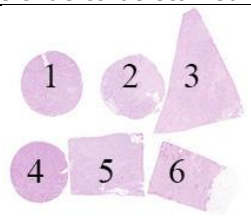
Assessment Run B37 2024
HER2 IHC

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 1-2+, 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+	0.71	1.8	Unamplified
Breast carcinoma, no. 2	1-2+	1.28	1.6	Unamplified
Breast carcinoma, no. 3	1-2+	1.49	4.1	Unamplified
Breast carcinoma, no. 4	0	1.09	1.2	Unamplified
Breast carcinoma, no. 5	2+	3.12	6.7	Amplified
Breast carcinoma, no. 6	3+	7.33	14.3	Amplified

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

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 was most reproducible.
- An alarmingly low scoring consensus between NordiQC and the participants was observed.

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, 2 and 3.
- Staining corresponding to score 0 in carcinoma no. 4.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 5.
- Staining corresponding to score 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. 5 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. 1-3.

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

Participation

Number of laboratories registered for HER2, run B37	429
Number of laboratories returning slides	405 (94%)

Results

At the date of 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 405 laboratories participated in this assessment and 89% achieved a sufficient mark (optimal or good).

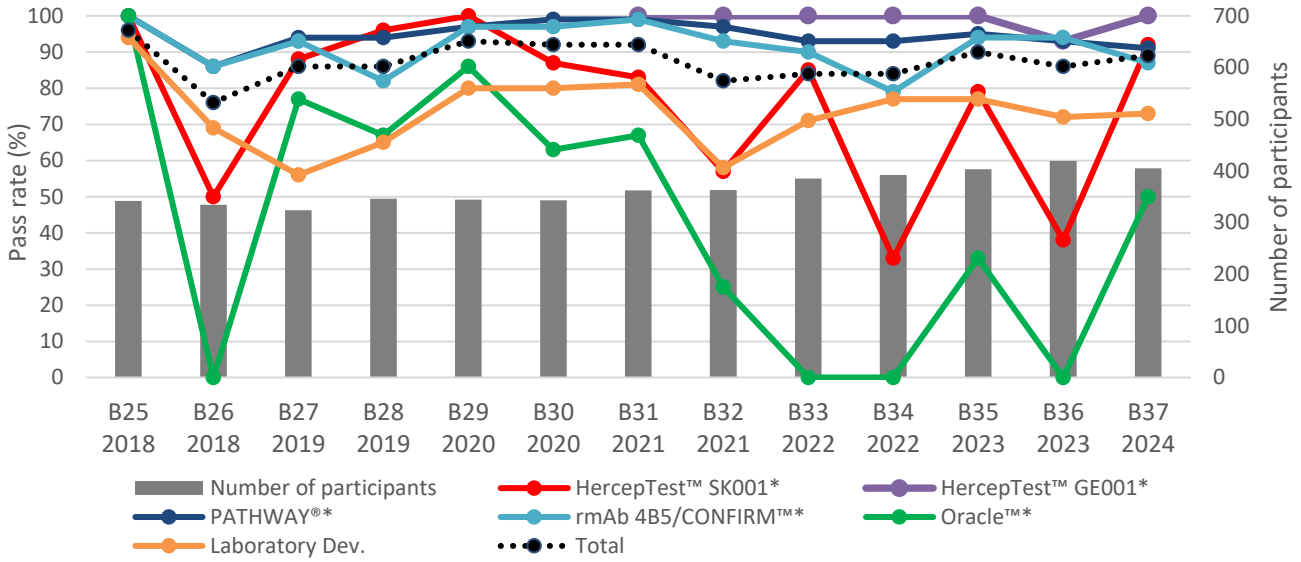
Conclusions

In this assessment, the **HercepTest™ GE001**, Dako/Agilent, for the Omnis platform provided the highest pass rate of 100% and 61% optimal results when using the vendor recommended protocol settings (VRPS). Compared to the previous run B36, the pass rate for the "classical**" HercepTest™ SK001**, Dako/Agilent, for the Autostainer Link 48 platform improved significantly from 38% to 92% and the proportion of optimal results improved from 23% to 31% (using VRPS).
The two widely used and established FDA-/CE-IVD approved HER2 IHC assays from Ventana/Roche, **PATHWAY® 790-2991** and **VENTANA HER2/4B5 790-4493**, respectively, gave a slightly inferior overall pass rate of 91% and 87% when used by VRPS, compared to 95% and 94%, respectively, in run B36.
Laboratory developed tests (LDT's) based on RTU Abs without predictive claim or based on concentrated Abs gave a pass rate of 73%, 31% optimal.
Although the CDx assay **Oracle™ TA9145**, Leica Biosystems, achieved a significantly higher pass rate of 80% compared to 14% in run B36, there were no optimal results.

Assessment marks for HER2 IHC CDx assays and HER2 LDTs (conc. Ab and RTU) are summarized in Tables 1a-1c (see pages 3-4).

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 2018-2024**



* pass rates using vendor recommended protocol settings

Table 1a. **Assessment marks for HER2 IHC assays and antibodies run B37**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
IVD approved HER2 assays	331	206	99	9	17	92%	62%
Concentrated antibodies	66	20	29	6	11	74%	30%
Ready-To-Use antibodies	8	3	2	2	1	63%	38%
Total	405	229	130	17	29		
Proportion		57%	32%	4%	7%	89%	

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]**	32	Ventana/Roche	17	12	3	0	91%	53%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)**[4]	108	Ventana/Roche	75	29	1	3	96%	69%
VENTANA HER2 rmab clone **4B5, 790-4493, (VRPS)[3]**	38	Ventana/Roche	24	9	1	4	87%	63%
VENTANA HER2 rmab clone **4B5, 790-4493, (LMPS)**[4]	104	Ventana/Roche	68	25	3	8	89%	65%
HercepTest™, pAb, **SK001, (VRPS)[3]**	13	Dako/Agilent	4	8	1	0	92%	31%
HercepTest™, pAb, **SK001, (LMPS)**[4]	2	Dako/Agilent	0	1	0	1	-	-
HercepTest™, rmAb **DG44, GE001, (VRPS)[3]**	28	Dako/Agilent	17	11	0	0	100%	61%
HercepTest™, rmAb **DG44, GE001, (LPMS)**[4]	1	Dako/Agilent	1	0	0	0	-	-
Oracle™ mAb clone **CB11, TA9145, (VRPS)[3]**	2	Leica Biosystems	0	1	0	1	-	-
Oracle™ mAb clone **CB11, TA9145, (LPMS)**[4]	3	Leica Biosystems	0	3	0	0	-	-
Total	331		206	99	9	17		
Proportion			62%	30%	3%	5%	92%	

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	Cell Marque	3	1	1	-	80%	60%
	2	Epitomics						
	1	Biocare						
rmAb clone **QR003**	1	DCS	2	-	-	-	-	-
	1	Quartett						
rmAb clone **SP3**	3	Thermo Fisher Scientific/Epredia	1	5	1	3	60%	10%
	2	Cell Marque						
	2	Master Diagnostica						
	2	Zytomed						
	1	Invitrogen						
pAb, **A0485**	49	Dako/Agilent	14	23	4	8	76%	29%
Total	66		20	29	6	11		
Proportion			30%	44%	9%	17%	74%	

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]
mAb clone **C1F7, CCM-0844**	1	Celnovte	-	1	-	-	-	-
rmAb clone **246G0D3, PA216**	1	Abcarta/Abcepta	-	-	1	-	-	-
rmAb clone **BP6020, I12182E-05**	1	Biolynx	-	-	-	1	-	-
rmAb clone **MXR011, RMA-1022**	2	Fuzhou Maixin	1	1	-	-	-	-
rmAb clone **SP3, MAD-000308QD**	2	Master Diagnostica	1	-	1	-	-	-
Ab clone **DA164, DMRD0203**	1	Shenzhen Dartmon Biotechnology	1	-	-	-	-	-
Total	8		3	2	2	1		
Proportion			38%	25%	25%	13%	63%	

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, 92 of 140 (66%) 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 as detection kit. Using these protocol settings, 108 of 115 (94%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 92 of 142 (65%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 20-64 min.) on BenchMark XT, GX, Ultra or Ultra plus, 12-40 min. incubation of the primary Ab and UltraView DAB as detection system. Using these protocol settings, 90 of 105 (86%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 4 of 15 (27%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 40 min. in the PT Link, 30 min. incubation of the primary Ab and SK001 as detection system. Using these protocol settings, 12 of 13 (92%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 18 of 29 (62%) 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, 28 of 28 (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**	29/32 (91%)	17/32 (53%)	94/98 (96%)	68/98 (69%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA 4B5, **790-4493**	33/38 (87%)	24/38 (63%)	87/97 (90%)	64/97 (66%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	12/13 (92%)	4/13 (31%)	1/2	0/2
Dako Omnis HercepTest™ rmAb DG44, **GE001**	28/28 (100%)	17/28 (61%)	1/1	0/1
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	1/2	0/2	3/3	0/3

* 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**: 14 of 48 (29%) protocols were assessed as optimal. Optimal protocols were typically based on HIER using either TRS low pH (Dako/Agilent) (7/21*), TRS High pH (Dako/Agilent) (3/6), CC1 (Ventana/Roche) (2/3) or Bond™ Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/6). The Ab was typically diluted in the range of 1:100-1,500 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 29 of 33 (88%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP3**: 3 of 5 (60%) protocols were assessed as optimal. All optimal protocols were based on HIER using either Tris/EDTA pH 9 (1/1) or BERS2 (Leica Biosystems) (2/3). The Ab was diluted in the range of 1:150-200 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**	0/3**	1/6 (17%)	3/5 (60%)	6/15 (40%)	2/3 -	-	2/6 (33%)	0/6
rmAb clone **EP3**	-	-	-	-	-	-	2/3	-

* 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, Ultra plus
3) Bond MAX, III, Prime

Comments

In this NordiQC assessment run B37 for HER2 IHC a slight increase in the overall pass rate to 89% was seen compared to 86% in the previous run B36 (2023) and being similar to the level of 90% achieved in run B35 (2023, 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 93% (43/46) 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. 6, 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. 5. This tumor was categorized as IHC 2+ in the NordiQC reference laboratory using the CE-IVD HER2 IHC assays: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (HER2 gene/chr17 ratio of 3,12 and average HER2 copy number ≥ 6.0 signals/cell) by FISH.

The remaining insufficient results were characterized by either an increased proportion of positive cells or excessive cytoplasmic staining reaction compromising the read-out and scoring of the specific HER2 membranous reaction.

78% of the participants (314/405) 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. 4% (17/405) of the participants used one of the approved assays on another platform than specified by the vendor, while the remaining 18% (n=74) used a laboratory developed test (LDT) based on a concentrated primary Ab or RTU format without a predictive claim. This segmentation has been relatively consistent in the last assessment runs.

The two Ventana/Roche assays **PATHWAY® 790-2991** and **VENTANA HER2 (4B5) 790-4493** were most widely applied and in total used by 70% of all participants (282/405). When applying the assays on the intended platforms, Ventana BenchMark, an overall pass rate (irrespective of protocol settings) of 92% (243/265) was observed and 65% (173/265) of the results evaluated as optimal. For both assays, the pass rate and proportion of optimal results were slightly superior using the assay by laboratory modified settings compared to the recommended protocol settings (see Tables 1b and 2).

Similar to runs B32 - B35, it was observed that 10% (27/265) 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 UltraView as recommended by Ventana/Roche. In this assessment, this modification resulted in a high pass rate of 100% (27/27). 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. 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.* In the same context 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.)

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 7% (n=29) 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% (28/28) was achieved, as seen in runs B31-B35. The proportion of optimal results was 61% compared to 50% in the previous run B36 and 72% in run B35. In this assessment the scores were downgraded from optimal to good due to an increased proportion and intensity of positive cells being visualized in tissue core no 4 which was expected to be negative and scored as 0 by the NordiQC reference IHC methods and vast majority of participants.

The "classic" Dako/Agilent **HercepTest™** CDx assay **SK001** for Dako Autostainer Link 48 provided a significantly higher pass rate of 87% (13/15) compared to 38% achieved in the previous HER2 run B36. When used according to VRPS, an even higher proportion of 92% (12/13) sufficient results was obtained. Although, as shown in Graph 1, a fluctuation of the pass rates for SK001 with VRPS has been observed in previous assessments, the proportion of optimal results has been relatively low, reaching 31% (4/13) in this assessment and ranging between 14-25% in runs B34-B36.
The two unsuccessful results were caused by a false negative staining reaction in the HER2 2+ amplified tumor, tissue core no. 5, and an overall too weak staining reaction leading to significantly reduced proportion of positive cells. No protocol-related causes were identified for the insufficient results and most likely caused by technical issues.

Slides from six TMA blocks was used in this run. It was observed that an overall pass rate of 70% was obtained in one of the TMA's used compared to 88-100% for the remaining five TMA's. The range of protocols applied for the six different TMAs were comparable and no plausible explanation for the difference has been identified. All NordiQC TMAs are meticulously verified at different levels (each 50th slide sectioned) by two reference IHC methods and all slides obtained the expected IHC HER2 level.

In this HER2 IHC assessment, 18% (74/405) 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 shown a slow, but steady decrease over the years with 18% being the lowest percentage to date over the course of NordiQC HER2 IHC assessments. Overall, the LDTs in run B37 provided a pass rate of 73% (54/74), 31% (23/74) being optimal.
The pAb **A0485** from Dako/Agilent is still the most widely applied Ab within a LDT being used by 12% (49/405) of the participants and gave an overall pass rate of 76% and 29% optimal results.

Scoring consensus B37
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 81% (326/405) of the participants returning slides.
For 132 of the 326 (40%) responding participants, scores for all the tissues in the multi-tissue sections were in concordance with the NordiQC assessor group using the ASCO/CAP 2023 scoring guidelines. This is a significant decrease compared to previous assessment runs as during this assessment a more precise scoring of every tissue core was carried out with an additional focus on HER2-low with perspective to score each tissue core in a specific subcategory being either 0, 1+ or 2+. Table 4 illustrates the distribution of scoring agreement between the participants and NordiQC assessor teams.

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+**
	0	330	13	4	1
	1+	72	502	150	2
	2+	4	42	468	27
	3+	0	1	6	326

Among laboratories with sufficient staining, 42% (124/294) 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. 1-3, which accounted for 81% (219/269) of all no-consensus cores, 57% (125/219) being overscored and 43% (94/219) being underscored by the participant compared to HER2 read-out given by the NordiQC assessor team (see Table 5).
Among participants with insufficient staining results, 22% (7/32) scored their HER2 IHC results in consensus with the NordiQC assessor group. For this group the disagreement was mainly related to the scoring of the breast carcinoma, tissue core no. 5, accounting alone for 36% (19/53) of all no-consensus cores (see Table 5). The score given by the participant based on the staining done at their facilities was 2+, however the same core was scored 0 or 1+ by the NordiQC assessor team in the same slide. The NordiQC assessment was primarily based on strict adherence to the ASCO/CAP guidelines but also to the level expected and characterized by the NordiQC HER2 IHC reference standard methods.

Table 5. **HER2 IHC scoring consensus results based on specific tissue cores**

	Core 1 1-2+	**Core 2 1-2+**	**Core 3 1-2+**	**Core 4 0**	**Core 5 2+ amp.**	**Core 6 3+ amp.**
All – consensus cores	241/325 (74%)	249/325 (77%)	237/326 (73%)	305/326 (94%)	275/326 (84%)	319/326 (98%)
All - no consensus cores with clinically relevant change	24/325 (7%)	33/325 (10%)	18/326 (6%)	19/326 (6%)	22/326 (7%)	2/326 (1%)
Sufficient staining - consensus cores	220/293 (75%)	226/294 (77%)	216/294 (73%)	277/294 (94%)	263/294 (89%)	292/294 (99%)
Sufficient staining - no consensus cores with clinically relevant change	22/293 (8%)	28/294 (10%)	12/294 (4%)	16/294 (5%)	4/294 (1%)	0/294 (0%)
Insufficient staining - consensus cores	22/32 (69%)	24/31 (77%)	22/32 (69%)	29/32 (91%)	13/32 (41%)	28/32 (88%)
Insufficient staining - no consensus cores with clinically relevant change	2/32 (6%)	5/31 (16%)	6/32 (19%)	9/32 (9%)	18/32 (56%)	2/32 (6%)

Figs. 1a and 1b – **optimal staining results**, same protocol

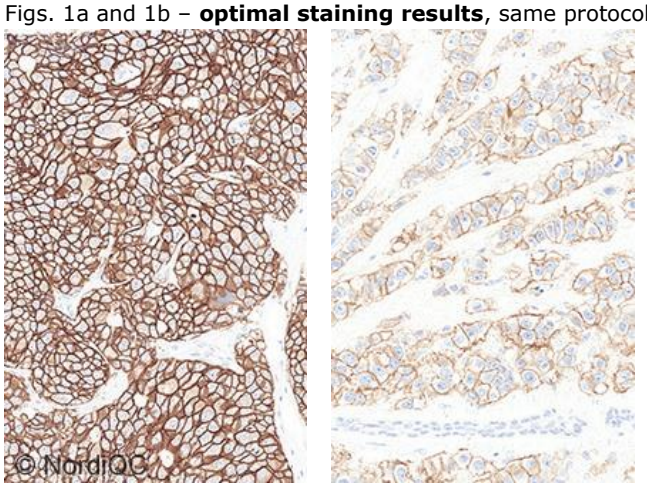


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of 3.12 and HER2 gene copy no of 6.7.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

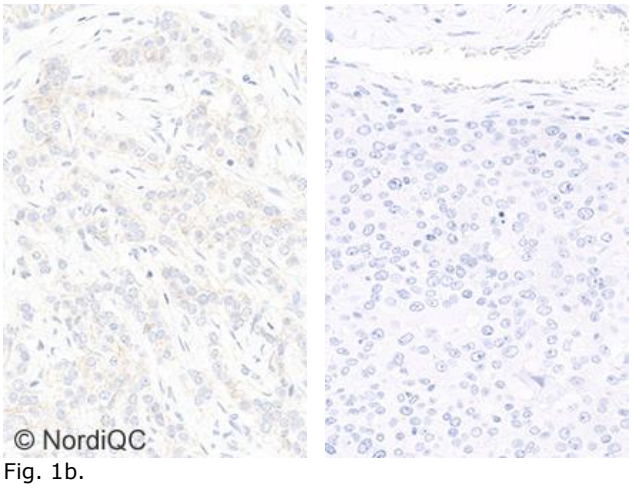


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 2, with a HER2/chr17 ratio of 1.28.
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER2 of the breast carcinoma, tissue core no. 4, with a HER2/chr17 ratio of 1.09.
No staining reaction is seen corresponding to 0.

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

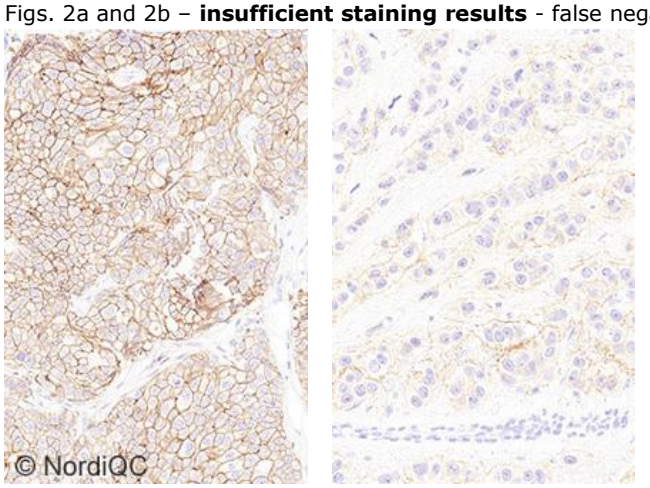


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of 3.12 and a HER2 gene copy no of 6.7.
>10% of the neoplastic cells show a weak, but incomplete membranous staining reaction corresponding to 1+.

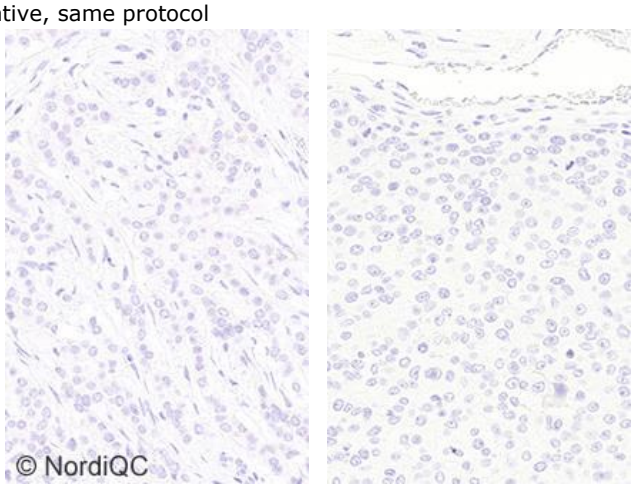
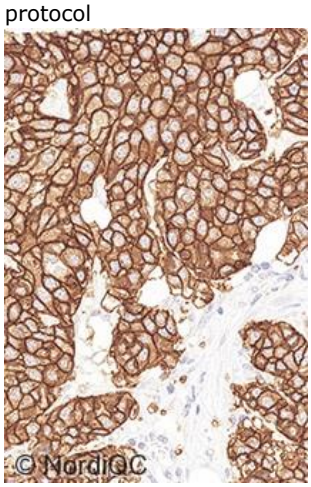


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 2, with a ratio HER2/chr17 of 1.28.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0 (the core was scored as 0 both by the participant and NordiQC), impacting HER2-low classification as the expected 1+ status changed to 0.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 4, with a HER2/chr17 ratio of 1.09.
No staining reaction is seen corresponding to 0.

Figs. 3a and 3b – **insufficient staining results** – excessive background, increased proportion of positive cells, same protocol



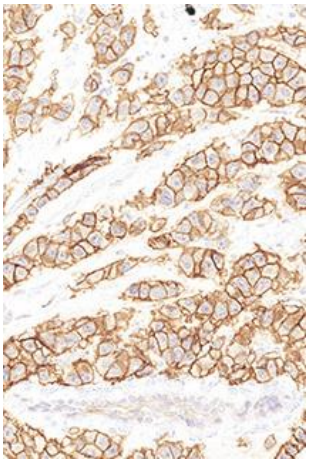
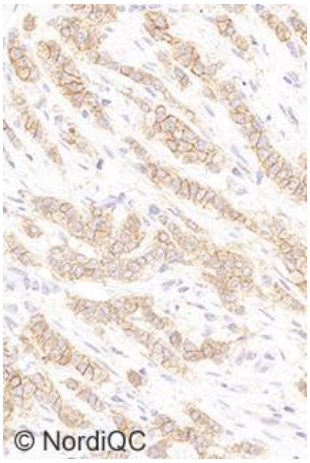


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 6, with a HER2/chr17 ratio of >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+, but also extended cytoplasmic staining reaction is present.
Right: Staining result for HER2 of the breast carcinoma, tissue core no. 5, with a HER2/chr17 ratio of 3.12 and a HER2 gene copy no of 6.7.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+



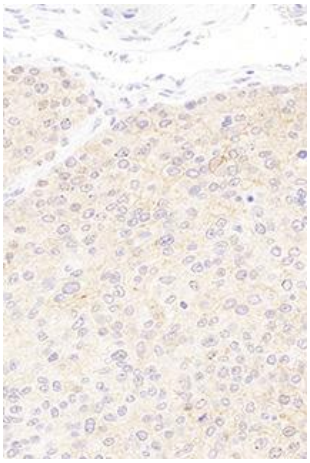


Fig. 3b.
Left: Staining result for HER2 of the breast carcinoma, tissue core no. 2, with a ratio HER2/chr17 of 1.28.
>10% of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+.
Right: **Insufficient staining result** for HER2 of the breast carcinoma, tissue core no. 4, with a HER2/chr17 ratio of 1.09.
>10% of the neoplastic cells show a faint and incomplete membranous staining reaction corresponding to 1+. An overall diffuse cytoplasmic staining reaction is seen and compromising the interpretation.

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