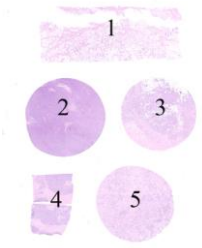
Assessment Run H5 2014

HER-2 ISH (BRISH or FISH)

Material

Table 1. **Content of the multi-block used for the NordiQC HER-2 ISH assessment, run H5**

	HER-2 IHC*	Dual - SISH**	FISH***
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17ratio¤**
1. Breast carcinoma	1+	1,0 – 1,2	1,0 – 1,2
2. Breast carcinoma	0	0,9 – 1,1	1,0 – 1,3
3. Breast carcinoma	3+	3,1 – 3,9	6,8 – 7,6
4. Breast carcinoma	2+	1,5 – 1,8	1,5 – 2,0
5. Breast carcinoma	2+	1,4 – 1,6	1,3 – 1,5



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

All tissues were fixed for 24 - 48 hours in 10% neutral buffered formalin according to the ASCO/CAP 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** was 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.

Tissue core slides no.1 and no. 3 were in many cases partially detached from the slides. This was most likely caused by inadequate adhesion of the tissues to the slides. The laboratories' technical assessment was not influenced or down-marked due to this issue.

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 carcinomas, no. 1, 2 and 5: non-amplified
- Breast ductal carcinoma no. 3: (highly) amplified
- Breast ductal carcinomas no. 4: non-amplified or equivocal

The most recent ASCO/CAP guidelines (2013) 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.

Results BRISH, technical assessment
In total 152 laboratories participated in this assessment. 111 laboratories performed BRISH and of these 85 (77%) achieved a sufficient mark. Results are summarized in Table 2.

Table 2**. Systems and assessment marks for BRISH HER-2**

Two colour HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422, 780-4422**	63	Ventana	34	15	12	2	78%	82%
INFORM™ HER-2 Dual ISH **780-4332+780-4331**	6	Ventana	1	4	1	0	83%	-
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	3	Ventana	3	0	0	0	-	-
DuoCISH pharmDx™ **SK109**	15	Dako	5	4	3	3	60%	67%
Zyto*Dot*® 2C **C-3022 / C-3023**	5	ZytoVision	3	2	0	0	100%	100%
Other	1		1	0	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	15	Ventana	7	4	1	3	73%	77%
Zyto*Dot*® **C-3003**	3	ZytoVision	1	1	1	0	-	-
Total	111		55	30	18	8	77%	-
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 HER-2 gene amplification status in all cores of the multitissue block could be obtained by all the different BRISH systems used by the participants. 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 caused by drying out during the staining process. 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 was rated as insufficient (poor or borderline).

Optimal protocol settings: Two-colour HER-2 systems
For the **INFORM™ Dual ISH systems 800-4422, 780-4422, 780-4332+780-4331** (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 - 16 min at 36-37˚C. The HER-2 SISH probe was typically applied for 6 hours at 50-52˚C, while the chr17 probe was applied for 2 hours at 42 - 44˚C. Using these protocol settings, sufficient results (optimal or good) were seen in 82% of the submitted protocols (49 of 60). 11 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. No reason for these insufficient results could be related to the protocol applied, reagents, system (being BenchMark XT, GX or Ultra) or any other parameters used by the participants.
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.

For the **DuoCISH™ system SK109** (Dako), protocol settings with optimal results were based on HIER in pre-treatment buffer in a waterbath for 10-20 min at 95 - 98°C or 10 min in a microwave oven and subsequently proteolysis in pepsin for 1-3 min at 37°C (both reagents included in the HER2 DuoCISH pharmDX kit SK109). The HER-2 and the 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 67% of the submitted protocols (6 of 9 laboratories). The insufficient result was characterized by a too weak staining of the HER-2 signals in both

neoplastic cells and normal stromal cells. No reason for the aberrant staining result could be identified from the submitted protocols.

For the **Zyto*Dot*® 2C system C-3022, C-3023** (ZytoVision) optimal results were obtained with proteolysis in pepsin for 2-5 min (room temperature or at 37°C), HIER in EDTA for 15 min at 98°C, hybridization at 37°C for 16-20 hours and visualization with the ZytoVision detection kit C-3022 or C-3044. Using these protocol settings, sufficient results were seen in 100% of the submitted protocols (5 of 5).

One-colour HER-2 systems

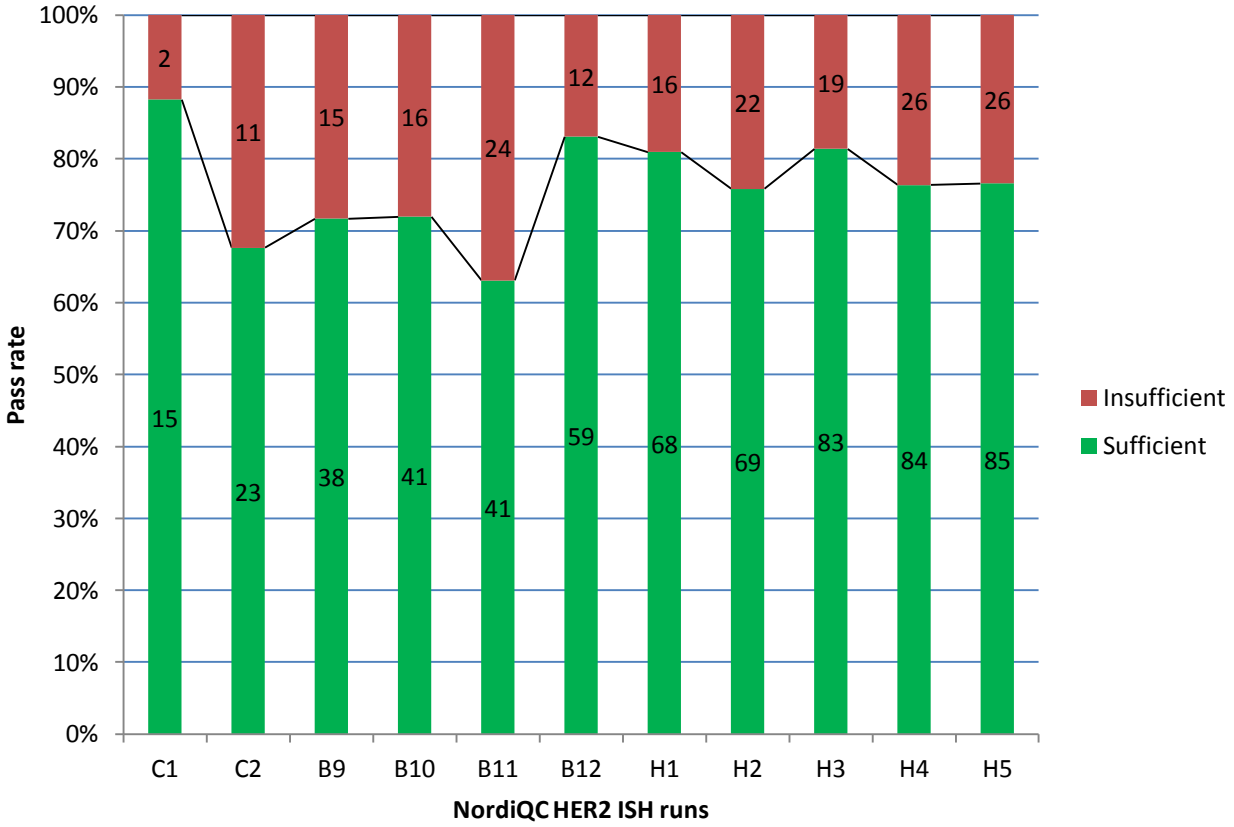
For the **INFORM™ HER-2 SISH 780-4332** (Ventana), optimal results were typically based on HIER in CC2 or Reaction buffer (RB) for 24-32 min at 90-95°C and proteolysis in P3 for 4 – 12 min at 37°C. The HER-2 SISH probe was typically applied for 6 hours at 50-52°C. Using these protocol settings sufficient results were seen in 77% of the submitted protocols (10 of 13).

For the **Zyto*Dot*® CISH system C-3003**, ZytoVision, optimal results were obtained with proteolysis in pepsin for 1½ min at room temperature, HIER in EDTA for 10 min at 96°C, hybridization at 37°C for 17 hours and visualization with the ZytoVision detection kit C-3003. Using these or similar protocol settings sufficient results were seen in 67% of the submitted protocols (2 of 3).

Performance history

This was the 11th assessment of HER-2 BRISH in NordiQC and a relatively consistent pass rate in the range of 76 – 83% has been obtained in the last 6 runs. Data is shown in table 3.

Table 3. **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**



HER-2 interpretation and scoring consensus:

Table 4. **NordiQC FISH amplification data**

	NordiQC FISH HER2/chr17 ratio	**NordiQC HER-2 amplification status**
1. Breast ductal carcinoma	1,0 – 1,2	Non-amplified
2. Breast ductal carcinoma	1,0 – 1,3	Non-amplified
3. Breast ductal carcinoma	6,8 – 7,6	Amplified
4. Breast ductal carcinoma	1,5 – 2,0*	Non-amplified / Equivocal
5. Breast ductal carcinoma	1,3 – 1,5	Non-amplified

*HER-2 gene copies in areas evaluated as ≥ 4 and < 6 per cell/nucleus.

Scoring sheets were completed by 142 of the 152 participating laboratories. These evaluations were compared to the HER-2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in Figs. 1 and 2. A slight difference regarding the concordance rate and evaluation of the HER-2 status was seen between the participants using FISH or BRISH. For laboratories performing FISH, the general consensus rate was 93% (38 of 41 laboratories) compared to 77% for the laboratories using BRISH (78 of 101 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, 2 and 3. The interpretation of HER-2 amplification status was more challenging for tissue core no. 4. This tumour was by the NordiQC reference laboratories evaluated as non-amplified or equivocal showing a ratio of 1,5 – 2,0 by FISH and 1,5 – 1,8 by BRISH. The number of HER-2 gene copies was encountered to be ≥ 4 and < 6 per cell/nucleus.
For laboratories performing FISH a general consensus rate of the interpretation in all five tumours compared to the NordiQC data of 92% was seen (38 of 41 laboratories). For laboratories performing BRISH a consensus rate of 84% was seen (84 of 101 laboratories).
The overall interpretation of amplification ratios and consensus rates of the participants are shown in table 5 and 6 (see page 5).

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 SISH (Ventana) and ZytoDot® (ZytoVision) could also be used to produce optimal HER-2 demonstration.
Retrieval settings – HIER and proteolysis - must be carefully balanced to provide a sufficient demonstration of HER-2 (and chr17 signals) and preserved morphology.
Despite optimal protocol settings being applied technical insufficient results could still be seen, indicating other issues influencing the quality of the BRISH assays.
Attention must be paid to interpretation in order to obtain correct HER-2 amplification status. Laboratories performing FISH obtained a slightly higher consensus rate to the NordiQC reference data compared to laboratories performing BRISH.

Table 5

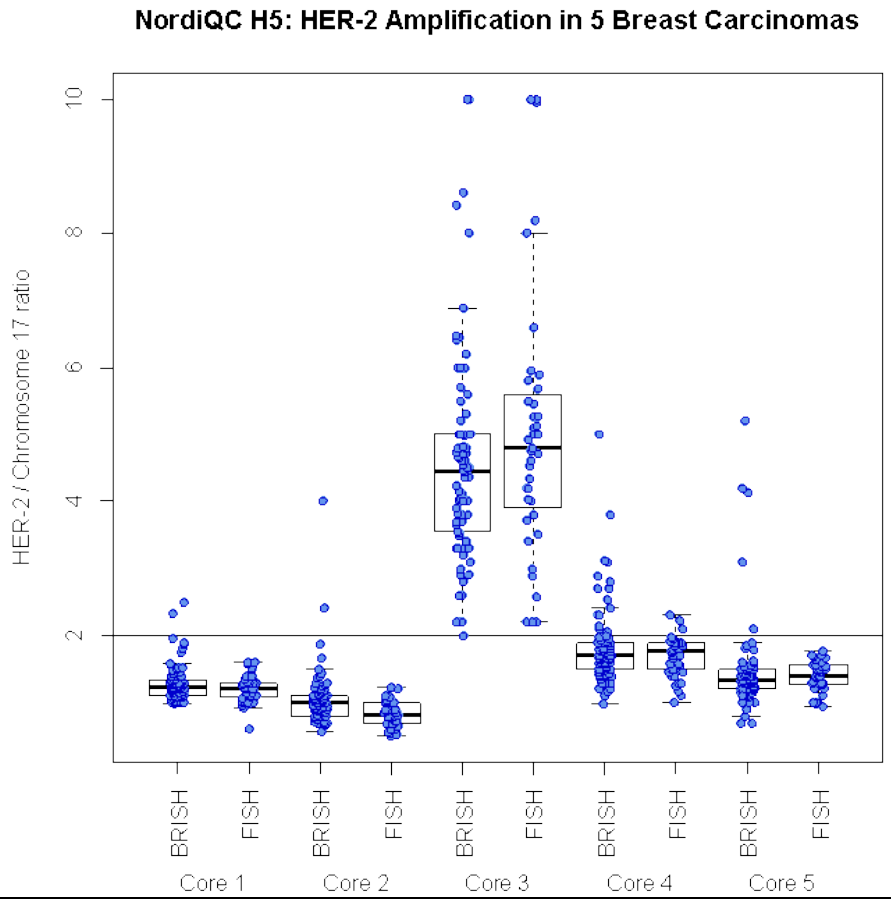
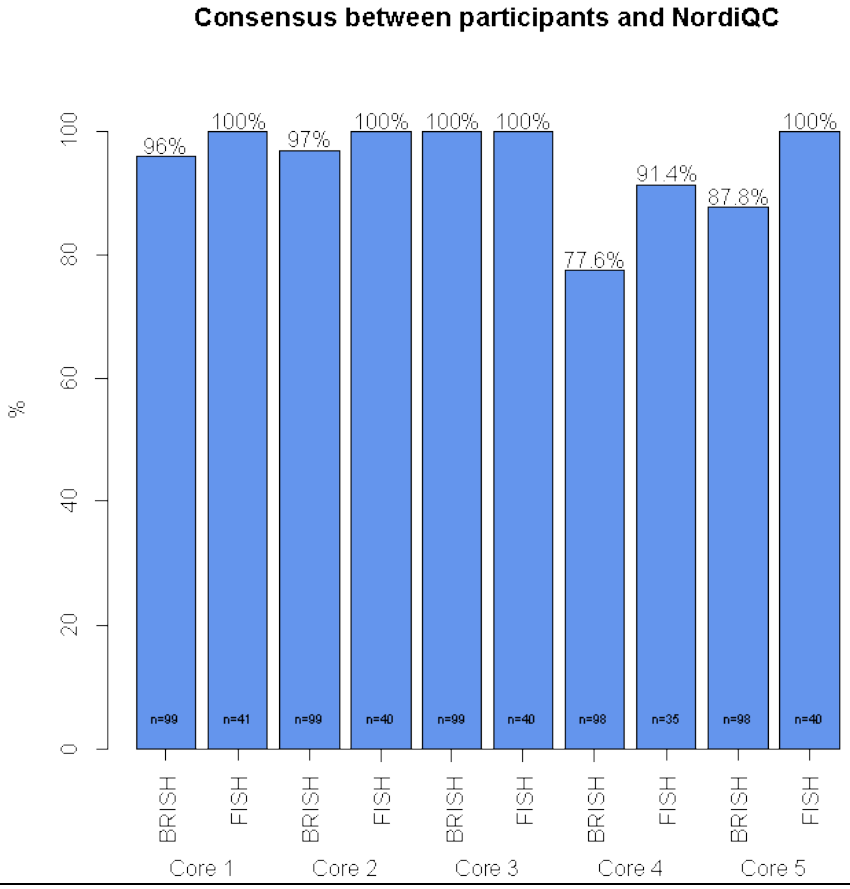


Table 6



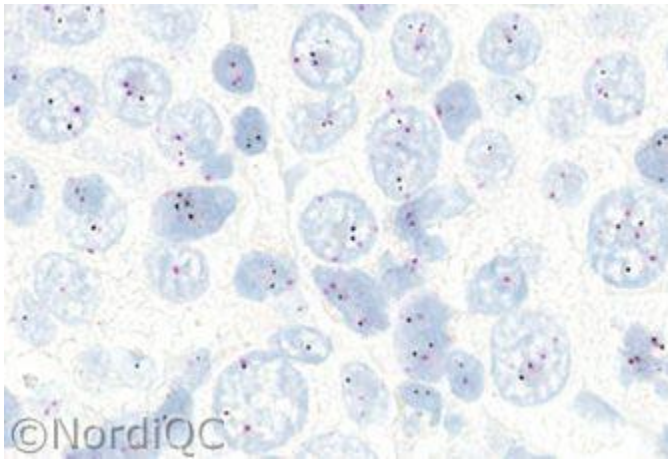


Fig. 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 2 without gene amplification: HER-2/chr17 ratio 1,0 – 1,3*. The HER-2 genes are stained black and chr17 red.

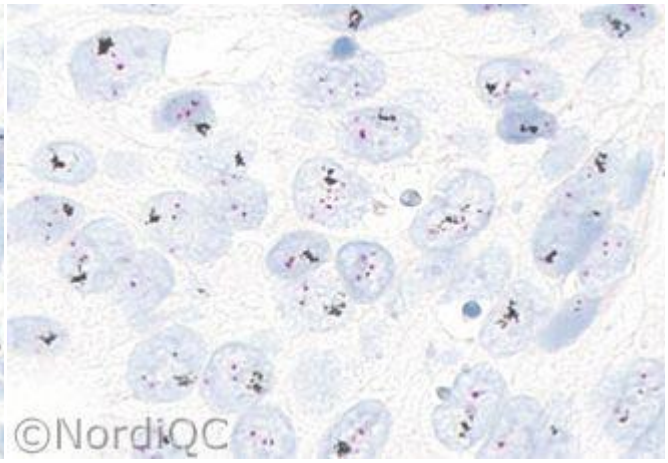


Fig. 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 3 with gene amplification: HER-2/chr17 ratio 6.8 – 7.6.The HER-2 genes are stained black and chr17 red. Some of the Her-2 genes are located in clusters.

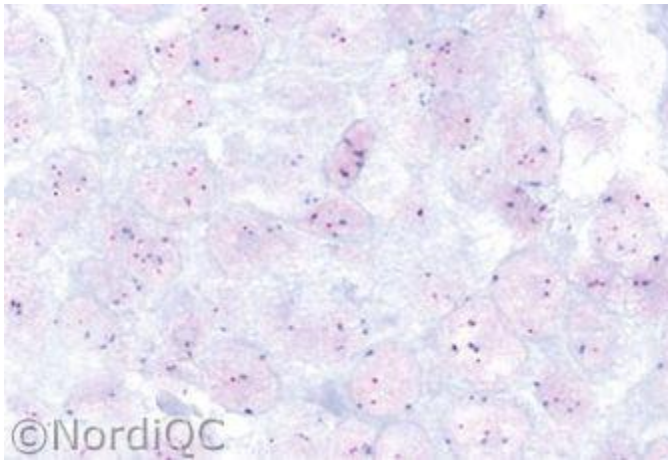


Fig. 2a
Optimal demonstration of the HER-2 gene status using the DuoCISH pharmDx™, Dako of the breast carcinoma no. 2 without gene amplification: HER-2/chr17 ratio 1,0 – 1,3. The HER-2 genes are stained red and chr17 blue.

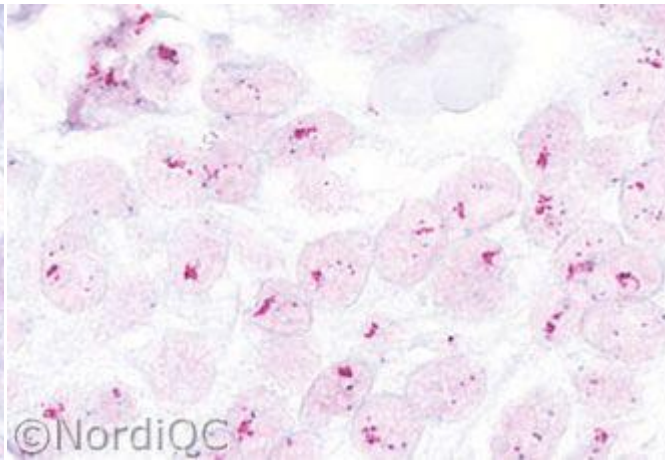


Fig. 2b
Optimal demonstration of the HER-2 gene status using the DuoCISH pharmDx™, Dako of the breast carcinoma no. 3 with gene amplification: HER-2/chr17 ratio 6,8 – 7,6. The HER-2 genes are stained red and chr17 blue. Some of the Her-2 genes are located in clusters.

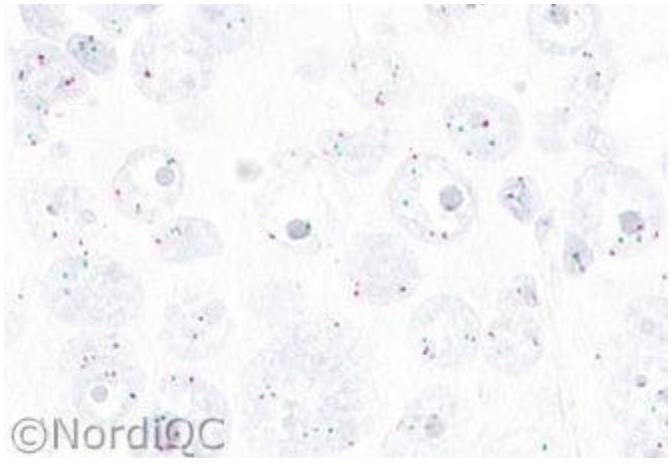


Fig. 3a
Optimal demonstration for the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 2 without gene amplification: HER-2/chr17 ratio 1,0 – 1,3. The HER-2 genes are stained green and chr. 17 red.

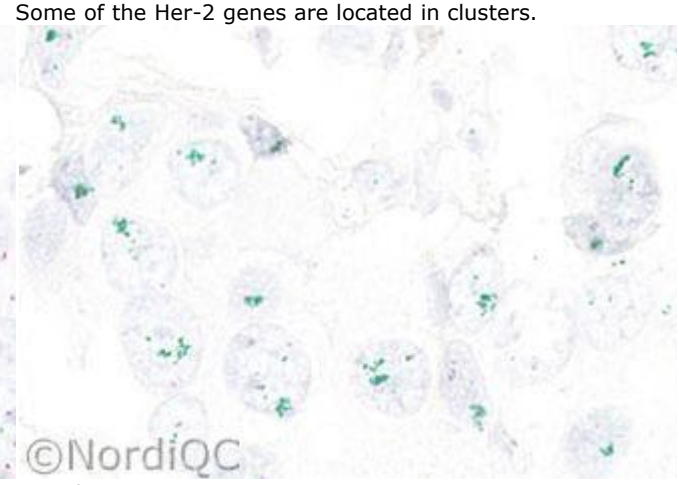


Fig. 3b
Optimal demonstration of the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 3 with gene amplification: HER-2/chr17 ratio 6,8 – 7,6. The HER-2 genes are stained green and chr. 17 red.

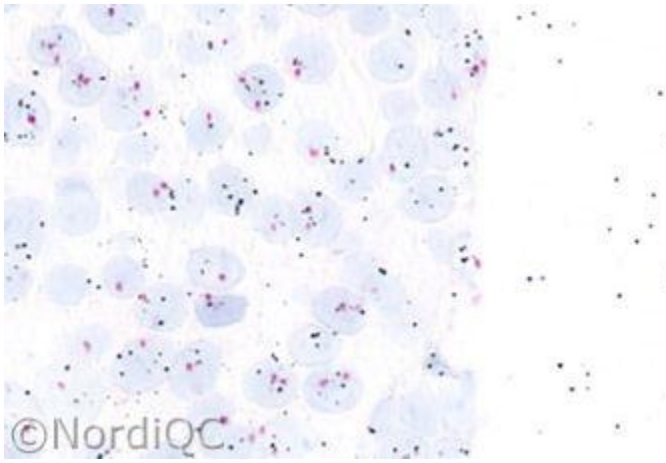


Fig. 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 5 without HER-2 gene amplification: HER-2/chr17 ratio 1,3 – 1,5. Due to silver precipitates both outside the cells and within in the nuclei, the HER2 gene status can not reliably be interpreted. Note the large precipitates at the right outside the tissue mimicking the HER-2 signals within the cells at the left. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.

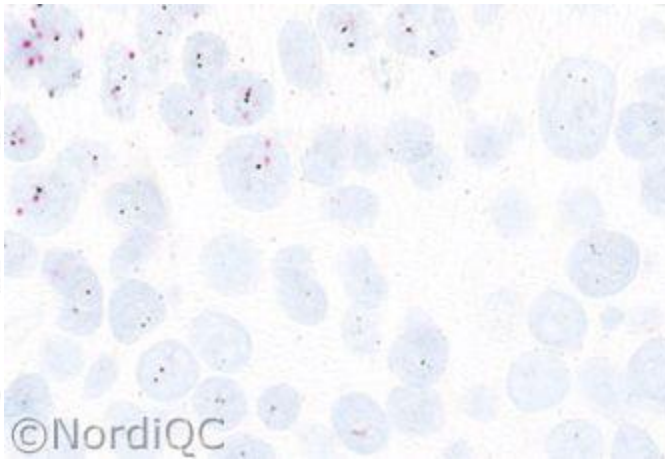


Fig. 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 5 without l gene amplification: HER-2/chr17 ratio 1,3 – 1,5*. The vast majority of the neoplastic cells are negative and only in scattered cells in the upper left corner HER-2 and chr17 signals can be identified. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument. Negative areas of < 25% of the tissue cores were accepted.

*HER2 FISH pharmDX™ Kit (Dako) and HER2 FISH (Zytovision), range of data from NordiQC.

SN/RR/MV/LE 11-04-2014