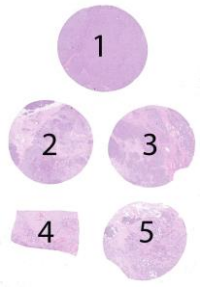
Assessment Run H10 2016

HER-2 ISH (BRISH or FISH)

Material

Table 1. **Content of the multi-block used for the NordiQC HER-2 ISH assessment, run H10**

	HER-2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	0	0.8	0.8 – 0.9	< 4
2. Breast carcinoma	2+	1.1	1.0 – 1.2	< 4, ≥ 4 < 6
3. Breast carcinoma	2+	1.5	1.3 – 1.5	< 4, ≥ 4 < 6
4. Breast carcinoma	2+	2.3	2.1 – 2.9	> 6
5. Breast carcinoma	3+	7.0	4.2 – 6.4	> 6



* PATHWAY® (Ventana), data from two reference labs.
** Inform HER-2 Dual ISH kit (Ventana), data from one reference lab.
*** HER2 FISH pharmDX™ Kit (Dako) and HER2 FISH (Zytovision), range of data from two reference lab.
¤HER2/chr17: HER-2 gene/chromosome 17 ratio

All tissues were fixed for 24 - 48 hours in 10% neutral buffered formalin according to the ASCO/CAP 2013 guidelines for tissue preparation of breast tissue for HER-2 ISH analysis.

HER-2 BRISH, Technical assessment

The main criteria for assessing a BRISH HER-2 analysis as technically **optimal** were the ability to interpret the signals and thus evaluate the HER2/chr17 ratios in all five tissues.

Staining was assessed as **good**, if the HER2/chr17 ratios could be evaluated in all five tissues, but the interpretation was slightly compromised e.g. due to excessive retrieval, weak or excessive counterstaining or focal negative areas.

Staining was assessed as **borderline** if one of the tissues could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

Staining was assessed as **poor** if two or more of the tissue cores could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

HER-2 BRISH and FISH interpretation

For both BRISH and FISH, participating laboratories were asked to submit a scoring sheet with their interpretation of the HER2/chr17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyze scoring consensus.

Consensus scores from the NordiQC FISH reference laboratories

- Breast ductal carcinoma no. 1: non-amplified
- Breast ductal carcinoma no. 2 and 3: non-amplified or equivocal
- Breast ductal carcinoma no. 4 and 5: amplified

The ASCO/CAP 2013 guidelines were applied for the interpretation of the HER-2 status

Unamplified: HER2/chr17 ratio < 2.0 using a dual probe assay or an average < 4 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Equivocal: HER2/chr17 ratio of < 2.0 using a dual probe assay with an average of ≥ 4 and < 6 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Amplified: HER2/chr17 ratio ≥ 2.0 using a dual probe assay or an average ≥ 4 HER-2 copies per cell/nucleus. Using a single probe assay an average of ≥ 6 HER-2 copies per cell/nucleus.

Participation

Number of laboratories registered for HER-2 BRISH	133
Number of laboratories returning slides	118 (89%)
Number of laboratories returning scoring sheet	110 (93%)
Number of laboratories registered for HER-2 FISH	61
Number of laboratories returning scoring sheet	55 (90%)

Results BRISH, technical assessment

In total, 118 laboratories participated in this assessment. 72 laboratories (61%) achieved a sufficient mark (optimal or good). Results are summarized in table 2.

Table 2**. HER-2 BRISH systems and assessment marks for BRISH HER-2 run H10.**

Two colour HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	88	Ventana	25	27	21	15	59%	67%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	12	Ventana	6	3	3	0	75%	82%
DuoCISH pharmDx™ **SK109**	4	Dako	3	0	0	1	-	-
Zyto*Dot*® 2C **C-3022 / C-3032**	5	ZytoVision	2	2	1	0	80%	80%
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	3	Ventana	1	0	2	0	-	-
Zyto*Dot*® **C-3003**	6	ZytoVision	1	2	1	2	50%	75%
Total	118		38	34	28	18	-	-
Proportion			32%	29%	24%	15%	61%	

1) Proportion of sufficient stains.
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Comments

In this assessment, optimal demonstration and evaluation of the HER-2 gene amplification status in all cores of the multi-tissue block could be obtained both by single and dual-colour systems as shown in table 2. Minor focal staining artefacts were accepted if they did not compromise the overall interpretation of each of the 5 individual tissue cores. Artefacts were silver precipitates, excessive background staining or negative areas most likely caused by technical issues as slides drying out during the staining process or inadequate washing etc. In this run and in concordance with the previous NordiQC runs, the ISH rejection criteria defined in the 2013 ASCO/CAP HER2 guidelines were applied. In brief, repeated test must be performed if more than 25% of the signals/cells cannot be interpreted due to the artefacts listed above. In these cases, the staining results were rated as insufficient (poor or borderline). For the most commonly used HER-2 BRISH assay, the INFORM™ HER-2 Dual ISH (Ventana), technical adequate result was thus provided in 67% of the laboratories using appropriate protocol settings being identified as essential for a technical optimal staining result.

Optimal protocol settings: Two-colour HER-2 systems

For the **INFORM™ Dual ISH system 800-4422** (Ventana), optimal demonstration of HER-2 BRISH was typically based on HIER in Cell Conditioning 2 (CC2) for 24-32 min. at 86-90°C and subsequent proteolysis in P3 for 8-20 min. at 36-37°C. The HER-2 and chr17 probe cocktail was typically applied for 6 hours at 44°C following denaturation at 80°C for 20 min.

Using these protocol settings, sufficient results (optimal or good) were seen in 67% of the submitted protocols (52 of 78). 26 laboratories used a protocol with optimal settings but, for unexplained reasons, completely false negative staining or excessive background staining (e.g. due to silver precipitates) were seen in the entire slide or in large areas comprising >25% of the neoplastic cells in one or more of the tissue cores. No reason for these insufficient results could be related to the applied protocols, reagents, platforms (BenchMark XT, GX or Ultra) or any other protocol parameter. This observation has been seen in the latest runs and might indicate a less robust and reproducible performance of the protocols on the used instruments.

The remaining insufficient results were characterized by impaired morphology hampering interpretation of the signals. This pattern was typically caused by excessive retrieval in e.g. P3 for 24–28 min. and/or

prolonged HIER and as a consequence the nuclei were almost totally digested and virtually no counterstaining could be seen.

12 laboratories used the **INFORM™ Dual ISH systems 800-4422** (Ventana) in combination with an immunohistochemical demonstration for **HER-2 PATHWAY®** (Ventana). An optimal demonstration of HER-2 BRISH using this assay was based on HIER in Cell Conditioning 2 (CC2) for 28-32 min. at 75-90°C and subsequent proteolysis in P2 for 8-20 min. at 36-37°C. The HER-2 and chr17 probe cocktail was typically applied for 6 hours at 44°C following a denaturation at 80°C for 4 min. HER-2 PATHWAY® was typically performed with iVIEW as detection system. Both BenchMark ULTRA and XT could be used as stainer platform.
Using these protocol settings, sufficient results were seen in 82% of the submitted protocols (9 of 11).

For the **DuoCISH™ system SK109** (Dako), protocol settings with optimal results were typically based on HIER in pre-treatment buffer in a waterbath or microwave oven for 10-15 min. at 95-100°C and subsequent proteolysis in pepsin for 2 min. at 37° C or 10 min. at RT (both reagents included in the HER2 DuoCISH pharmDX kit SK109). The HER-2 and chr17 probes were applied for 14–20 hours at 45°C and visualized by the detection reagents provided in the DuoCISH™ kit SK109.
Using these settings, sufficient (optimal) results were seen in 3 of 4 of the submitted protocols.

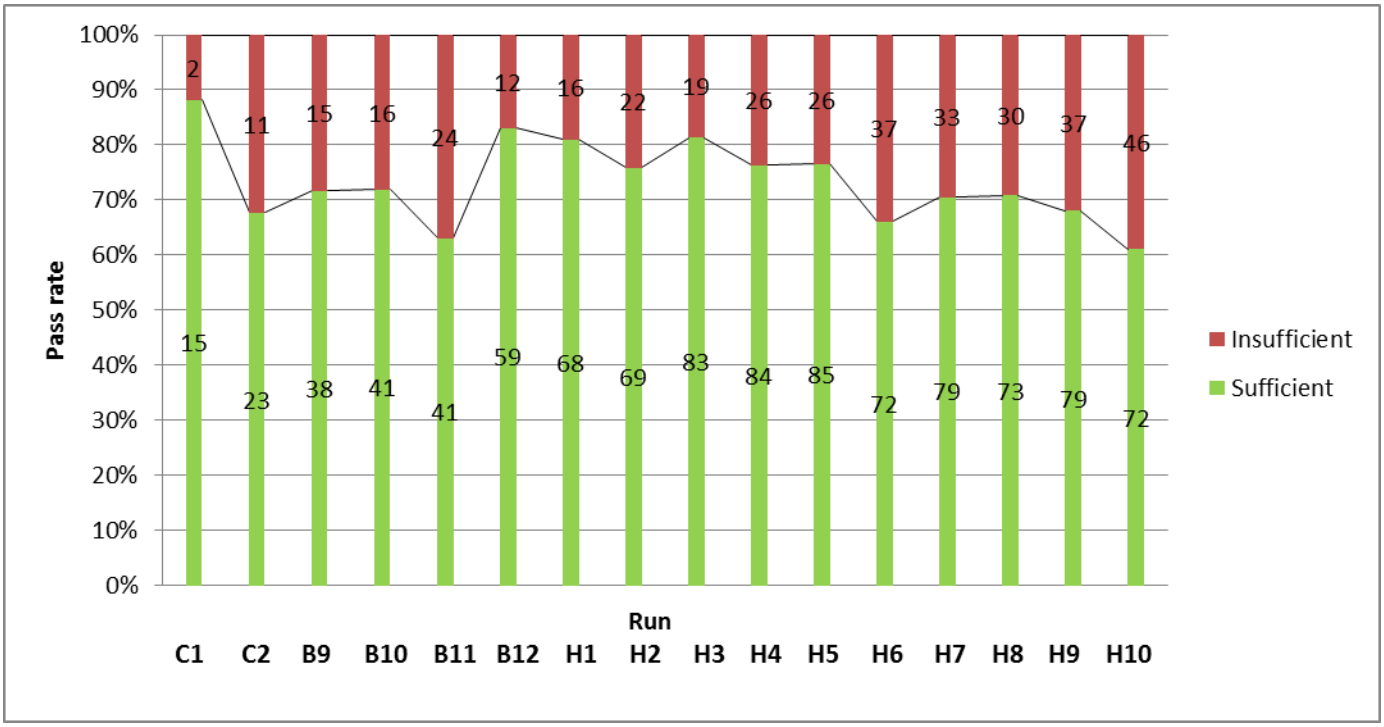
For the **Zyto*Dot*® 2C system C-3022 / C-3032** (ZytoVision), protocol settings with optimal results were typically based on HIER in EDTA pH 8 in a waterbath or hot plate for 15 min. at 95-98°C, proteolysis in pepsin for 2-5 min. at room temperature, hybridization at 37°C for 16-18 hours and visualization with the ZytoVision detection kit C-3022. Using these settings, sufficient results were seen in 80% of the submitted protocols (4 of 5).

One-colour HER-2 systems
One protocol using the **INFORM™ SISH system 780-4332** (Ventana) gave an optimal result.
The protocol was based on HIER in CC2 for 28 min. at 85°C and subsequent proteolysis in P3 for 8 min. at 36°C. The HER-2 SISH probe was applied for 6 hours at 50°C.
Using these or similar protocol settings, sufficient results were seen in 1 of 2 of the submitted protocols.

One protocol using the **Zyto*Dot*® CISH system C-3003** (ZytoVision) gave an optimal result.
The protocol was based on HIER in EDTA pH 8 in a waterbath for 15 min. at 100°C, proteolysis in pepsin for 2 min. at room temperature, hybridization at 37°C overnight and visualization with the ZytoVision detection kit C-3003. Using these or similar protocol settings, sufficient results were seen in 75% of the submitted protocols (3 of 4).

Performance history
This was the sixteenth assessment of HER-2 BRISH in NordiQC and a consistent pass rate at a relatively low level has been observed in the latest runs. Data is shown in Fig 1.

Fig. 1. **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**



HER-2 ISH interpretation and scoring consensus:

Table 3. **NordiQC FISH amplification data**

	NordiQC FISH HER2/chr17 ratio	NordiQC FISH HER2 copies	NordiQC HER-2 amplification status
1. Breast ductal carcinoma	0.8 – 0.9	< 4	Non-amplified
2. Breast ductal carcinoma	1.0 – 1.2	< 4, ≥ 4 < 6	Non-amplified / Equivocal
3. Breast ductal carcinoma	1.3 – 1.5	< 4, ≥ 4 < 6	Non-amplified / Equivocal
4. Breast ductal carcinoma	2.1 – 2.9	> 6	Amplified
5. Breast ductal carcinoma	4.2 – 6.4	> 6	Amplified

* data from 2 different NordiQC reference laboratories.

Scoring sheets were completed by 165 of the 179 participating laboratories. These evaluations were compared to the HER-2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in Figs. 2 and 3. For laboratories performing FISH, the consensus rate was 80% (44 of 55 laboratories) and 78% (86 of 110 laboratories) for laboratories using BRISH.

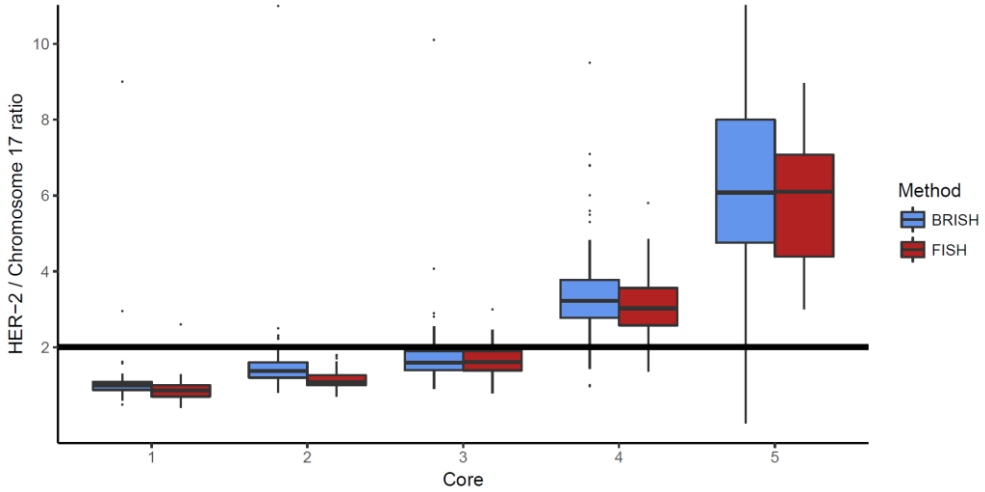
In general, for both BRISH and FISH, high consensus rates were observed between participants and NordiQC regarding the HER-2 amplification status in the breast carcinomas tissue cores no. 1, 4 and 5. The interpretation of HER-2 amplification status was more challenging for the tissue core no. 2 and 3.
For both BRISH and FISH, disagreement of the interpretation of the HER-2 amplification status between the participants and NordiQC data were related to "overrating" of the HER-2 status and thus classification of an aberrant positive result compared to the NordiQC reference data and the majority of other participants.
Tumour no. 2 was by the NordiQC reference laboratories classified as non-amplified with a HER-2 ratio of 1.0 – 1.2, but simultaneously showed polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies was identified. Consequently, it was accepted to classify the tumour as both as non-amplified and equivocal using the ASCO/CAP 2013 guidelines. This tumour was, by some laboratories using BRISH classified as amplified.
Tumour no. 3 was by the NordiQC laboratories characterized to be non-amplified or equivocal. The tumour showed HER-2 ratio in the range of 1.3 – 1.5 and in areas a level of ≥ 4 but less < 6 HER-2 gene copies

was identified. This tumour was, both by laboratories using BRISH and FISH, frequently classified as amplified.

Participants using FISH tended to have marginally higher level of consensus than participants using BRISH. In general one could speculate that the technical quality of the BRISH staining reaction, as excessive background staining, inadequate counterstaining, chromogene or silver precipitates would compromise the interpretation. However, it was observed that the consensus rates of laboratories producing results assessed as technically sufficient (optimal and good) and insufficient (borderline and poor) were almost identical (78% and 80%, respectively). This was most likely caused by the ISH rejection criteria applied by NordiQC in the assessment. The criteria (defined in the 2013 ASCO/CAP HER2 guidelines) require retest, if more than 25% of the signals/cells cannot be interpreted due to artefacts such as silver precipates, excessive background or negative areas. The material in the assessment consisted of breast tumours with relatively homogenous HER2 expression which permitted correct evaluation even in slides with large negative areas. This is not always the scenario in diagnostic settings with heterogeneous tumours or evaluation in specific "hot-spot areas" identified by HER2 IHC.

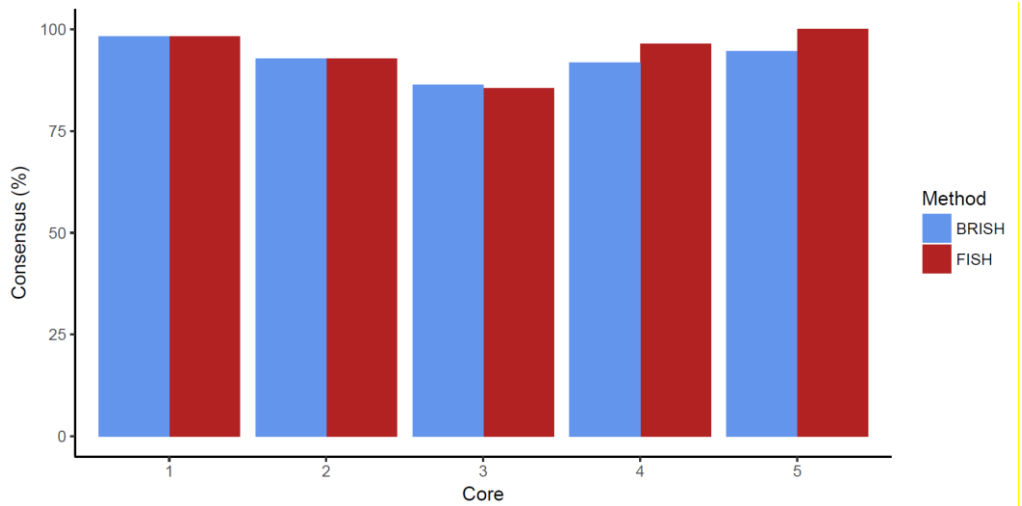
Participants overall interpretation of amplification ratios and consensus rates are shown in Figs. 2 and 3.

Fig. 2



NordiQC HER-2 ISH run H10: participants interpretation of amplification status

Fig. 3



NordiQC HER-2 ISH run H10: consensus between participants and NordiQC

No technical evaluation of FISH protocols was performed. Table 4 shows the FISH assay used by the participants and concordance level to the NordiQC data observed. In this matter it has to be emphasized that it was not possible to identify the cause of an aberrant interpretation of the HER-2 status whether this was related to the technical performance of the FISH assay or the interpretation by the observer(s).

Table 4. **FISH assays used and level of consensus HER-2 status to NordiQC reference data**

	Number	Consensus rate
Dako, K5731	13	77% (10/13)
Dako, GM333	2	100% (2/2)
Leica, TA9217	3	100% (3/3)
Pathvysion/Abbot, 6N4630 / 30-161060	16	81% (13/16)
ZytoVision, Z2015 / Z2020/ Z2077	11	73% (8/11)
Other	10	80% (8/10)

Conclusion

In this assessment and in concordance with previous NordiQC HER-2 ISH runs, technical optimal demonstration of HER-2 BRISH could be obtained by the commercially available two-colour HER-2 systems **INFORM™ HER-2 Dual ISH** (Ventana), **DuoCISH™**(Dako) and **ZytoDot® 2C** (ZytoVision).
The single-colour HER-2 systems **INFORM™ HER-2 ISH** (Ventana) and **ZytoDot®** (ZytoVision) could also be used to produce a technical optimal HER-2 demonstration.
For all systems, retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient demonstration of HER-2 (and chr17 signals) and preserved morphology.
Despite optimal protocol settings being applied, a high proportion of technical insufficient results were seen, indicating that other issues are influencing the quality of the BRISH assays. Especially the capability of present instrumentation to provide reproducible performance of the protocols might be a central factor.
It was thus observed that the most commonly used HER-2 BRISH assay, INFORM™ HER-2 Dual ISH (Ventana), only provided a pass rate of 61% and a technical adequate result despite using appropriate and well characterized protocol settings.
Attention must be paid to interpretation in order to obtain correct HER-2 amplification status.
Laboratories performing FISH achieved a slightly higher consensus rate for the interpretation of HER-2 amplification status compared to laboratories performing BRISH.

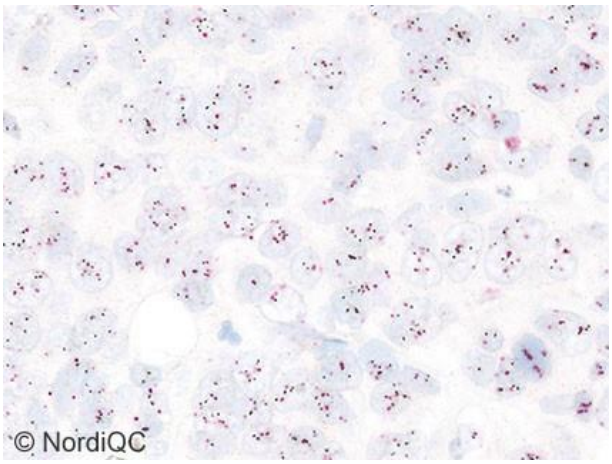


Fig. 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 2 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.0 – 1.2*.
The HER-2 genes are stained black and chr. 17 red.
The signals are distinctively demonstrated.
Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies is identified.
NordiQC and the vast majority of participants interpreted this tumour as negative or equivocal.

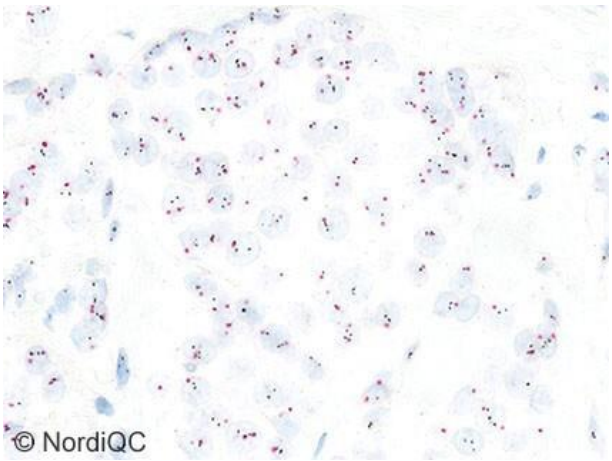


Fig. 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 3 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.3 - 1.5*.
The HER-2 genes are stained black and chr. 17 red.
The HER-2 signals are distinctively demonstrated.
NordiQC and the vast majority of participants interpreted this tumour as negative or equivocal.

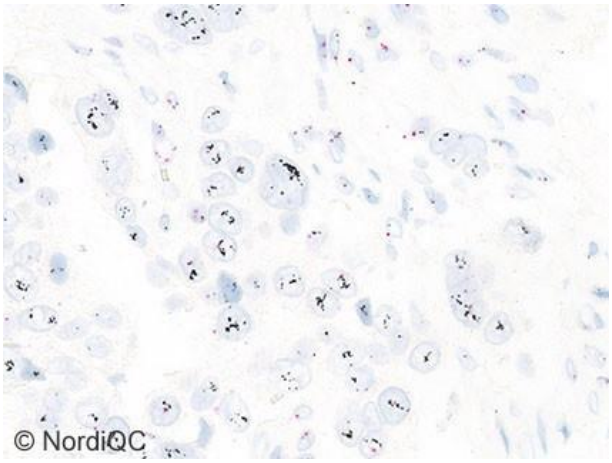


Fig. 2a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 5 with high level HER-2 gene amplification: HER-2/chr. 17 ratio > 4.2 – 6.4*.
The HER-2 genes are stained black and chr. 17 red.
The signals are distinctively demonstrated and many HER2 signals are located in large clusters.
NordiQC and virtually all participants interpreted this tumour as positive, highly amplified.

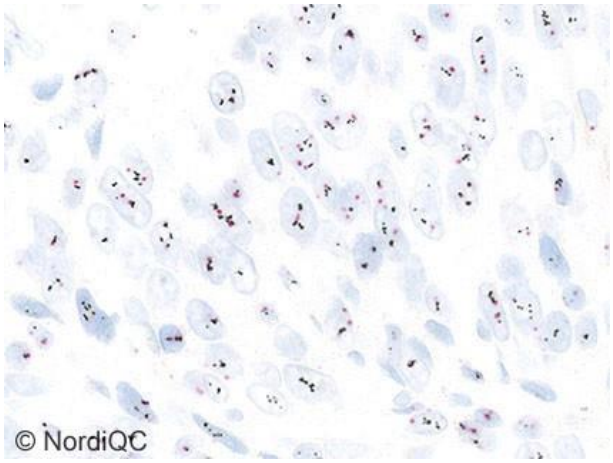


Fig. 2b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 4 with low level HER-2 gene amplification: HER-2/chr. 17 ratio > 2.1 – 2.9*.
The HER-2 genes are stained black and chr. 17 red.
The signals are distinctively demonstrated and some HER2 signals are located in small clusters.
NordiQC and the vast majority all participants interpreted this tumour as positive, low amplified.

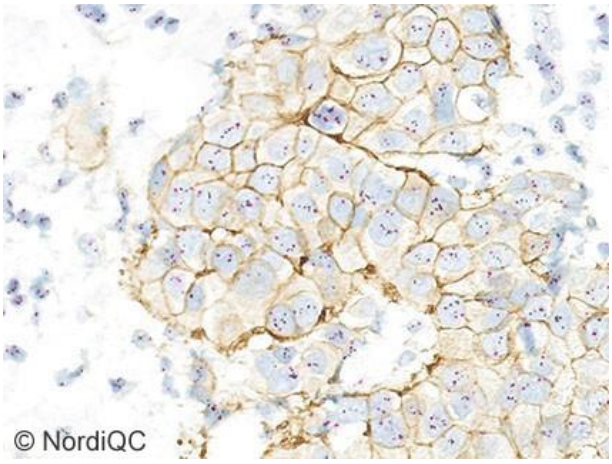


Fig. 3a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, in combination with HER2 IHC using PATHWAY, Ventana, of the breast carcinoma no. 3 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.3 – 1.5*.
The gene protein assay (GPA) label the HER-2 genes black, chr. 17 red and HER-2 protein brown.
The IHC level is interpreted as 2+ and the GPA assay visualizes IHC hot-spots to evaluate the HER-2 gene status precisely.
The participant interpreted this tumour as negative.

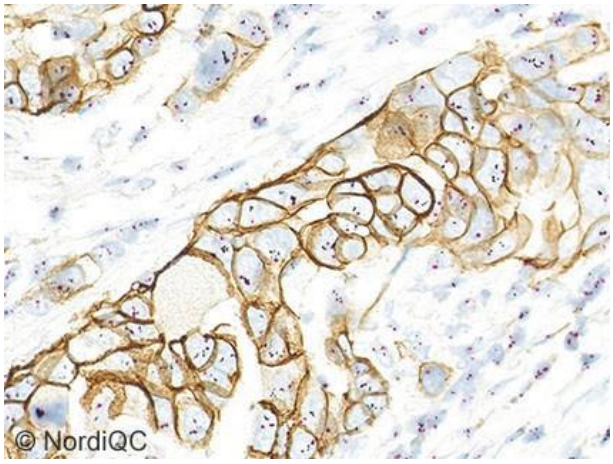


Fig. 3b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, in combination with HER2 IHC using PATHWAY, Ventana, of the breast carcinoma no. 4 with low level HER-2 gene amplification: HER-2/chr. 17 ratio > 2.1 – 2.9*.
The gene protein assay (GPA) label the HER-2 genes black, chr. 17 red and HER-2 protein brown.
The IHC level is interpreted as 3+ and the GPA assay visualizes the HER2 IHC overexpression and the HER-2 gene status simultanously.
The participant interpreted this tumour as positive, low amplified.

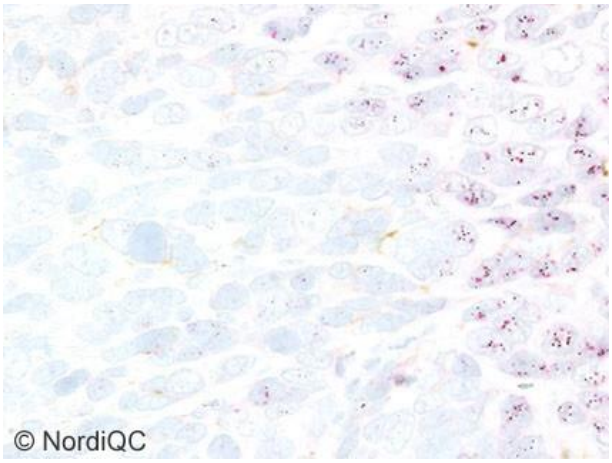


Fig. 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 3 without gene amplification: HER-2/chr. 17 ratio 1.2 – 1.5*.
The vast majority of the neoplastic cells are negative and only in scattered cells HER-2 and chr. 17 signals can be identified. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1 and 2.
Negative areas of < 25% in each of the tissue cores were accepted.
The laboratory reported the result as technically insufficient and new test required.

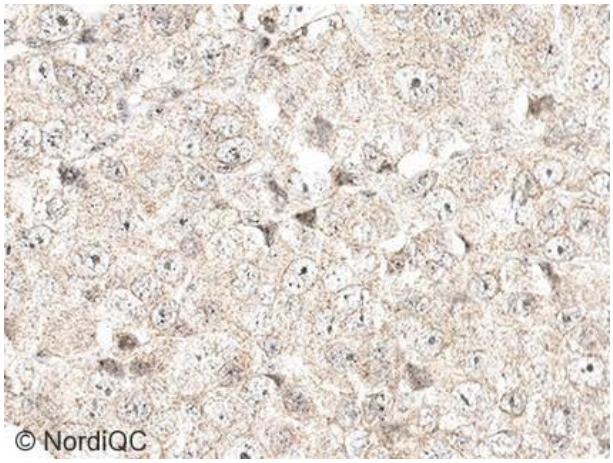


Fig. 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana, of the breast carcinoma no. 3 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.2 – 1.5*.
Due to extensive silver precipitates the HER-2 gene status cannot reliably be interpreted. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1 and 2.
The laboratory reported the result as technically insufficient and new test required.

SN/LE/MV/RR 06.12.2016