessed as Optimal). 3a right and 3b right same protocol (assessed as Good).

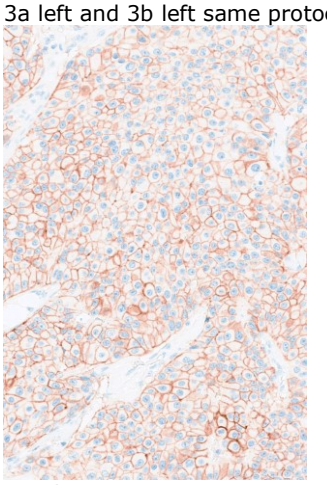


Fig. 3a.
Left: Expected staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 3.33.
>10% of the neoplastic cells show a weak but complete membranous staining reaction corresponding to 2+.
The proportion and intensity of cells demonstrated is reduced but still the result is considered acceptable for HER2 classical overexpression.

However, the reduced analytical sensitivity of the protocol applied in Fig. 3a right has an impact on the accuracy for HER-Low, see Fig. 3b.

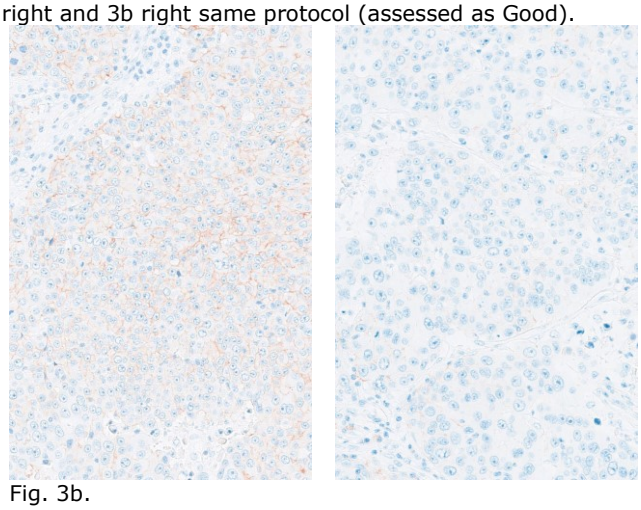


Fig. 3b.
Left: Expected staining result for HER2 of the breast carcinoma, tissue core no. 4, with a ratio HER2/chr17 of 1.35.
>10% of the neoplastic cells show a weak, partial membranous staining reaction corresponding to 1+.
Right: **Insufficient** staining result for HER2 of the breast carcinoma, tissue core no. 4, with a HER2/chr17 ratio of 1.35.
Only very few neoplastic cells show a very faint and partial membranous staining reaction corresponding to a 0 score.

Both the participant and NordiQC scored the result in Fig. 3b right as 0.

The tumour was thus categorized as HER 0 impacting HER2-Low classification as the expected 1+ status changed to 0.

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