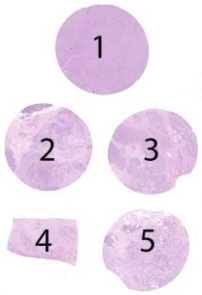
Assessment Run H9 2016

HER-2 ISH

Material

Table 1. **Content of the multi-tissue block used for the NordiQC HER-2 ISH assessment, run H9**

	HER-2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	0	0.8	0.9 – 1.0	< 4
2. Breast carcinoma	2+	1.1	1.0 – 1.3	< 4, ≥ 4 < 6
3. Breast carcinoma	2+	1.5	1.3 – 1.9	< 4, ≥ 4 < 6
4. Breast carcinoma	2+	2.3	2.2 – 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 one 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.

The staining reaction 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.

The staining reaction 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.

The staining reaction 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	126
Number of laboratories returning slides	116 (90%)
Number of laboratories returning scoring sheet	104 (92%)
Number of laboratories registered for HER-2 FISH	61
Number of laboratories returning scoring sheet	54 (89%)

Results BRISH, technical assessment

In total, 116 laboratories participated in this assessment. 79 laboratories (68%) achieved a sufficient mark (optimal or good). Results are summarized in Table 2.

Table 2**. HER-2 BRISH assays and assessment marks for BRISH HER-2 run H9.**

Two colour HER-2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	86	Ventana	32	22	17	15	63%	73%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	7	Ventana	5	1	1	0	86%	86%
DuoCISH pharmDx™ **SK109**	6	Dako	3	3	0	0	100%	100%
Zyto*Dot*® 2C **C-3022 / C-3032**	6	ZytoVision	4	1	0	1	83%	100%
One colour HER-2 assays								
INFORM™ HER-2 SISH **780-4332**	5	Ventana	3	2	0	0	100%	100%
Zyto*Dot*® **C-3003**	6	ZytoVision	3	0	2	1	50%	75%
Total	116		50	29	20	17	68%	-
Proportion								

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 multitissue 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 such as slides drying out during the staining process or inadequate washing etc. In this run, 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 artefacts. In these cases, the staining result was 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 73% using appropriate protocol settings being identified as essential for a technical optimal staining result.

Optimal protocol settings: Two-colour HER-2 assays

For the **INFORM™ Dual ISH assay 800-4422** (Ventana), optimal demonstration of HER-2 BRISH typically was based on HIER in Cell Conditioning 2 (CC2) for 24-32 min. at 86-90°C and subsequent proteolysis in P3 for 8 - 16 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 20 min.

Using these protocol settings, sufficient results (optimal or good) were seen in 73% of the submitted protocols (50 of 69). 19 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 of 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. This pattern was typically caused by excessive retrieval and as a consequence the nuclei were almost totally digested and virtually no counterstaining could be seen.

Seven laboratories used the **INFORM™ Dual ISH assay 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 16-32 min. at 75-90°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 a denaturation at 80°C for 4-20 min. HER-2 PATHWAY® was 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 86% of the submitted protocols (6 of 7).

For the **DuoCISH™ assay 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 2-3 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 protocol settings, sufficient results were seen in 100% of the submitted protocols (6 of 6).

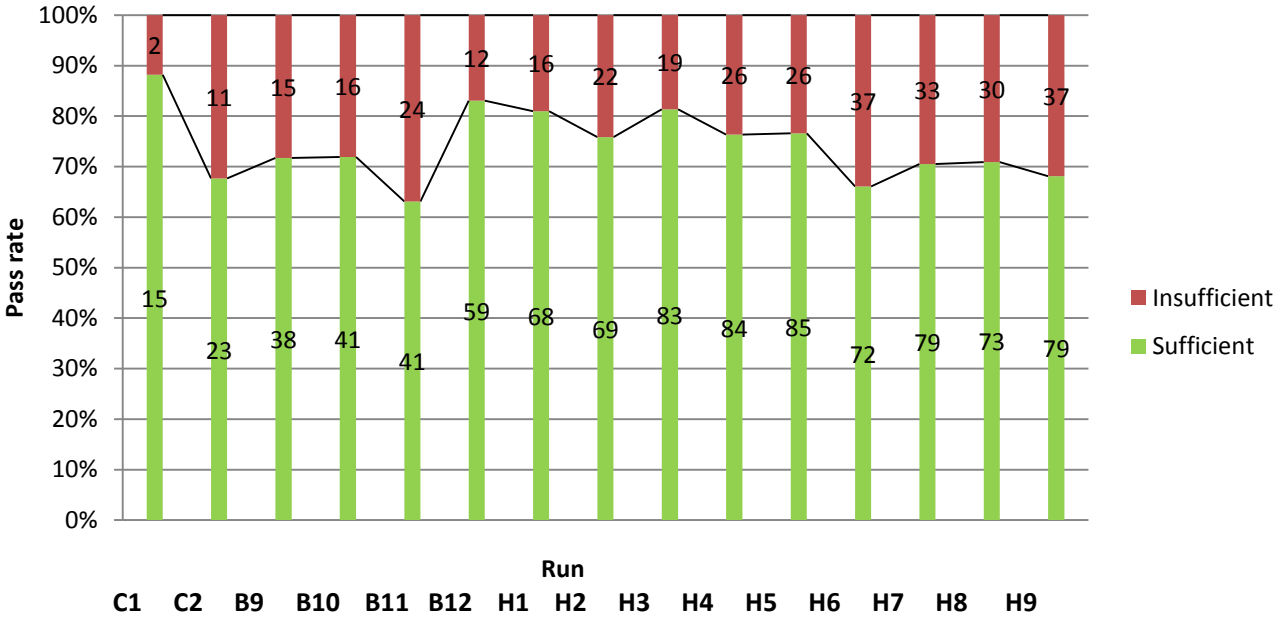
For the **Zyto*Dot*® 2C assay C-3022 / C-3032** (ZytoVision), protocol settings with optimal results were typically based on HIER in EDTA pH 8 in a waterbath or hot plate for 15 min. at 95-98°C, proteolysis in pepsin for 2-5 min at room temperature, hybridization at 37°C for 16-18 hours and visualization with the ZytoVision detection kit C-3022. Using these settings, sufficient results were seen in 100% of the submitted protocols (5 of 5).

One-colour HER-2 assays
For the **INFORM™ SISH assay 780-4332** (Ventana), protocol settings with optimal results were typically based on HIER in CC2 for 28 - 36 min. at 86-93°C and subsequent proteolysis in P3 for 8-12 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 100% of the submitted protocols (4 of 4).

For the **Zyto*Dot*® CISH assay C-3003**, ZytoVision, protocol settings with optimal results were typically based on HIER in EDTA pH 8 in a waterbath for 15 min. at 95-98°C, proteolysis in pepsin for 2-4 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 15th assessment of HER-2 BRISH in NordiQC and a relatively consistent pass rate 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	0.9 – 1.0	< 4	Non-amplified
2. Breast ductal carcinoma	1.0 – 1.3	< 4, ≥ 4 < 6	Non-amplified / Equivocal
3. Breast ductal carcinoma	1.3 – 1.9	< 4, ≥ 4 < 6	Non-amplified / Equivocal
4. Breast ductal carcinoma	2.2 – 2.9	> 6	Amplified
5. Breast ductal carcinoma	4.2 – 6.4	> 6	Amplified

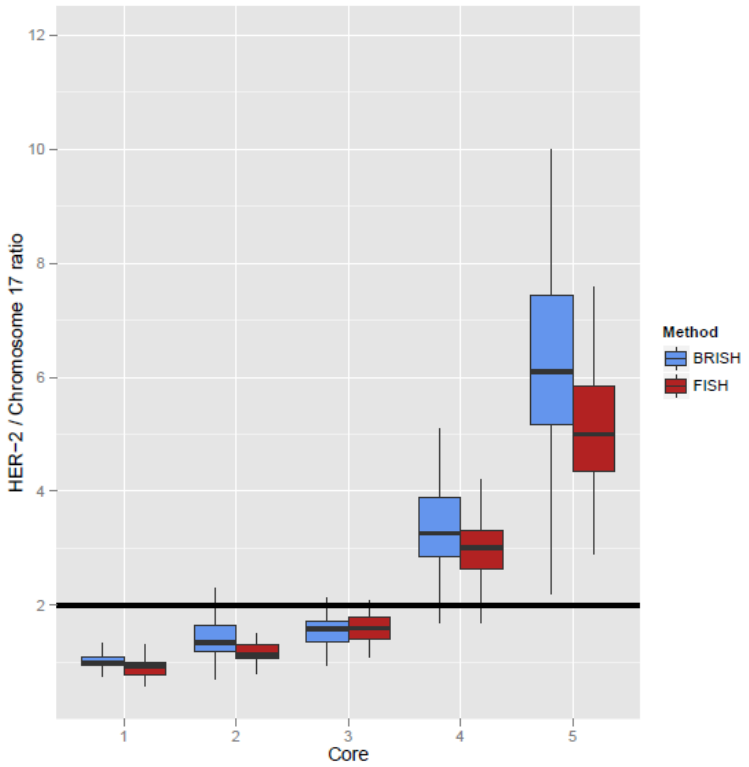
* data from 2 different NordiQC reference laboratories.

Scoring sheets were completed by 158 of the 177 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 85% (46 of 54 laboratories) and 77% for the laboratories using BRISH (80 of 104 laboratories).

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 tissue cores no. 2 and 3. Tumour no. 2 was by the NordiQC reference laboratories classified as non-amplified with a HER-2 ratio of 1.0 – 1.3, 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, in particular by laboratories using BRISH frequently 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 – 1.9 and in areas a level of ≥ 4 but less < 6 HER-2 gene copies was identified.
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. This was, as mentioned above, more frequently observed for laboratories performing BRISH compared to FISH and most pronounced in tumour core no 2.
The reduced precision of the interpretation of the HER-2 status using BRISH compared to FISH was in part shown to be related to the technical quality of the protocol applied. For the results assessed as technically sufficient (optimal and good), a consensus rate for the interpretation of 80% was seen, whereas a consensus rate of 68% was seen for results assessed as insufficient (borderline and poor). Excessive background staining, inadequate counterstaining, chromogene or silver precipitates most likely compromised the interpretation.

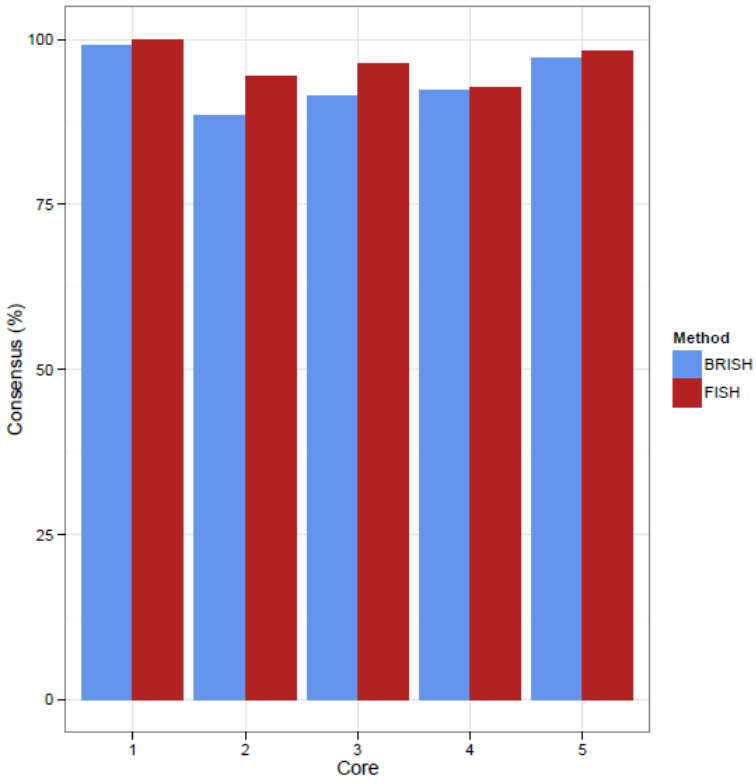
Participants overall interpretation of amplification ratios and consensus rates are shown in Figs. 2 and 3.

Fig. 2



NordiQC HER-2 ISH run H9: participants interpretation of amplification status

Fig. 3



NordiQC HER-2 ISH run H9: 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, K5331	2	50%
Dako, K5731	10	80%
Dako, GM333	3	100%
Leica, TA9217	4	100%
Kreatech, KBI 10701	3	100%
Pathvysion/Abbot, 6N4630 / 30-161060	17	94%
ZytoVision, Z2015 / Z2020/ Z2077	11	82%
Other	4	75%

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 technique to provide a reproducible performance of the protocols might be a central factor. 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.

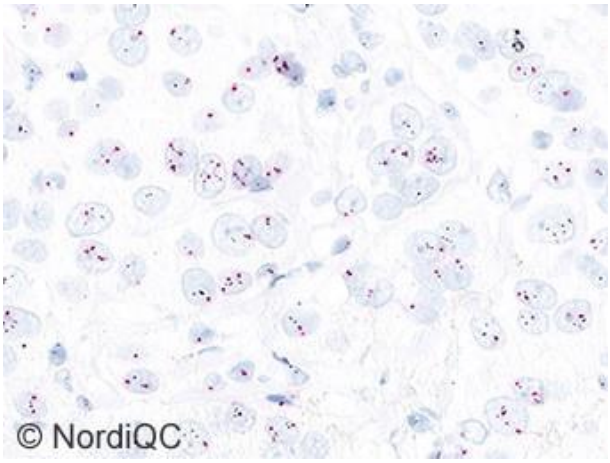


Fig. 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 3 without HER-2 gene amplification:
HER-2/chr. 17 ratio 1.3 – 1.9*.
The HER-2 genes are stained black and chr. 17 red.
The signals are distinctively demonstrated.
Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies is identified.

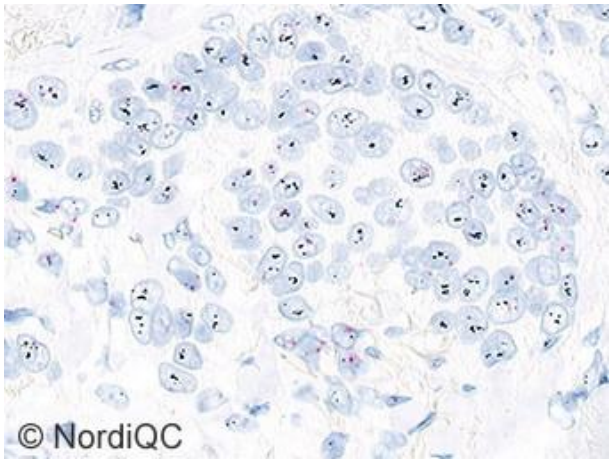


Fig. 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 5 with HER-2 gene amplification:
HER-2/chr. 17 ratio >6.
The HER-2 genes are stained black and chr. 17 red.
The HER-2 signals are mostly located in clusters and distinctively demonstrated.

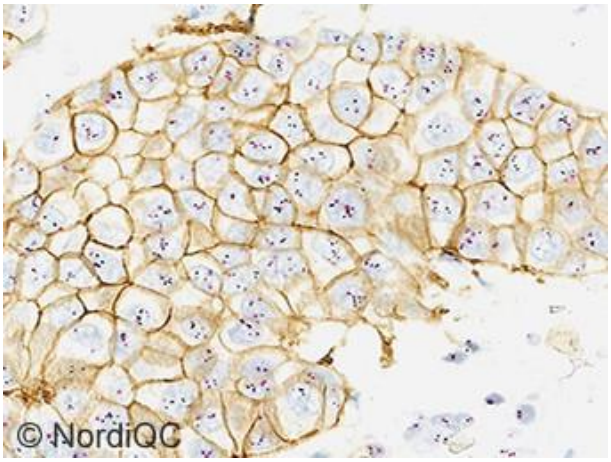


Fig. 2a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana in combination with HER2 IHC using PATHWAY, Ventana of the breast carcinoma no. 3 without HER-2 gene amplification:
HER-2/chr. 17 ratio 1.3 – 1.9*.
The gene protein assay (GPA) label the HER-2 genes black, chr. 17 red and HER-2 protein brown.
The IHC level is interpreted as 2+ and the GPA assay visualizes IHC hot-spots to evaluate the HER-2 gene status precisely.

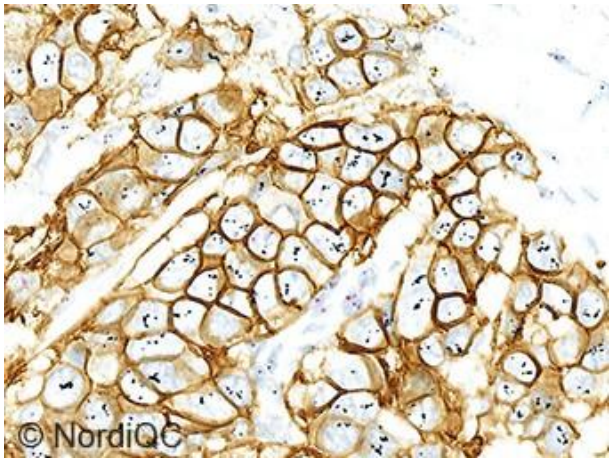


Fig. 2b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana in combination with HER2 IHC using PATHWAY, Ventana of the breast carcinoma no. 5 with HER-2 gene amplification.
The HER-2 genes are stained black, chr. 17 red and HER-2 protein brown.
The IHC level is interpreted as 3+ and the GPA assay visualizes the HER-2 genes in clusters.

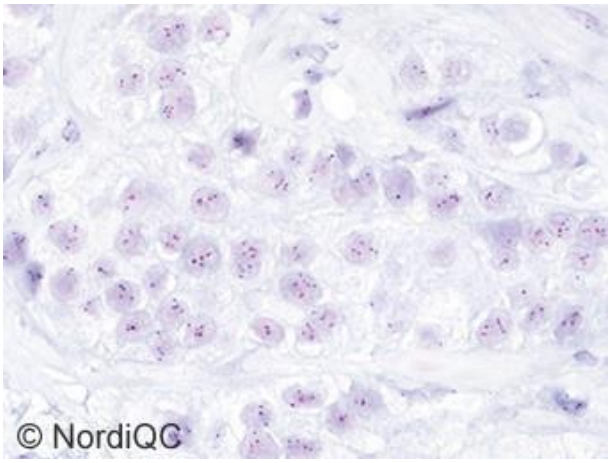


Fig. 3a
Optimal demonstration of the HER-2 gene status using the DuoCISH pharmDx™ kit cat. no. SK109, Dako of the breast carcinoma no. 3 without gene amplification:
HER-2/chr. 17 ratio 1.3 – 1.9.
The HER-2 genes are stained red and chr. 17 blue.
Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies is identified.

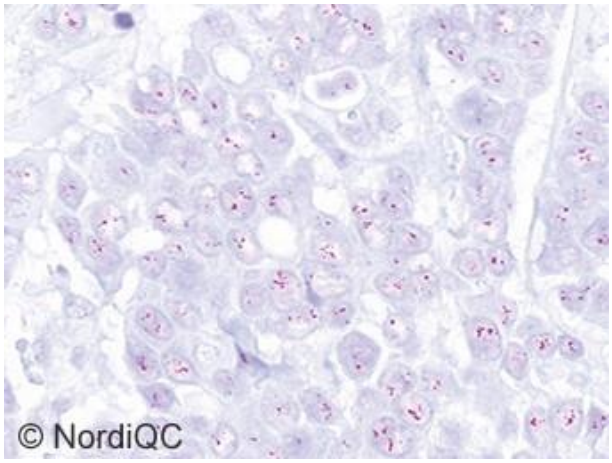


Fig. 3b
Optimal demonstration of the HER-2 gene status using the DuoCISH pharmDx™ kit cat. no. SK109, Dako of the breast carcinoma no. 2 with gene amplification:
HER-2/chr. 17 ratio >6.
The HER-2 genes are stained red and chr. 17 blue.
The HER-2 signals are mostly located in clusters and distinctively demonstrated.

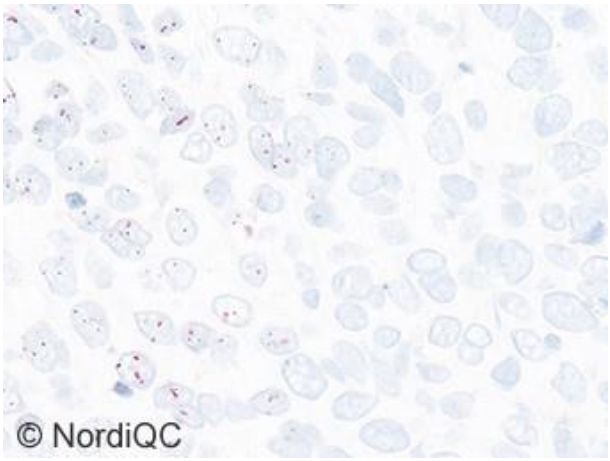


Fig. 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 0.9 – 1.0*.
The vast majority of the neoplastic cells are negative and only in scattered cells HER-2 and chr. 17 signals 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. 1a and 1b.
Negative areas of < 25% in each of the tissue cores were accepted.

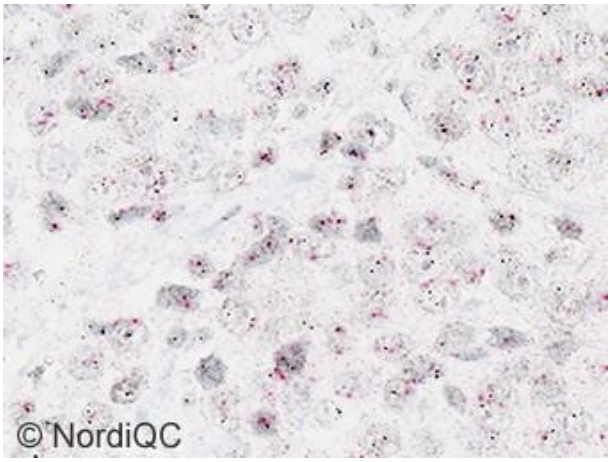


Fig. 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 3 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.3 – 1.9*.
Due to extensive silver precipitates the HER-2 gene status cannot reliably be interpreted. This aberrant 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. 1a and 1b.
The laboratory reported the result as technically insufficient and new test required.

SN/LE/MV/RR 18.04.2016