Assessment Run B31 2021

HER2 IHC

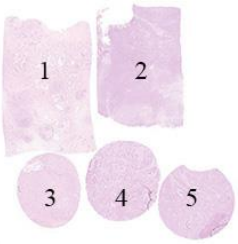
Purpose

Evaluation of the analytical accuracy of HER2 IHC tests performed by the NordiQC participants for demonstration and establishment of the HER2 protein expression 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****
1. Breast carcinoma, no. 1	2+	2.4–2.6 (amplified)
2. Breast carcinoma, no. 2	1-2+	1.1–1.5 (unamplified)
3. Breast carcinoma, no. 3	0-1+	1.3–1.5 (unamplified
4. Breast carcinoma, no. 4	3+	>6.0 (clusters) (amplified)
5. Breast carcinoma, no. 5	3+	>6.0 (clusters) (amplified)



* HER2 immunohistochemical score (see table below) as achieved by using the two FDA / CE-IVD approved HER2 IHC assays, HercepTest™ (SK001, Dako/Agilent) and PATHWAY® (790-2991, Ventana/Roche), in NordiQC reference laboratories.
** HER2 gene/chromosome 17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision) in NordiQC reference laboratories.
# Same donor materials as in run B30

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. 3.
- Staining corresponding to score 1+ or 2+ in carcinoma no. 2.
- Staining corresponding to score 2+ or 3+ in carcinoma no. 1.
- Staining corresponding to score 3+ in carcinoma no. 4 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. 4 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. 1 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. 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+ 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 B31	405
Number of laboratories returning slides	362 (89%)

The number of laboratories returning slides has decreased in this run B31 compared to previous assessments, due to the COVID-19 pandemic and associated postal delays. All slides returned after the assessment were assessed and received advice if the result being insufficient but the results were not included in this report.

Results

In total 362 laboratories participated in this assessment and 92% achieved a sufficient mark (optimal or good).

The overall pass rate was identical to the level seen in the latest assessment Run B30, 2020
In this assessment, the two FDA-/CE-IVD approved HER2 IHC assays from Ventana/Roche, PATHWAY® 790-2991 and HER2/4B5 790-4493 and HercepTest™ GE001, Dako/Agilent, were most successful and provided a high pass rate superior to both HercepTest™ (SK001, Dako/Agilent), Oracle™ (Leica Biosystems) and LDTs as illustrated in Graph 1.
Assessment marks for IHC HER2 assays and HER2 antibodies are summarized in Table 1.

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

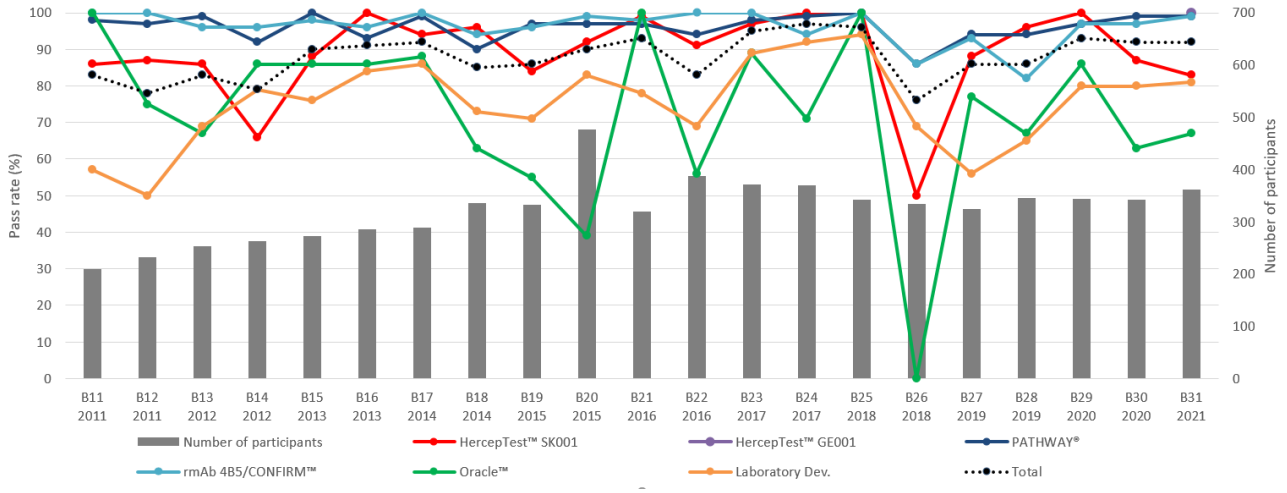


Table 1. **Assessment marks for IHC assays and antibodies run B31, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[4]**	24	Ventana/Roche	22	2	-	-	100%	92%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	114	Ventana/Roche	106	7	-	1	99%	93%
rmAb clone **4B5, 790-4493, (VRPS)[4]**	15	Ventana/Roche	15	-	-	-	100%	100%
rmAb clone **4B5, 790-4493, (LMPS)[5]**	75	Ventana/Roche	70	4	1	-	99%	93%
HercepTest™, pAb **SK001, (VRPS)[4]**	21	Dako/Agilent	11	6	-	4	81%	52%
HercepTest™, pAb **SK001, (LMPS)[5]**	9	Dako/Agilent	6	1	-	2	78%	67%
HercepTest™, rmAb **DG44 GE001, (VRPS)[4]**	10	Dako/Agilent	8	2	-	-	100%	80%
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	2	Leica Biosystems	-	1	-	1	-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	4	Leica Biosystems	1	2	-	1	-	-

Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CB11**	1	Leica Biosystems	-	-	-	1	-	-
rmAb clone **BP6020**	1	Bailing Biotechnology	-	1	-	-	-	-
rmAb clone **EP3**	2 1 1 1 1	Cell Marque Epitomics BioGenex Biocare Medical Diagnostic Biosystems	3	1	-	2	67%	50%
rmAb clone **SP3**	7 4 3 1	Thermo Fisher Scientific Cell Marque Zytomed enQuire	-	8	4	3	53%	0%
rmAb clone **ZR5**	1	Zeta	-	1	-	-	-	-
pAb, **A0485**	52	Dako/Agilent	29	17	2	4	88%	56%
mAb clone **IHC042**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **RM228**	1	RevMab Bioscience	1	-	-	-	-	-
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Ab clone **MXR001** Kit-0043	2	Maixin	-	2	-	-	-	-
mAb clone **CB11, PA0983**	1	Leica	-	-	-	1	-	-
Ab clone **GR011, 8362-C010**	3	Sakura Finetek	-	3	-	-	-	-
rmAb clone **SP3, MAD-000308QD**	1	Master Diagnostica	-	1	-	-	-	-
rmAb clone **SP3, 237R-17**	3	Cell Marque	-	3	-	-	-	-
Total	362		273	62	7	20		
Proportion			75%	17%	2%	6%	92%	

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, 128 of 138 (93%) 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 16-64 min.) on BenchMark XT, GX or Ultra, 12-32 min. incubation of the primary Ab and iView or UltraView as detection kit. Using these protocol settings, 133 of 134 (99%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 85 of 90 (94%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-64 min.) on BenchMark XT, GT or Ultra, 12-32 min. incubation of the primary Ab and UltraView as detection system. Using these protocol settings, 88 of 89 (99%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 17 of 30 (57%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in HercepTest™ epitope retrieval solution at 97-99°C for 20-40 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, 18 of 23 (78%) laboratories produced a sufficient staining result.

HercepTest™ rmAb clone **DG44** (GE001, Dako/Agilent): In total, 8 of 10 (80%) protocols were assessed as optimal. Protocols with optimal results were all based on the recommended protocol settings using 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, 10 of 10 (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**	24/24 (100%)	22/24 (92%)	109/110 (99%)	103/110 (94%)
Ventana BenchMark XT, GX, Ultra rmAb 4B5, **790-4493**	15/15 (100%)	15/15 (100%)	73/74 (99%)	69/74 (93%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	17/21 (81%)	11/21 (52%)	1/3	1/3
Dako Omnis HercepTest™ rmAb DG44, **GE001**	10/10 (100%)	8/10 (80%)	-	-
Leica Bond MAX, III Oracle™ mAb CB11, **TA9145**	1/2	0/2	3/4	1/4

* 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**: 29 of 52 (56%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) low pH (Dako/Agilent) (16/25*), TRS High pH (Dako/Agilent) (8/15), CC1 (Ventana/Roche) (3/6), Novocastra Epitope Retrieval Solution pH 6 (Leica) (1/1) or unknown (1/1). The Ab was diluted in the range of 1:100-1,600 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 44 of 48 (92%) 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 Abs 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 Bond III / Max**	
	TRS pH High	TRS pH Low pH	TRS High pH	TRS Low pH	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
pAb clone **A0485**	3/9** (33%)	4/8 (50%)	5/6 (83%)	12/17 (71%)	3/6 (50%)	-	0/3	-

* 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 B31 for HER2, an overall and very satisfactory pass rate of 92% was observed which was identical to the level seen in the latest run, B30 2020.
The insufficient results were primarily characterized by a false negative staining reaction being observed in 74% (20 of 27). Virtually all laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinomas, tissue cores no. 4 and 5, with high level gene amplification, whereas 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. 1. This tumour was categorized as IHC 2+ in the NordiQC reference laboratories using the two FDA/CE-IVD HER2 IHC assays: PATHWAY® (Ventana/Roche) and HercepTest™ (Dako/Agilent) and showed HER2 gene amplification (ratio 2.4-2.6) by FISH.
In 7% (2 of 27) of the insufficient results a false positive staining reaction was seen, characterized by a 3+ IHC result in the breast carcinoma, tissue core no. 2, expected to show a 1+ or 2+ IHC result and was not HER2 gene amplified.

In the remaining insufficient results, a poor signal-to-noise ratio was seen and characterized by an excessive cytoplasmic staining reaction compromising the interpretation of the specific HER2 membranous reaction.

73% of the participants (n=263) used FDA/CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY® (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica) on the specified stainer with predictive claim for HER2 status in breast cancer. 11 laboratories used an approved assay on another platform than specified by the vendor, while the remaining laboratories used a laboratory developed test (LDT) based on a concentrated primary Ab or a RTU format without a predictive claim.

The Ventana/Roche PATHWAY® HER2 IHC assays 790-2991 and 790-4493 were used by 63% of all participants (n=228). Overall, a pass rate of 99% was observed and 93% were optimal. In both the previous and 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. Despite this observation, it is still highly recommended to use the assays strictly in concordance to the instructions and guidelines provided by the vendor, as e.g. in run B28 it was shown that both the pass rate and proportion of optimal results were reduced for laboratories modifying the protocols. More data can be found at;
https://www.nordiqc.org/downloads/assessments/123_11.pdf

In contrast to run B29, it was observed that an increased number of participants used OptiView or UltraView with amplification for the HER2 IHC assays 790-2991 and 790-4493 substituting iView or UltraView as recommended by Ventana/Roche. In this run 11% of the laboratories used one of the two HER2 CDx assays in combination with either OptiView or UltraView with amplification, which was the same level seen in run B28. In run B28 this modification frequently induced an insufficient result characterized by a false positive HER2 reaction in a 2+, HER2 gene unamplified breast carcinoma. 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 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 Dako/Agilent HercepTest™ CDx assay SK001 for Dako Autostainer Link 48 provided an overall pass rate of 83% and was used by 30 participants. The vast majority of laboratories used the IHC CDx assay in concordance with the recommended protocol settings from Dako/Agilent, with a pass rate of 81%, 52% optimal (see Table 2).

The recently launched Dako/Agilent HercepTest™ CDx assay for Dako Omnis based on the rmAb clone DG44 provided a pass rate of 100%, 80% optimal results, and was used by 10 participants. As seen in Table 2, all laboratories used the assay by vendor recommended protocol settings.

In this HER2 IHC assessment, 24% 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. Overall, the LDTs provided a pass rate of 81% (71 of 88) and 39% optimal (34 of 88).

The pAb A0485 from Dako/Agilent was most widely used and applied with optimal protocol settings as described above, a pass rate of 88% was obtained.
Slight surprisingly, the rmAb clone SP3 as concentrate was found less successful. In this assessment run B31, no optimal results were obtained as shown in Table 1 irrespectively applying similar protocol settings as e.g for the pAb A0485.

In this assessment, the FDA-/CE-IVD approved HER2 IHC CDx assays PATHWAY® /4B5 from Ventana/Roche and HercepTest™, GE001 Dako/Agilent were most successful and provided a high pass rate superior to both other CDx assays as HercepTest™, SK001 Dako/Agilent and Oracle™, Leica Biosystems and also LDTs as illustrated in Graph 1.
The proportion of laboratories using the FDA-/CE-IVD approved HER2 IHC assays and LDTs is very consistent. In this run, 24% of the participants (n=88) used LDTs compared to 23-31% in the latest assessments.

Scoring consensus B31
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% (305 of 362) of the participants returning slides.
For 229 of the 305 (75%) 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. This was on par to the level of 77% observed in run B30 but lower compared to run B29 where 93% of the scores were in consensus with the NordiQC assessor group.
Among laboratories with sufficient staining, 78% (222 of 284) of the scoring read-outs were in agreement with the NordiQC assessors. Disagreement was primarily related to the scoring of the HER2 status in breast carcinoma, tissue core no. 5. This was characterized as 3+ both by the NordiQC reference standard methods and by the vast majority of all participants. The membranes of neoplastic cells in the tumour, however were less intense compared to the breast carcinoma, tissue core no. 4, being very intense, but both tumours should be scored as 3+, accordingly to the ASCO/CAP 2018 scoring guidelines.
Among participants with insufficient staining results, 37% were in consensus with the NordiQC assessor group (7 of 19). For this group the disagreement primarily was related to the scoring of the breast carcinoma, tissue core no. 1. The results submitted to NordiQC was scored as 1+ by NordiQC assessor team and typically 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 two HER2 IHC reference standard methods.

Conclusion
The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/4B5** 790-2991/790-4493 from Ventana/Roche and **HercepTest™**, GE001 Dako/Agilent were in this assessment the most accurate and successful assays for the semi-quantitative IHC determination of HER2 protein expression in breast carcinoma.
Laboratory developed assays based on concentrated formats especially rmAb clone SP3 provided a lower pass rate and reduced proportion of optimal results.
Inclusion of 2+ tumours with and without HER2 gene amplification in the control material for both EQA and internal quality control seems to be essential to evaluate accuracy, precision and reproducibility of the HER2 IHC assays used by laboratories.

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 – false positive, same protocol

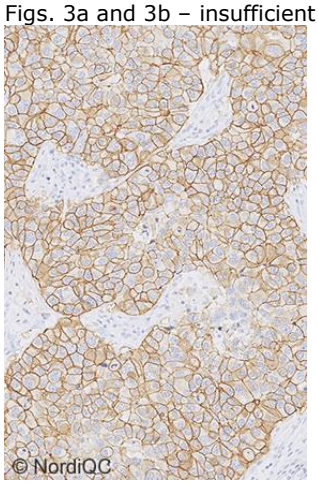


Fig. 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0. > 10% of the neoplastic cells show a strong and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 2.4-2.6. > 10% of the neoplastic cells show a weak to moderate and complete membranous staining reaction corresponding to 2+.

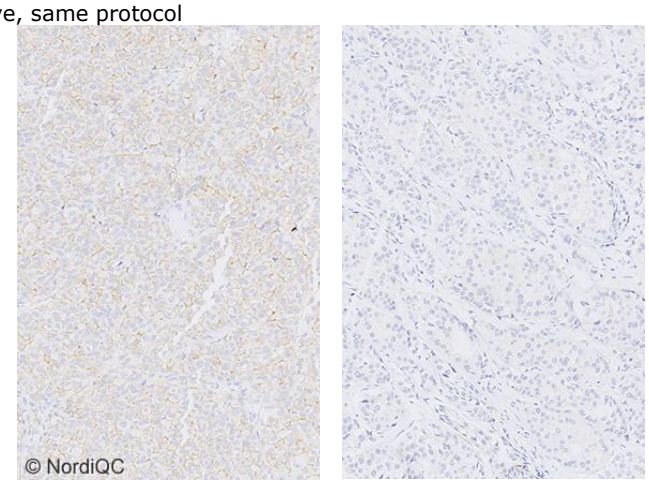
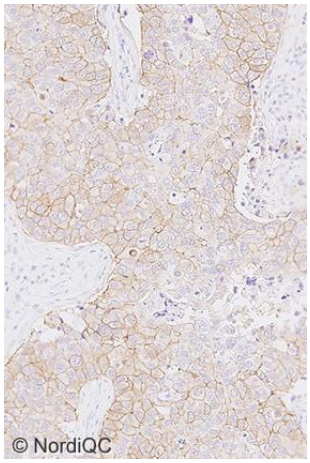


Fig. 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5. > 10% of the neoplastic cells show a weak complete membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 3 with a HER2 / chr17 ratio of 1.3-1.5. < 10% of the neoplastic cells show a faint, partial membranous staining reaction corresponding to 0.



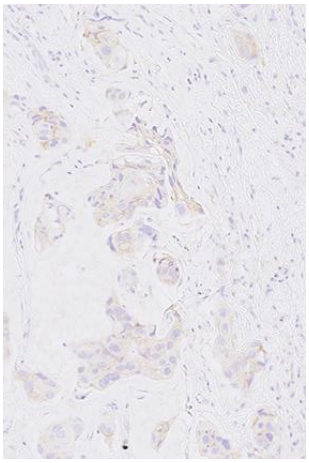
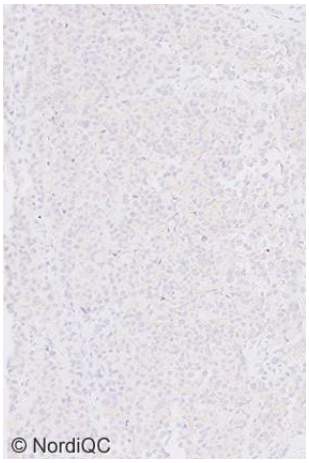
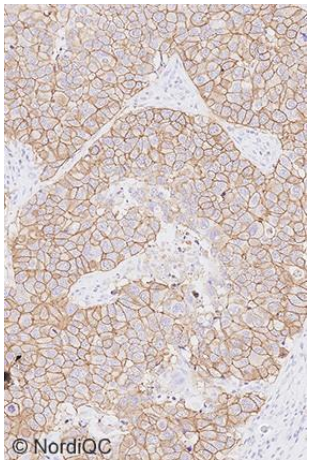


Fig. 2a.
Left: Staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show a strong complete membranous staining reaction corresponding to 2+.
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 2.4-2.6
> 10% of the neoplastic cells show a weak to moderate, incomplete membranous staining reaction corresponding to 1+ (the core was scored as 1+ both by the participant and NordiQC).



Fi.g 2b.
Left: Staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5.
< 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. 3 with a HER2 / chr17 ratio of 1.3-1.5.
No staining reaction is seen corresponding to 0.



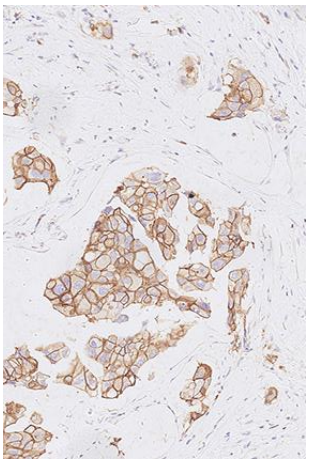
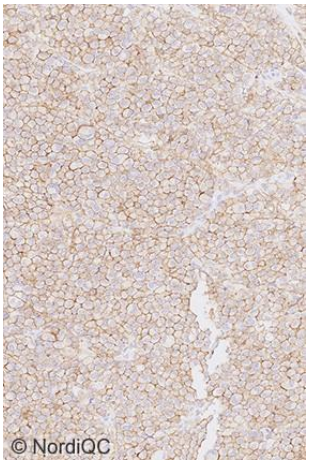


Fig. 3a.
Left: Staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Staining result for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 2.4-2.6
The membranes of the neoplastic cells are showing a 3+ reaction.
However, compare with Figs. 3b – insufficient results obtained.



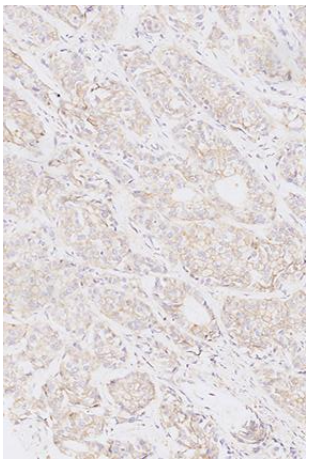


Fig. 3b.
Left: **Insufficient staining result** for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5.
> 10% of the neoplastic cells show a strong complete membranous staining reaction corresponding to 3+ (the core was scored as 3+ both by the participant and NordiQC).
Right: Insufficient Staining result for HER2 of the breast carcinoma no. 3 with a HER2 / chr17 ratio of 1.3-1.5.
This tumour was by the NordiQC reference standard methods characterized as 0-1+ and by the protocol applied giving a 2+ status and additional need to reflex for ISH.

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