Assessment Run B34 2022

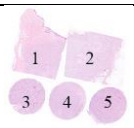
HER2 IHC

Purpose

Evaluation of the analytical accuracy of HER2 IHC tests performed by the NordiQC participants for demonstration and establishment of the HER2 protein 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 five breast carcinomas with the dynamic and critical relevant expression levels of HER2. The obtained score in NordiQC is indicative of the performance of the IHC tests used by the participants, but due to the limited number and composition of samples, internal validation and extended quality control, e.g. regularly measuring the HER2 results, is necessary and recommended.

Material

The slide to be stained for HER2 comprised the following 5 materials:

	**IHC: HER2 Score* (0, 1+, 2+, 3+)**	**FISH: HER2 gene/chr 17 ratio****	**FISH: HER2 gene copy no.****	**FISH HER2 gene amplification status**
Breast carcinoma, no. 1	3+	4.9	6.9	Amplified
Breast carcinoma, no. 2	2+	2.9	4.6	Amplified
Breast carcinoma, no. 3	1-2+	1.1	1.4	Unamplified
Breast carcinoma, no. 4	0-1+	1.0	1.5	Unamplified
Breast carcinoma, no. 5	3+	>6 (clusters)	10.9	Amplified

* HER2 immunohistochemical score (see table below) as achieved by using three FDA / CE-IVD approved HER2 IHC assays, HercepTest™ (SK001 and GE001, Dako/Agilent) and PATHWAY® (790-2991, Ventana/Roche), in the NordiQC reference laboratorie.
** HER2 gene/chromosome 17 ratio achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision) in NordiQC reference laboratory.

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

IHC scoring system according to the 2018 ASCO/CAP guidelines:

Score 0	No staining is observed **or** membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumor cells.
Score 1+	Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells.
Score 2+	Weak to moderate complete membrane staining observed in >10% of tumor cells.
Score 3+	Circumferential membrane staining that is complete, intense, and in >10% of tumor cells*.

**Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.*

Criteria for assessing a HER2 staining as **optimal** were:

- Staining corresponding to score 0 or 1+ in carcinoma no. 4.
- Staining corresponding to score 0, 1+ or 2+ in carcinoma no. 3.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 2.
- Staining corresponding to score 3+ in carcinomas no. 1 and 5.
- No or only weak cytoplasmic reaction that did not interfere with the interpretation.

Staining was assessed as **good**, if (1) the HER2 gene amplified tumours no. 1 and 5 showed a 2+ reaction and the other breast carcinomas showed reaction pattern as described above (equivocal 2+ IHC staining should always be analyzed by ISH according to the ASCO/CAP guidelines) ***or*** (2) a less distinct and/or reduced number of neoplastic cells were demonstrated in the HER2 2+ gene amplified tumour no. 2 compared to the NordiQC reference standards determined by HercepTest™ and PATHWAY® ***or*** (3) a 2+ reaction was seen in the HER2 gene unamplified 0/1+ tumour no. 4.

Staining was assessed as **borderline,** if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive counterstaining or impaired morphology hampering the interpretation.

Staining was assessed as **poor** in case of a false negative staining (e.g., the IHC 3+ tumours or the 2+ tumour with HER2 gene amplification showing a 0 or 1+ reaction) ***or*** a false positive staining (e.g. the IHC 2+ tumour without HER2 gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B34	417
Number of laboratories returning slides	392 (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 392 laboratories participated in this assessment and 84% achieved a sufficient mark (optimal or good).

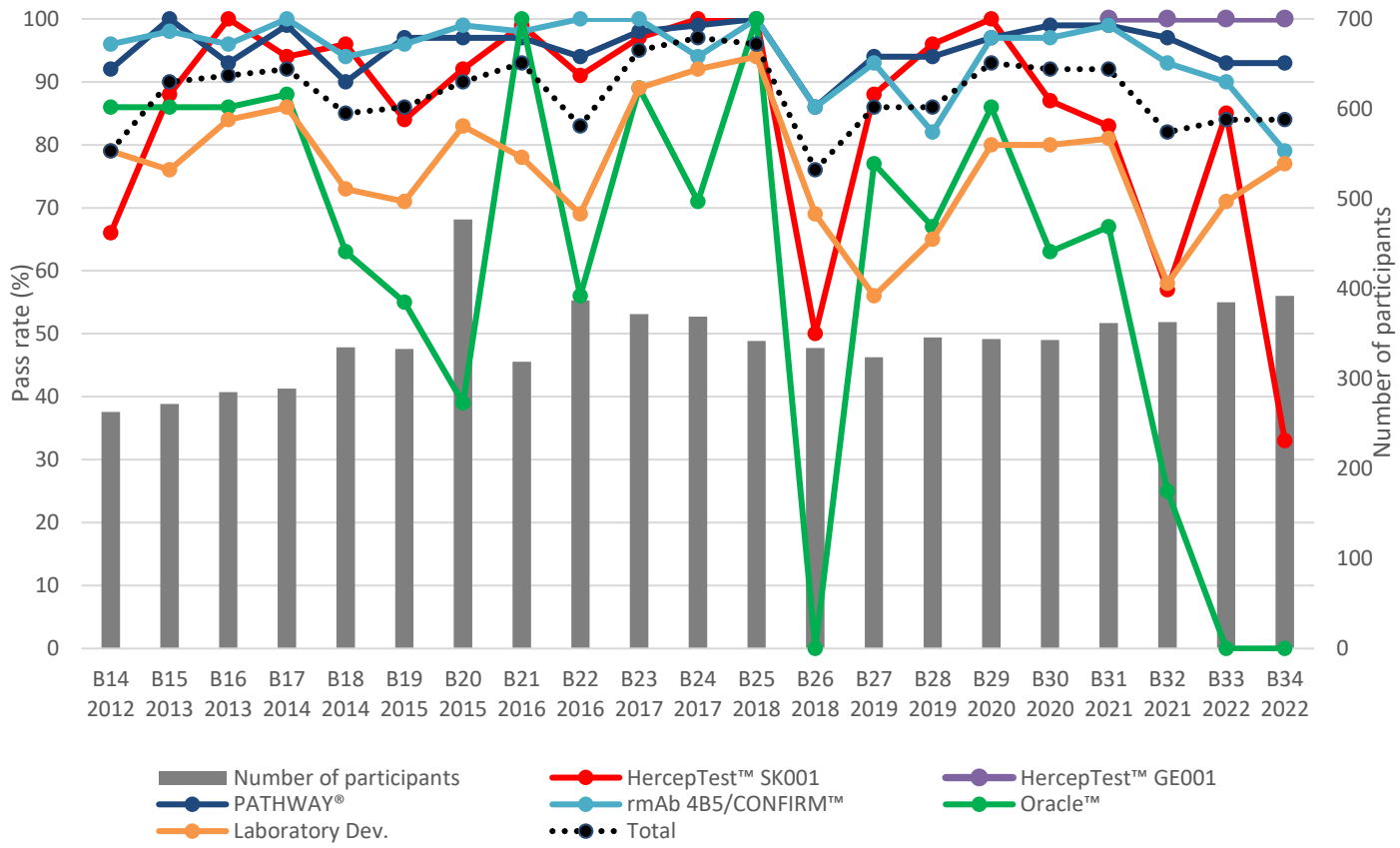
Conclusions

The overall pass rate was identical to the two latest runs B32 and B33 and slightly reduced compared to the level seen in the three previous assessment runs B29-B31.
In this assessment, the recently launched **HercepTest™ GE001**, Dako/Agilent, for the Omnis platform provided the best performance with a pass rate of 100% and 91% optimal results 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 33%.
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 an overall pass rate of 93% and 79% 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 77%, being significantly superior to the two CDx assays **HercepTest™ SK001**, Dako/Agilent and **Oracle™**, Leica Biosystems.

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

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 2012-2022**



* pass rates using vendor recommended protocol settings

Table 1. **Assessment marks for IHC assays and antibodies run B34, 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	21	5	-	2	93%	75%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	125	Ventana/Roche	93	19	3	10	90%	74%
VENTANA HER2 (**4B5**), **790-4493, (VRPS)[4]**	19	Ventana/Roche	14	1	1	3	79%	74%
VENTANA HER2 (**4B5**), **790-4493, (LMPS)[5]**	87	Ventana/Roche	71	6	2	8	89%	82%
HercepTest™, pAb **SK001, (VRPS)[4]**	12	Dako/Agilent	3	1	1	7	33%	25%
HercepTest™, pAb **SK001, (LMPS)[5]**	5	Dako/Agilent	2	1	1	1	60%	40%
HercepTest™, rmAb **DG44 GE001, (VRPS)[4]**	22	Dako/Agilent	20	2	-	-	100%	91%
HercepTest™, rmAb **DG44 GE001, (LPMS)[5]**	1	Dako/Agilent	1	-	-	-	-	-
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	2	Leica Biosystems	-	-	-	2	-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	7	Leica Biosystems	3	-	1	3	43%	43%
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **UMAB36**	4	Origene	1	1	1	1		
rmAb clone **BP6020**	2	Bailing Biotechnology	2	-	-	-	-	-
rmAb clone **EP3**	1 2	Cell Marque Biocare	3	-	-	-	-	-
rmAb clone **QR3**	1	Quartett	1	-	-	-		
rmAb clone **SP3**	4 4 3 2 1 1	Cell Marque Zytomed Thermo Fisher Scientific Master Diagnostica DCS Unknown	5	4	2	4	60%	33%
rmAb clone **RM228**	1	RevMab Bioscience	-	-	-	1		
rmAb clone **ZR218**	1	Zeta Corporation	-	-	-	1		
pAb, **A0485**	49	Dako/Agilent	30	12	-	7	86%	61%
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Ab clone **246G0D3, PA216**	1	Abcarta/Abcepta	1	-	-	-		
Ab clone **EAB-007, 01.09.70.19.01.01**	1	Zybio	-	-	1	-		
Ab clone **MXR001, RMA-1022**	2	Fuzhou Maixin	2	-	-	-	-	-
rmAb clone **EP3 AN726**	1	BioGenex	1	-	-	-		
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	1	-	-	-	-	-
rmAb clone **SP3, 237R-17**	2	Cell Marque	-	1	1	-	-	-
Total	392		275	53	14	50		
Proportion			70%	14%	3%	13%	84%	

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

Detailed Analysis
IVD approved assays

PATHWAY® rmAb clone **4B5** (790-2991, Ventana/Roche): In total, 114 of 153 (75%) 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 XT, GX or Ultra, 12-36 min. incubation of the primary Ab and UltraView as detection kit. Using these protocol settings, 107 of 122 (88%) laboratories produced a sufficient staining result (optimal or good).

Ventana HER2 rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 85 of 106 (80%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 30-64 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView as detection system. Using these protocol settings, 64 of 74 (86%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 5 of 17 (29%) 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 a water bath or PT Link, 30 min. incubation of the primary Ab and SK001 Polymer as detection system. Using these protocol settings, 4 of 12 (33%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 21 of 23 (91%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in HercepTest™ epitope retrieval solution at 97°C for 30 min., 10 min. incubation of the primary Ab and GE001 Polymer as detection system. Using these protocol settings, 22 of 22 (100%) laboratories produced a sufficient staining result.

Oracle™ mAb clone **CB11** (TA9145, Leica Biosystems): In total, 3 of 9 (33%) protocols were assessed as optimal. Protocols with optimal results were based on HIER in Bond Epitope Retrieval Solution 2 (BERS2) at 100°C for 20-30 min., 15 min. incubation of the primary Ab and Bond Polymer as detection system. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

Table 2 summarizes the proportion of sufficient and optimal marks for the most commonly used IVD approved assays. The performance was evaluated both as "true" plug-and-play systems performed accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the specific IHC stainer device are included.

Table 2. **Comparison of pass rates for vendor recommended and laboratory modified protocols**

CDx assay	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra PATHWAY® rmAb 4B5, **790-2991**	26/28 (93%)	21/28 (75%)	107/120 (89%)	86/120 (74%)
Ventana BenchMark XT, GX, Ultra VENTANA 4B5, **790-4493**	15/19 (79%)	14/19 (74%)	73/84 (87%)	67/84 (80%)
Dako Autostainer Link 48+ HercepTest™ pAb, **SK001**	4/12 (33%)	3/12 (25%)	2/3	1/3
Dako Omnis HercepTest™ rmAb DG44, **GE001**	22/22 (100%)	21/22 (91%)	1/1	1/1
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	0/2	0/2	3/7 (43%)	3/7 (43%)

* 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**: 30 of 49 (61%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) low pH (Dako/Agilent) (13/25*), TRS High pH (Dako/Agilent) (9/11), CC1 (Ventana/Roche) (2/3), BERS2 pH 9 (Leica Biosystems) (3/5), Bond Epitope Retrieval Solution 1 (BERS1) pH 6 (Leica Biosystems) (1/3) or Novocastra low pH 6 (1/1). 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, 39 of 46 (85%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone, **EP3**: 3 of 3 (100%) 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 BERS2 pH 9 (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 GX / XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS High pH	TRS Low pH	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
pAb clone **A0485**	4/5** (80%)	1/7 (14%)	5/6 (83%)	12/18 (67%)	2/3	-	3/5 (60%)	1/3

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

Comments
In this NordiQC assessment B34 for HER2, a pass rate of 84% was obtained and virtually identical to the levels seen in the latest runs B32 (82%) and B33 (84%) but slightly reduced compared to the level seen in the assessment runs B29-B31 (see Graph 1).

The insufficient results were primarily characterized by a too weak or false negative staining reaction being observed in 84% (54 of 64 results). Virtually all laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinomas, tissue cores no. 1 and 5, with high level gene amplification, whereas too weak or false negative staining results were particularly and most critically observed as a 0/1+ IHC staining reaction in the HER2 gene amplified breast carcinoma, tissue core no. 2. This tumour was categorized as IHC 2+ in the NordiQC reference laboratorie using the FDA/CE-IVD HER2 IHC assays: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (ratio 2.9) by FISH.
In the remaining insufficient results, these were characterized by e.g. a poor signal-to-noise ratio, impaired morphology and/or excessive cytoplasmic staining reaction compromising the read-out and scoring of the specific HER2 membranous reaction.

76% of the participants (n=298) used one of the FDA/CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY®, VENTANA HER2 (4B5) (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica Biosystems) on the specified stainer with predictive claim for HER2 status in breast cancer. 3% (n=10) of the participants used one of approved assays on another platform than specified by the vendor, while the remaining 21% (n=84) 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 PATHWAY® HER2 IHC assays 790-2991 and VENTANA HER2 (4B5) 790-4493 were most widely used and in total used by 66% of all participants (n=259). When applying the assays on the intended platform, Ventana BenchMark, an overall pass rate (irrespective of protocol settings) of 88% was observed and 75% of the results evaluated as optimal.
Similar to previous assessments, it was noticed that the majority of laboratories (81%; 204/251) used the two assays by modified protocol settings as shown in Tables 1 and 2. For the PATHWAY® HER2 IHC assay 790-2991, the pass rate and proportion of optimal results was superior, when applied in concordance to the instructions and guidelines provided by the vendor, whereas for the VENTANA HER2 (4B5) assay 790-4493, the pass rate and proportion of optimal results were superior using the assay by laboratory modified settings compared to the recommended protocol settings (see Tables 1 and 2).

Comparable to both run B32 and B33, it was observed that 12% of the participants used OptiView or UltraView with amplification for the Ventana/Roche PATHWAY® HER2 IHC assay 790-2991 and VENTANA HER2 (4B5) 790-4493, substituting iView or UltraView as recommended by Ventana/Roche. In this run, this modification was found very successful providing a pass rate of 97% (28/29) and superior to the vendor recommended protocol settings. Of particular attention, the prevalence of false negative results primarily in the HER2 IHC 2+ gene amplified tumor was significantly reduced. 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 and/or potentially also increase the number of HER2 2+ cases on a daily basis hereby extending the number of cases reflexed to ISH for final HER2 status. This underlines that modifications of CDx assays should be meticulously validated by the end-users on a large cohort of breast carcinomas (e.g. n=100). This has been addressed by ASCO/CAP in both the 2013 guidelines for HER2 testing and the 2020 guidelines for ER/PR testing and in particular in detail in the publication by Torlakovic

et al; "*Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine Part 3: Technical Validation of Immunohistochemistry", AIMM 2017;25:151–159.*

The recently launched Dako/Agilent HercepTest™ CDx assay GE001 for Dako Omnis based on the rmAb clone DG44 was the most widely used "non-Ventana" CDx assay and used by 6% (n=23) of all participants. As seen in Tables 1 and 2, the majority of all laboratories used the assay by vendor recommended protocol settings and when used as "plug-and-play" a pass rate of 100% was obtained, 91% being optimal and as such the most successful assay in this assessment run.

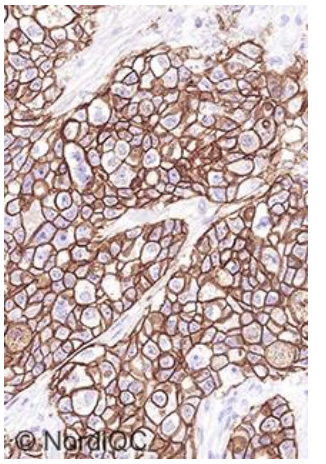
The "classic" Dako/Agilent HercepTest™ CDx assay SK001 for Dako Autostainer Link 48 provided an inferior and unexpected low pass rate of 33% when used in concordance with the recommended protocol settings from Dako/Agilent. This was a significant reduced level compared to the result obtained in run B33 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. runs B26 and B32. 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.

In this HER2 IHC assessment, 21% of the participants used LDTs based on concentrated Ab formats or generic RTU Abs without intended use or predictive claim for HER2 demonstration in breast carcinoma to guide decision with treatment with Herceptin or similar drugs. The proportion of laboratories using LDTs and FDA-/CE-IVD approved HER2 IHC assays seems to be very consistent. In the two latest assessment runs B32 and B33, 22% of the participants used LDTs compared to 23-31% in previous assessments.

Overall, the LDTs in run B34 provided a pass rate of 77%, 56% being optimal. In this assessment LDT's for HER2 IHC status with focus on overexpression showed an improvement compared to previous data obtained in runs B32 and B33 and de facto superior to both the CDx assays HercepTest™, SK001 Dako/Agilent and Oracle™, Leica Biosystems.
The pAb A0485 from Dako/Agilent is still the most widely applied Ab within a LDT being used by 13% (n=49 of 392) of the participants and gave an overall pass rate of 86% and 61% optimal results.

Scoring consensus B33
Laboratories were requested to submit scores (0, 1+, 2+ or 3+) on the NordiQC homepage of their own HER2 stained slides. This was done by 84% (329 of 392) of the participants returning slides.
For 260 of the 329 (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 2018 scoring guidelines.
Among laboratories with sufficient staining, 84% (227 of 271) 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. 2. Tissue core no. 2 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+. The tumour showed a mixed growth pattern of solid tumour nest but also in many areas with a micropapillary growth pattern. In the former, the classical 2+ pattern with a complete membranous staining reaction and in the latter showing a more basolateral staining pattern.
Among participants with insufficient staining results, 74% scored their HER2 IHC results in consensus with the NordiQC assessor group (43 of 58). For this group the disagreement mainly was also related to the scoring of the breast carcinoma, tissue core no. 2. The results submitted to NordiQC was scored as 0 or 1+ by NordiQC assessor team and as 2+ by the participant. The NordiQC assessment was primarily based on strict adherence to the ASCO/CAP guidelines but also to the level expected and characterized by the 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



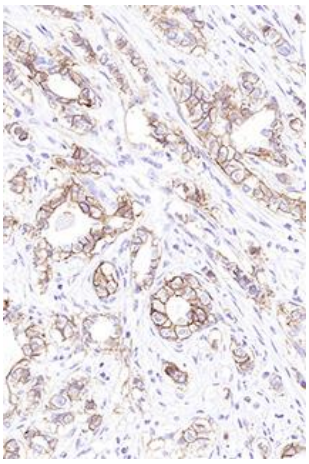
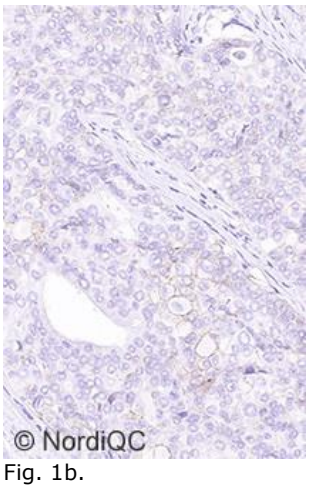


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.9.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 2.9.
>10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+. In the areas with micropapillary growth pattern a more basolateral staining pattern is seen.



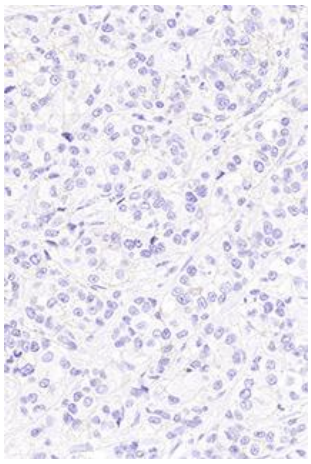
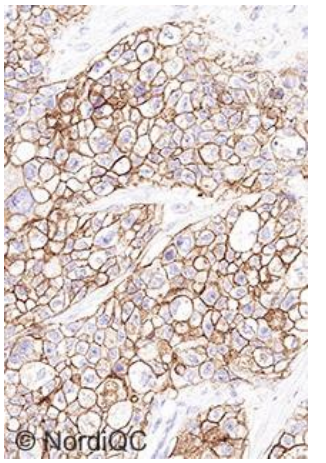


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.1.
>10% of the neoplastic cells show a weak incomplete membranous staining reaction corresponding to 1+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.0.
<10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.



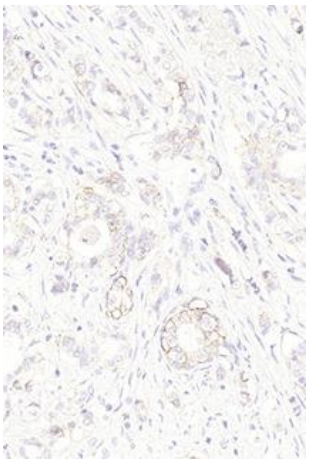
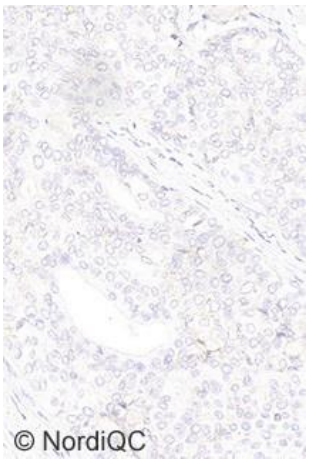


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.9.
>10% of the neoplastic cells show a strong complete membranous staining reaction corresponding to 3+.
Right: **Insufficient and false negative staining result** for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 2.9
>10% of the neoplastic cells show a weak to moderate, but incomplete membranous staining reaction corresponding to 1+ (the core was scored as 1+ both by the participant and NordiQC).



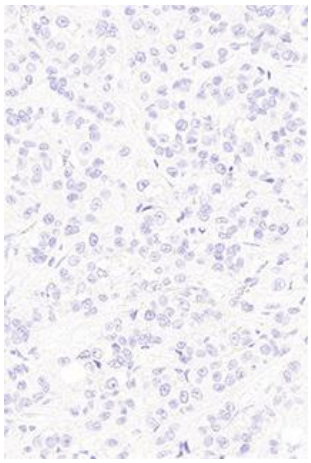
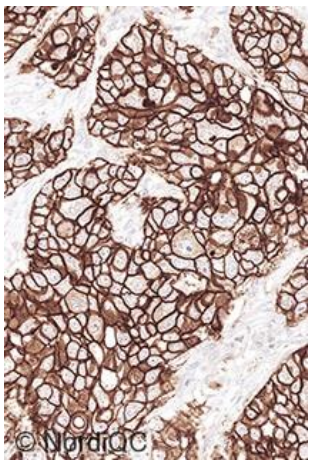


Fig. 2b.
Left: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.1.
<10% of the neoplastic cells show a weak partial membranous staining reaction corresponding to 0.
Right: Staining result for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.0.
No staining reaction is seen corresponding to 0.



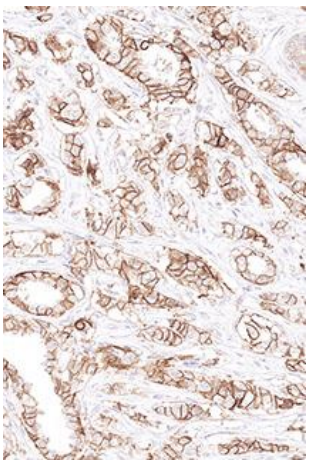
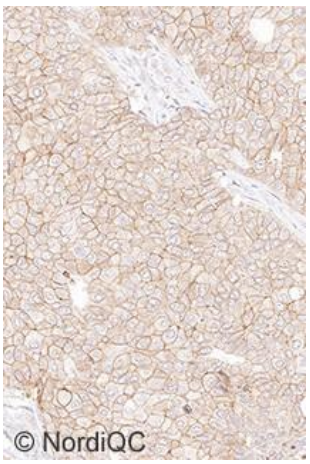


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 4.9.
>10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 2.9.
>10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+. An excessive cytoplasmic staining reaction is seen, but the scoring is not compromised. The tumour was score 3+ both by the NordiQC assessor group and the participant.



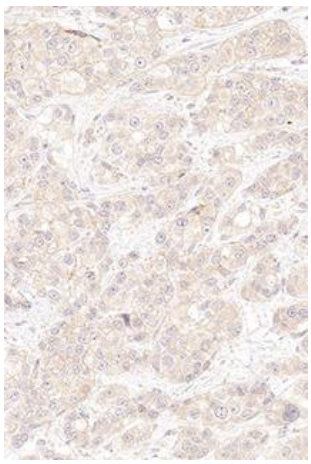


Fig. 3b.
Left: Staining result for HER2 of the breast carcinoma no. 3 with a ratio of HER2 / chr17 of 1.1.
>10% of the neoplastic cells show a moderate and complete membranous staining reaction corresponding to 2+ (the core was scored as 2+ both by the participant and NordiQC).
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.0.
A diffuse cytoplasmic staining reaction is observed hampering the read-out of the specific HER2 expression.

SN/LE 13.12.2022