Assessment Run B36 2023

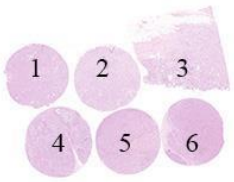
HER2 IHC

Purpose

Evaluation of the analytical accuracy of HER2 IHC tests performed by the NordiQC participants for demonstration and establishment of the HER2 protein overexpression level in breast carcinomas. The HER2 IHC assays PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) were used as reference standard methods, and accuracy was evaluated in six breast carcinomas with the dynamic and critical relevant expression levels of HER2. The obtained score in NordiQC is indicative of the performance of the IHC tests used by the participants, but due to the limited number and composition of samples, internal validation and extended quality control, e.g. regularly measuring the HER2 results, is necessary and recommended.

Material

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

	**IHC: HER2 Score* (0, 1+, 2+, 3+)**	**FISH: HER2 gene/chr 17 ratio****	**FISH: HER2 gene copy no.****	**FISH HER2 gene amplification status**
Breast carcinoma, no. 1	1-2+	1.74	3.65	Unamplified
Breast carcinoma, no. 2	1-2+	1.03	1.5	Unamplified
Breast carcinoma, no. 3	3+	>6	8.8	Amplified
Breast carcinoma, no. 4	0-2+***	1.36	2.65	Unamplified
Breast carcinoma, no. 5	0	0.69	1.2	Unamplified
Breast carcinoma, no. 6	2+	2.53	4.79	Amplified

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

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

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

IHC scoring system according to the 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 2.
- Staining corresponding to score 3+ in carcinoma no. 3.
- Staining corresponding to score 0, 1+ or 2+ in carcinoma no. 4.
- Staining corresponding to score 0 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. 3 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by FISH/BRISH according to the ASCO 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 2+ reaction was seen in the HER2 gene unamplified 0/1+ tumor no. 5.

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 B36	444
Number of laboratories returning slides	419 (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 419 laboratories participated in this assessment and 86% achieved a sufficient mark (optimal or good).

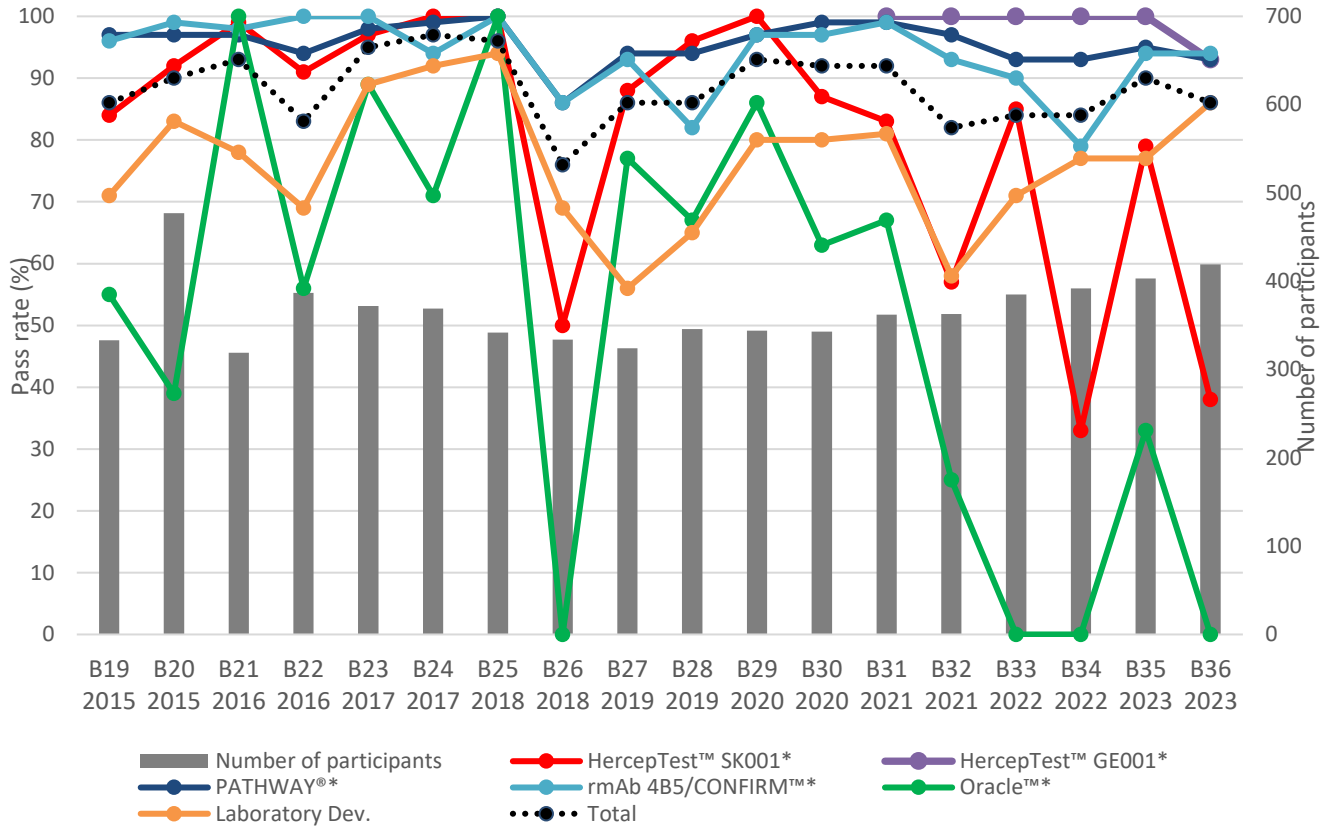
Conclusions

The overall pass rate decreased compared to the last run B35 and is nearly at the level seen in the three previous assessment runs B32-B34.
In this assessment, the three most widely used FDA-/CE-IVD approved HER2 IHC assays **PATHWAY® 790-2991** (Ventana/Roche), **VENTANA HER2/4B5 790-4493** (Ventana/Roche) and 2nd generation **HercepTest™ GE001** (Dako/Agilent) provided an almost identical high pass rate of 93-94% when using the vendor recommended protocol settings (VRPS).
In contrast the "classical" HercepTest™ SK001, Dako/Agilent, for the Autostainer Link 48 platform gave a very low pass rate of 38% (using VRPS).
Laboratory developed tests (LDT's) based on RTU Abs without predictive claim or based on concentrated Abs gave a pass rate of 72%, 28% optimal.
As seen in the latest assessments, the CDx assay **Oracle™**, Leica Biosystems was less successful showing a pass rate of 14%, no optimal results.

Assessment marks for IHC HER2 CDx assays and HER2 LDTs are summarized in Table 1 (see page 4).

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

Graph 1. **Pass rates of the HER2 IHC assessments in the NordiQC breast cancer module 2015-2023**



* pass rates using vendor recommended protocol settings

Table 1. **Assessment marks for IHC assays and antibodies run B36, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[4]**	28	Ventana/Roche	15	11	-	2	93%	54%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	116	Ventana/Roche	72	36	1	7	93%	62%
VENTANA HER2 rmab clone **4B5, 790-4493, (VRPS)[4]**	36	Ventana/Roche	23	11	1	1	94%	64%
VENTANA HER2 rmab clone **4B5, 790-4493, (LMPS)[5]**	103	Ventana/Roche	69	26	-	8	92%	67%
HercepTest™, pAb, **SK001, (VRPS)[4]**	8	Dako/Agilent	2	1	-	5	38%	25%
HercepTest™, pAb, **SK001, (LMPS)[5]**	5	Dako/Agilent	1	1	-	3	40%	20%
HercepTest™, rmAb **DG44, GE001, (VRPS)[4]**	30	Dako/Agilent	15	13	-	2	93%	50%
HercepTest™, rmAb **DG44, GE001, (LPMS)[5]**	1	Dako/Agilent	-	1	-	-	-	-
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	4	Leica Biosystems	-	-	-	4	-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	3	Leica Biosystems	-	1	-	2	-	-
Antibodies[3] for laboratory developed HER2 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CB11**	1 1	Zytomed Leica	-	-	1	1	-	-
rmAb clone **EP3**	1 1 2	Biocare Cell Marque Epitomics	3	1	-	-	-	-
rmAb clone **QR003**	1 1	DCS Quartett	1	1	-	-	-	-
rmAb clone **SP3**	1 1 3 3 1	Cell Marque Invitrogen Master Diagnostica Thermo Fisher Scientific/Epredia Zytomed	-	4	2	3	44%	0%
rmAb clone **ZR5**	1	Zeta Corporation	-	1	-	-	-	-
pAb, **A0485**	55	Dako/Agilent	17	27	2	9	80%	31%
Antibodies for laboratory developed HER2 assays, RTU	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 **EP3, AN726**	1	BioGenex	-	-	1	-	-	-
rmAb clone **EP3, 8388-C010**	2	Sakura Finetek	1	-	-	1	-	-
rmAb clone **MXR011, RMA-1022**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **SP3, MAD-000308QD**	2	Master Diagnostica	-	1	1	-	-	-
rmAb clone **SP3, 237R-17**	2	Cell Marque	-	-	-	2	-	-
Ab clone **DA164, DMRD0203**	1	Shenzhen Dartmon Biotechnology	-	-	-	1	-	-
Total	419		221	138	9	51		
Proportion			53%	33%	2%	12%	86%	

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

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): In total, 87 of 144 (60%) 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 32-64 min.) on BenchMark XT, Ultra or Ultra Plus, 12-32 min. incubation of the primary Ab and UltraView as detection kit. Using these protocol settings, 109 of 117 (93%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 92 of 110 (84%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 24-64 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, 98 of 104 (94%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 3 of 13 (23%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 40 min. in the PT Link, 30 min. incubation of the primary Ab and SK001 as detection system. Using these protocol settings, 3 of 8 (38%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 15 of 31 (48%) 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 30 (93%) 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**	26/28 (93%)	15/28 (54%)	98/106 (92%)	63/106 (59%)
Ventana BenchMark GX, XT, Ultra, Ultra Plus VENTANA 4B5, **790-4493**	34/36 (94%)	23/36 (64%)	91/98 (93%)	66/98 (67%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	3/8 (38%)	2/8 (25%)	0/3	0/3
Dako Omnis HercepTest™ rmAb DG44, **GE001**	28/30 (93%)	15/30 (50%)	1/1	0/1
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	0/4	0/4	1/3	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**: 17 of 55 (31%) protocols were assessed as optimal. Optimal protocols were typically based on HIER using either Target Retrieval Solution (TRS) low pH (Dako/Agilent) (9/27*), TRS High pH (Dako/Agilent) (4/10) or CC1 (Ventana/Roche) (3/5). The Ab was typically diluted in the range of 1:200-1000 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 29 of 35 (83%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP3**: 3 of 4 (75%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Tris/EDTA pH 9 (1/1), CC1 (Ventana/Roche) (1/1) or Tissue-Tek Genie High pH Antigen Retrieval solution (Sakura Fintek) (1/1). The Ab was diluted in the range of 1:70-200 depending on the level of the total technical sensitivity of the protocol employed.

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

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

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS High pH	TRS Low pH	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
pAb clone **A0485**	1/5** (20%)	3/9 (33%)	3/5 (60%)	6/18 (33%)	3/5 (60%)	-	1/5 (20%)	0/6

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Comments
In this NordiQC assessment B36 for HER2 a slight decrease in the overall pass rate to 86% was seen compared to 90% in the previous run B35 (2023) and being similar to the level of 84% achieved in runs B33 and B34 (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% (54 of 60) of the results. 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 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: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (HER2/chr17 ratio >4) by FISH.
The remaining insufficient results were characterized by either a poor signal-to-noise ratio, false positive staining reaction, excessive cytoplasmic staining reaction and/or excessive counterstaining compromising the read-out and scoring of the specific HER2 membranous reaction.

76% of the participants (317/419) used one of the CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY® (Ventana/Roche), VENTANA HER2 (4B5) (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica Biosystems) on the specified stainer with predictive claim for HER2 status in breast cancer. 4% (17/419) of the participants used one of the approved assays on another platform than specified by the vendor, while the remaining 20% (n=85) used a laboratory developed test (LDT) based on a concentrated primary Ab or a RTU format without a predictive claim. This segmentation has been relatively consistent in the last assessment runs.

The two Ventana/Roche assays PATHWAY® 790-2991 and VENTANA HER2 (4B5) 790-4493 were most widely applied and in total used by 68% of all participants (283/419). When applying the assays on the intended platform, Ventana BenchMark, an overall pass rate (irrespective of protocol settings) of 93% (254/273) was observed and 63% (171/273) of the results evaluated as optimal. In this assessment, the pass rates and proportion of optimal results for laboratories using these two IHC assays as "plug-and-play" and strictly compliant to the recommended protocol settings or using modified protocols were fully comparable as seen in Table 1 and 2.

Similar to runs B32 - B35, it was observed that 11% 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 similar pass rate of 93% (28/30) to the vendor recommended protocol settings. However, this observation must be carefully evaluated as in previous assessment runs e.g. run B28, this modification frequently induced an insufficient result characterized by a false positive 3+ HER2 reaction in a 2+ HER2 gene unamplified breast carcinoma. In addition, 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.*

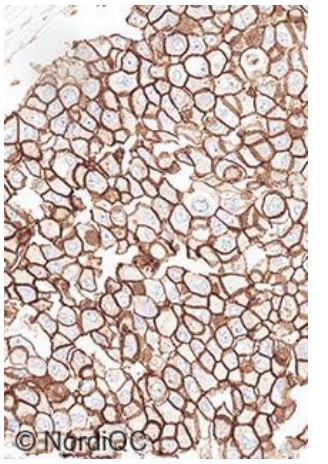
The most recently launched Dako/Agilent HercepTest™ CDx assay GE001 for Dako Omnis based on the rmAb clone DG44 was the most widely used "non-Ventana" CDx assay and was used by 7% (n=31) of all participants. As seen in Tables 1 and 2, the vast majority of laboratories used the assay by vendor recommended protocol settings and when used as "plug-and-play" a pass rate of 93% (28/30) was achieved which was reduced compared to 100% obtained in all of the previous runs B31-B35 that have

taken place after the launch of the product. The proportion of optimal results was decreased to 50% compared to 72% in the previous run B35 and 91% in run B34. In this assessment the scores were downgraded from optimal to good mainly due to a reduced proportion and intensity of positive cells being visualized but tumours still in correct category for HER2 status. The Insufficient results were caused by a false negative staining reaction in the HER2 2+ gene amplified tumor, tissue core no. 6, compared to the levels characterized 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 low pass rate of 38% and 25% optimal results when used in concordance with the recommended protocol settings. This was a significant reduction compared to the result obtained in run B35 for this assay, but as shown in Graph 1, a fluctuation of the pass rates for SK001 has been observed in previous assessments, e.g. the results were very similar in run B34. No single parameter causing the low pass rate e.g. lot no of HercepTest SK001 can be identified and data must be interpreted with caution due to relatively few data points. All unsuccessful results were caused by a false negative staining reaction in the HER2 2+ amplified tumor, tissue core no. 6.

In this HER2 IHC assessment, 20% (85/419) 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 and CE-IVD approved HER2 IHC assays seems to be very consistent. In the latest assessment runs B32 to B35, 20-22% of the participants used LDTs. Overall, the LDTs in run B36 provided a pass rate of 72% (61/85), 28% (24/85) being optimal.
The pAb A0485 from Dako/Agilent is still the most widely applied Ab within a LDT being used by 13% (55/419) of the participants and gave an overall pass rate of 80% and 31% optimal results.

Scoring consensus B36
Laboratories were requested to submit scores (0, 1+, 2+ or 3+) on the NordiQC homepage of their own HER2 stained slides. This was done by 80% (335 of 419) of the participants returning slides.
For 263 of the 335 (79%) 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.
Among laboratories with sufficient staining, 83% (241 of 291) of the scoring read-outs were in agreement with the NordiQC assessors. Disagreement was primarily related to the scoring of the HER2 status in the breast carcinoma, tissue core no. 6, which was characterized as 2+ both by the NordiQC reference standard methods and by the vast majority of all participants, but a minor proportion of participants scored this as 1+.
Among participants with insufficient staining results, 50% (22 of 44) scored their HER2 IHC results in consensus with the NordiQC assessor group. For this group the disagreement was also mainly related to the scoring of the breast carcinoma, tissue core no. 6. The score given by the participant was 2+ based on the staining done at their facilities, however the same core was scored 0 or 1+ by the NordiQC assessor team. The NordiQC assessment was primarily based on strict adherence to the ASCO/CAP guidelines but also to the level expected and characterized by the NordiQC HER2 IHC reference standard methods.

Figs. 1a and 1b – optimal staining results, same protocol
Figs. 2a and 2b – insufficient staining results - false negative, same protocol
Figs. 3a and 3b – insufficient staining results – excessive background reaction, same protocol



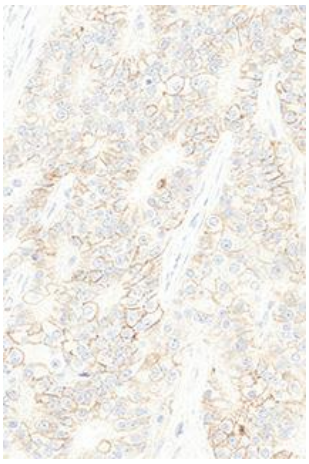
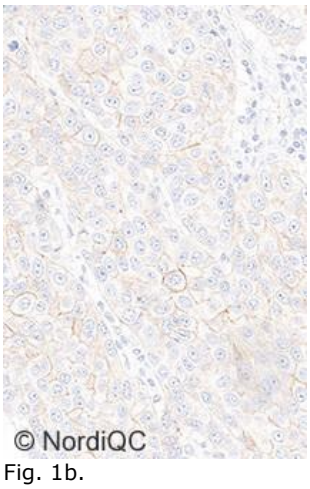


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 6 with a ratio of HER2 / chr17 of 2.53.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+. In the areas with tubular growth pattern a more basolateral staining pattern is seen.



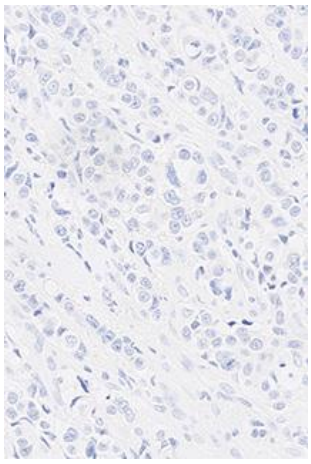
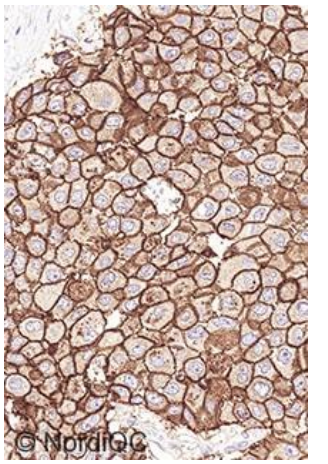


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 1.74.
>10% of the neoplastic cells show a weak, but incomplete membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 5 with a HER2 / chr17 ratio of 0.69.
No staining reaction is seen corresponding to 0.



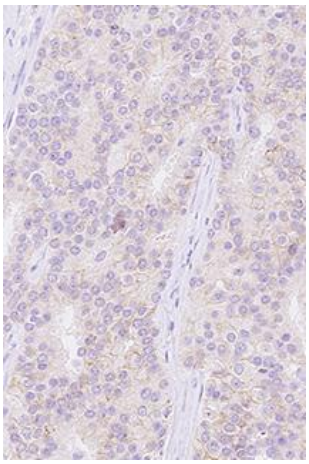
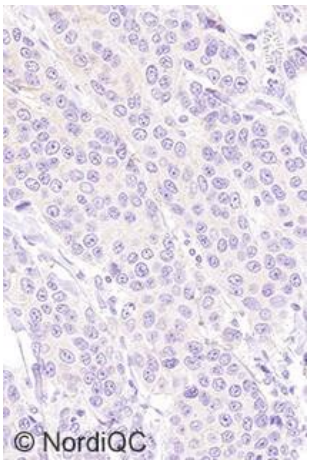


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of >6.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma no. 6 with a ratio of HER2 / chr17 of 2.53.
>10% of the neoplastic cells show a weak, but incomplete membranous staining reaction corresponding to 1+ (the core was scored as 1+ both by the participant and NordiQC).



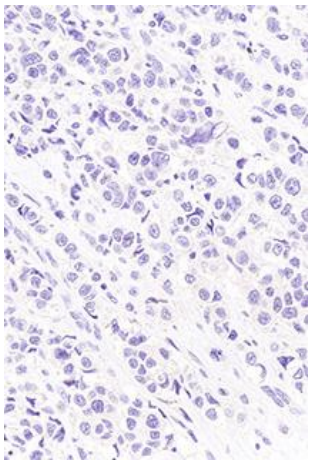


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 1.74.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.
Right: Staining result for HER2 of the breast carcinoma no. 5 with a HER2 / chr17 ratio of 0.69.
No staining reaction is seen corresponding to 0.

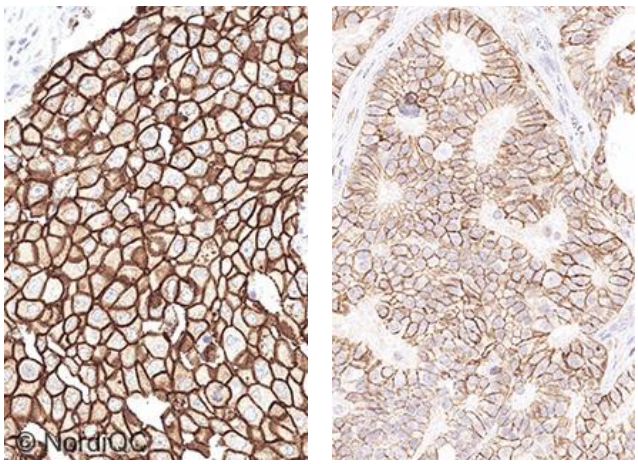


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of >6.
>10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast carcinoma no. 6 with a ratio of HER2 / chr17 of 2.53.
>10% of the neoplastic cells show a moderate to strong and complete membranous staining reaction corresponding to 3+ (Using the ASCO/CAP 2023 guidelines for 3+; Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population).

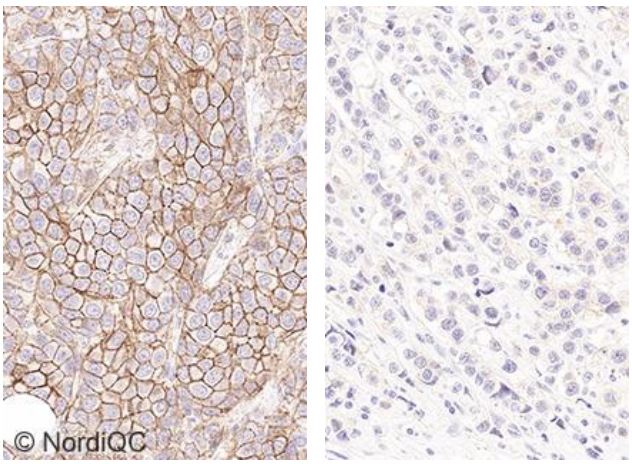


Fig. 3b.
Left: **Insufficient and false positive staining result** for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 1.74.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
(Using the ASCO/CAP 2023 guidelines for 3+; Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population).
Right: Staining result for HER2 of the breast carcinoma no. 5 with a HER2 / chr17 ratio of 0.69.
>10% of the neoplastic cells show a faint and incomplete membranous staining reaction corresponding to 1+.

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