Assessment Run B29 2020

HER2 IHC

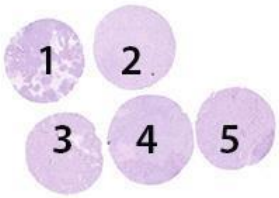
Purpose

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) and HercepTest™ (Dako) 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	3+	> 6.0 (clusters) (amplified)
4. Breast carcinoma, no. 4	0-1+	1.3 – 1.5 (unamplified)
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™ (Dako) and PATHWAY® (Ventana), in NordiQC reference laboratories.
** HER2 gene/chromosome 17 ratios achieved using Zyto*Light* ® SPEC HER2/CEN 17 Dual Color FISH (Zytovision)

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 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. 3 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. 3 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®.

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 gene amplification showing a 0 or 1+ reaction) or a false positive staining (e.g. the IHC 2+ tumour without gene amplification showing a 3+ reaction).

Participation

Number of laboratories registered for HER2, run B29	362
Number of laboratories returning slides	344 (95%)

Results: 344 laboratories participated in this assessment and 93% achieved a sufficient mark (optimal or good). Assessment marks for IHC HER2 assays and HER2 antibodies are summarized in Table 1.

Table 1. **Assessment marks for IHC assays and antibodies run B29, HER2 IHC**

IVD approved HER2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
PATHWAY® rmAb clone **4B5, 790-2991, (VRPS)[4]**	41	Ventana/Roche	35	6	-	-	100%	85%
PATHWAY® rmAb clone **4B5, 790-2991, (LMPS)[5]**	103	Ventana/Roche	88	11	-	4	96%	85%
rmAb clone **4B5, 790-4493, (VRPS)[4]**	23	Ventana/Roche	21	-	-	2	91%	91%
rmAb clone **4B5, 790-4493, (LMPS)[5]**	55	Ventana/Roche	53	2	-	-	100%	96%
HercepTest™ **SK001, (VRPS)[4]**	19	Dako/Agilent	17	2	-	-	100%	90%
HercepTest™ **SK001, (LMPS)[5]**	10	Dako/Agilent	6	4	-	-	100%	60%
Oracle™ mAb clone **CB11, TA9145, (VRPS)[4]**	3	Leica	1	1	1		-	-
Oracle™ mAb clone **CB11, TA9145, (LMPS)[5]**	4	Leica	1	3	-		-	-
Antibodies[3] for laboratory developed HER2 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **C1F7**	1	Celnovte	-	-	-	1		
mAb clone **CB11**	5 1	Leica/Novocastra Zytomed	-	1	4	1	17%	-
mAb clone **e2-4001**	1	Thermo Fisher Scientific	1	-	-	-		
rmAb clone **BSR44**	1	Nordic Biosite	1	-	-	-	-	-
rmAb clone **EP3**	1 1 1	Cell Marque Biocare Epitomics	2	1	-	-	-	-
rmAb clone **SP3**	9 5 3 1 1 1 1	Thermo Fisher Scientific Cell Marque Zytomed Spring Biosystems Invitrogen enquire Master Diagnostica	5	12	1	3	85%	24%
rmAb clone **RM228**	1	RevMAb Biosciences	1	-	-	-	-	-
rmAb clone **ZR5**	1	Zeta	-	-	1	-		
pAb, **A0485**	43	Dako/Agilent	33	8	1	1	95%	77%
Antibodies for laboratory developed HER2 assays, RTU		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **CB11, PA0571, PA0983**	3	Leica	-	-	1	2	-	-
Ab clone **GR011, 8362-C010**	1	Sakura Finetek	1	-	-	-	-	-
rmAb clone **EP3, RMPD049R**	1	Diagnostic Biosystems	-	-	-	1	-	-
rmAb clone **EP3, AN726**	1	BioGenex	-	1	-	-	-	-
rmAb clone **SP3, 237R**	2	Cell Marque	1	-	-	1	-	-
Total	344		267	52	9	16	-	-
Proportion			78%	15%	3%	5%	93%	-

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, 123 of 144 (85%) 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, 12-48 min. incubation of the primary Ab and iView or UltraView as detection kit. Using these protocol settings, 107 of 109 (98%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **4B5** (790-4493, Ventana/Roche): In total, 74 of 78 (95%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 16-64 min.) on BenchMark XT, GT or Ultra, 12-48 min. incubation of the primary Ab and iView or UltraView as detection system. Using these protocol settings, 65 of 67 (97%) laboratories produced a sufficient staining result.

HercepTest™ pAb (SK001, Dako/Agilent): In total, 23 of 29 (79%) 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, 22 of 22 (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**	41/41 (100%)	35/41 (85%)	96/100 (96%)	85/100 (85%)
Ventana BenchMark XT, GX, Ultra rmAb 4B5, **790-4493**	21/23 (91%)	21/23 (91%)	54/54 (100%)	53/54 (98%)
Dako Autostainer Link 48+ HercepTest™ pAb **SK001**	19/19 (100%)	17/19 (90%)	5/5 (100%)	4/5 (80%)
Leica Bond MAX, III Oracle™ mAb CB11 **TA9145**	2/3	1/3	4/4	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**: 33 of 43 (77%) protocols were assessed as optimal. Optimal protocols were based on HIER using either Target Retrieval Solution (TRS) low pH (Dako) (20/24*), TRS High pH (Dako) (7/8), CC1 (Ventana) (3/3), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/2) or Novocastra Epitope Retrieval Solution pH 6 (Leica) (1/1). The pAb A0485 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, 37 of 38 (97%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP3**: 5 of 21 (24%) protocols were assessed as optimal. Optimal protocols were based on HIER using CC1 (Ventana) (2/4), TRS High pH (Dako) (1/4), TRS Low pH (Dako) (1/1) or EDTA/EGTA pH 8 (1/1). The rmAb clone SP3 was diluted in the range of 1:40-100 depending on the level of the total technical sensitivity of the protocol employed. Using these protocol settings, 9 of 11 (82%) laboratories produced a sufficient staining result.

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

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

Concentrated antibodies	**Dako Agilent Autostainer**		**Dako Agilent Omnis**		**Ventana/Roche BenchMark 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**	3/4**	7/10 (70%)	4/4	13/14 (93%)	3/3	-	2/2	-
rmAb clone **SP3**	1/2	1/1	0/2	-	2/4	-	0/10	-

* 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 B29 for HER2, an overall pass rate of 93% was observed which was slightly higher than the recent assessments. The insufficient results were typically characterized by either a false negative staining reaction and/or a poor signal-to-noise ratio compromising the interpretation. Virtually all laboratories were able to demonstrate the expected HER2 3+ staining reaction in the breast carcinomas, tissue cores no. 3 and 5, with high level gene amplification, whereas false negative staining results were particularly and most critically observed as a 0/1+ IHC 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) and HercepTest™ (Dako) and showed HER2 gene amplification (ratio 2.4-2.6) by FISH.
The poor signal-to-noise ratio was typically characterized by an excessive cytoplasmic staining reaction compromising the interpretation of the specific HER2 membranous reaction.
In contrast to the previous assessment run B28, where 40% of insufficient results were related to false positive results, only 4% were observed to be false positive in this run B29. The difference most likely caused by different materials included in the two runs, but also to a reduced number of laboratories using OptiView or UltraView with amplification kit for the Ventana/Roche PATHWAY® HER2 IHC assays 790-2991 and 790-4493 and hereby following the recommendations provided in the last assessment run B28.

75% of the participants (n=258) used FDA/CE-IVD approved companion diagnostic (CDx) HER2 IHC assays as PATHWAY® (Ventana/Roche), HercepTest™ (Dako/Agilent) and Oracle™ (Leica), while the remaining 25% of laboratories used a laboratory developed test (LDT) based on a concentrated primary Ab or a RTU format being optimized for the IHC system used by the laboratory.

The Ventana/Roche PATHWAY® HER2 IHC assays 790-2991 and 790-4493 were used by 65% of all participants (n=222). Overall, a pass rate of 97% was observed and 89% were 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. Despite this observation, it is still highly recommended to use the assays strictly in concordance to the instructions and guidelines provided, as e.g. in run B28 it was shown that the pass rate and proportion of optimal results were reduced for laboratories modifying the protocols, see https://www.nordiqc.org/downloads/assessments/123_11.pdf
In this run, 29% used the Ventana HER2 CDx assays as "plug-and-play" systems compared to 24% in run B29.
It was observed that a reduced number of participants used OptiView and UltraView with amplification for the HER2 IHC assays 790-2991 and 790-4493 substituting iView or UltraView as recommended by Ventana/Roche. OptiView or use of amplification kit for UltraView will typically amplify the analytical sensitivity of the IHC system 3-4 times compared to UltraView using otherwise identical protocol settings and consequently induce a potential risk of inaccurate HER2 IHC results. This was in particular the main reason for the relatively high proportion of false positive results in run B28.

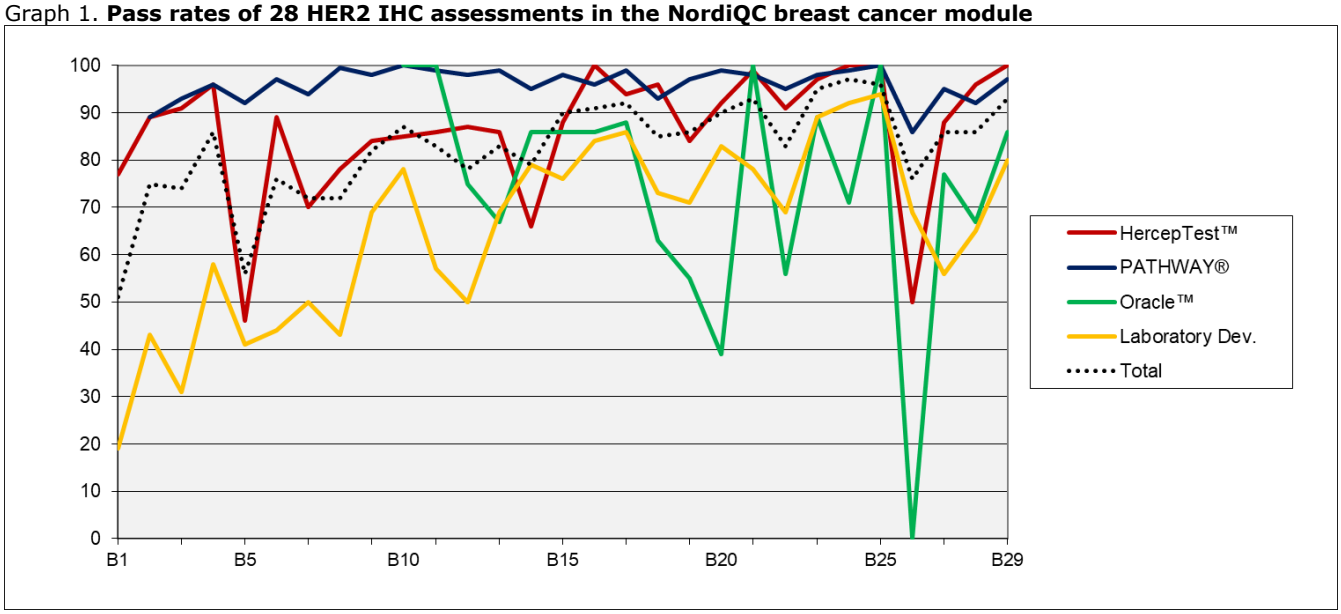
The Dako/Agilent HercepTest™ CDx assay SK001 provided an overall pass rate of 100% and was used by 29 participants. The vast majority of laboratories used the IHC assay in concordance with the recommended protocol settings from Dako/Agilent. No significant differences were observed for the laboratories using the IHC assay SK001 as "plug-and-play" versus laboratories modifying the protocols – see Table 1 and 2.

In this HER2 assessment, 25% of the participants used LDTs based on concentrated Ab formats or generic RTU Abs without intended use or 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 79% (68 of 86) and 52% optimal (45 of 86).

The pAb A0485 from Dako was most widely used and applied with optimal protocol settings as described above, a pass rate of 83% was obtained.

In this assessment, the FDA-/CE-IVD approved HER2 IHC assays HercepTest™ and PATHWAY® /4B5 were most successful and provided a high pass rate superior to Oracle™ and 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, 25% of the participants (n=86) used LDTs compared to 23-31% in the latest assessments.

Graph 1. **Pass rates of 28 HER2 IHC assessments in the NordiQC breast cancer module**



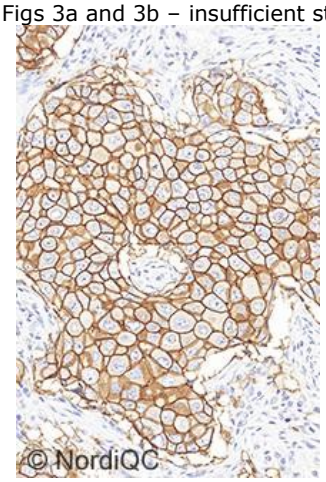
Scoring consensus B29

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% (290 of 344) of the participants returning slides.
For 271 of the 290 (93%) 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 interpretation guidelines. This was significantly higher than the previous run B28 where 71% (207 of 292) of the scores were in consensus with the NordiQC assessor group. No reason for this increase could be extrapolated from the data. Among laboratories with sufficient staining, 96% (263 of 273) of the interpretations were in agreement with the NordiQC assessors. Among participants with insufficient staining, 47% were in consensus with the NordiQC assessor group (8 of 17).

Conclusion

The FDA-/CE-IVD approved HER2 IHC assays **PATHWAY®/4B5** 790-2991/790-4493 (Ventana/Roche) and **HercepTest™** SK001 (Dako) 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 tests based on concentrated formats or generic RTU formats without intended use provided a lower pass-rate and a 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 is essential to evaluate accuracy, precision and reproducibility of the IHC HER2 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 – poor signal-to-noise ratio, same protocol



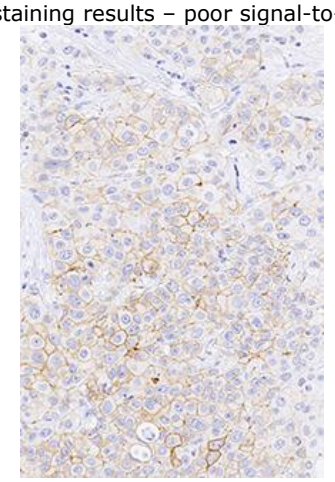
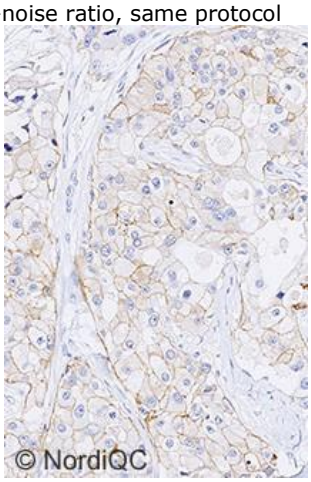


Fig 1a.
Left: Optimal staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 1 with a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



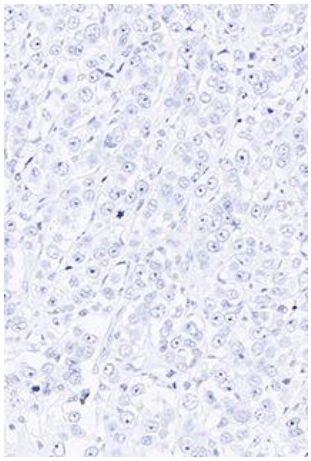
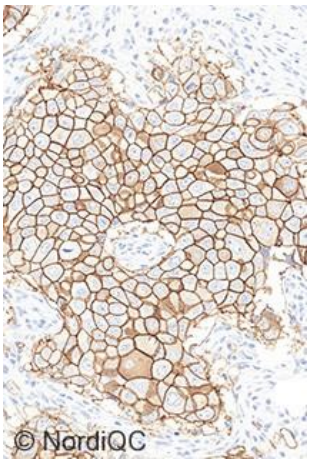


Fig 1b.
Left: Optimal staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5.
> 10% of the neoplastic cells show a weak-moderate membranous staining reaction corresponding to 2+.
Right: Optimal staining result for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.3-1.5.
< 10% of the neoplastic cells show a faint, incomplete membranous staining reaction corresponding to 0.



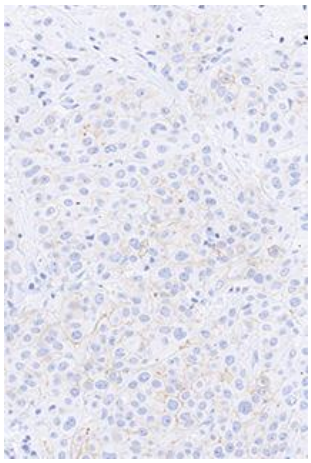
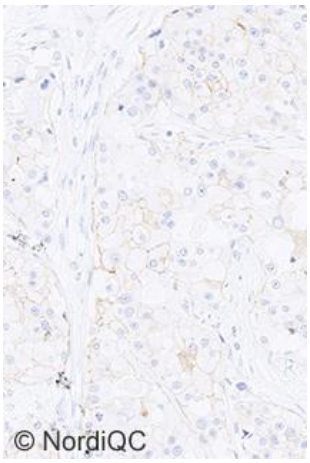


Fig 2a.
Left: Staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 1 with 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).



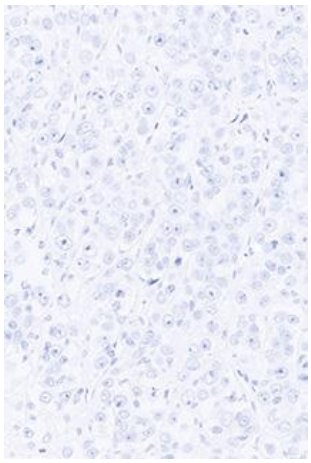
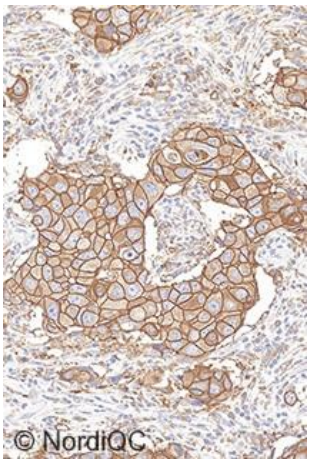


Fig 2b.
Left: Staining result for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5.
≥ 10% of the neoplastic cells show a weak to moderate, incomplete membranous staining reaction corresponding to 1+
Right: Staining result for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.3-1.5.
No staining reaction is seen corresponding to 0.



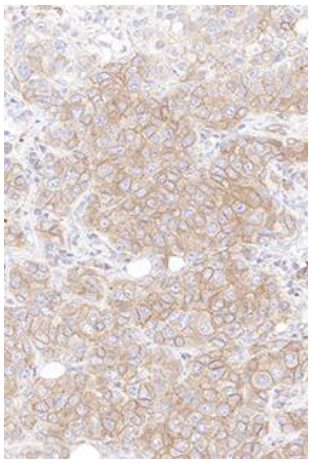
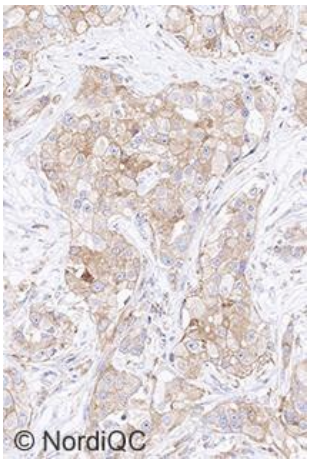


Fig 3a.
Left: Staining result for HER2 of the breast carcinoma no. 5 with a ratio of HER2 / chr17 of > 6.0.
> 10% of the neoplastic cells show an intense and complete membranous staining reaction corresponding to 3+.
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 1 with a ratio of HER2 / chr17 of 2.4-2.6
An excessive cytoplasmic staining reaction complicates and impedes the interpretation and level of the specific membranous staining reaction.



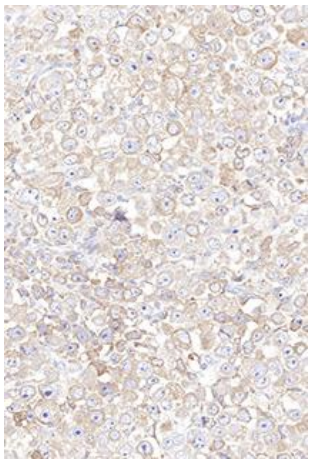


Fig 3b.
Left: **Insufficient staining result** for HER2 of the breast carcinoma no. 2 with a ratio of HER2 / chr17 of 1.1-1.5.
An excessive cytoplasmic staining reaction complicates and impedes the interpretation and level of the specific membranous staining reaction.
Right: **Insufficient staining result** for HER2 of the breast carcinoma no. 4 with a HER2 / chr17 ratio of 1.3-1.5.
An excessive cytoplasmic staining reaction complicates and impedes the interpretation and level of the specific membranous staining reaction.

SN/LE/RR 07.04.2020