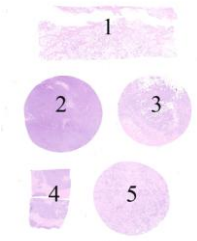
Assessment Run H6 2014

HER-2 ISH (BRISH or FISH)

Material

Table 1. **Content of the multi-block used for the NordiQC HER-2 ISH assessment, run H6**

	HER-2 IHC*	Dual - SISH**	FISH***
	IHC score	**HER2/chr17¤**	**HER2/chr17¤**
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 ratio

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** 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.

Participation

Number of laboratories registered for HER-2 BRISH	125
Number of laboratories returning slides	109 (87%)

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 146 laboratories participated in this assessment. 109 laboratories performed BRISH and of these 85 (66%) 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**	80	Ventana	35	17	16	12	65%	69%
INFORM™ HER-2 Dual ISH **780-4332+780-4331**	4	Ventana	2	0	1	1	-	-
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER2 IHC**	3	Ventana	2	0	0	1	-	-
DuoCISH pharmDx™ **SK109**	8	Dako	3	3	1	1	75%	86%
DuoCISH **SK108 + K5331**	1	Dako	0	0	1	0		
Zyto*Dot*® 2C **C-3022 / C-3032**	6	ZytoVision	4	0	1	1	80%	-
Other	2		2	0	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	2	Ventana	0	2	0	0	-	-
Zyto*Dot*® **C-3003**	3	ZytoVision	1	1	1	0	-	-
Total	109		49	23	21	16	66%	-
Proportion			45%	21%	19%	15%		

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). For the most commonly used HER-2 BRISH assay, the INFORM™ HER-2 Dual ISH (Ventana) a technical adequate result was thus provided in 69% using appropriate protocol settings otherwise being identified for a technical optimal staining result.

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 69% of the submitted protocols (49 of 71). 22 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) in the entire slide or in large areas of one or more of the tissue cores were seen. No reason for these insufficient results could be related to the protocol applied, reagents, platform (being BenchMark XT, GX or Ultra) or any other parameters used by the participants. 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.

For the **DuoCISH™ system SK109** (Dako), protocol settings with optimal results were based on HIER in pre-treatment buffer in a waterbath or microwave oven for 10 min. at 95 - 98°C and subsequent 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 86% of the submitted protocols (6 of 7 laboratories). The insufficient result was characterized by a too weak staining of the HER-2 signals and an impaired morphology. No reason for the aberrant staining result could be identified from the submitted protocol.

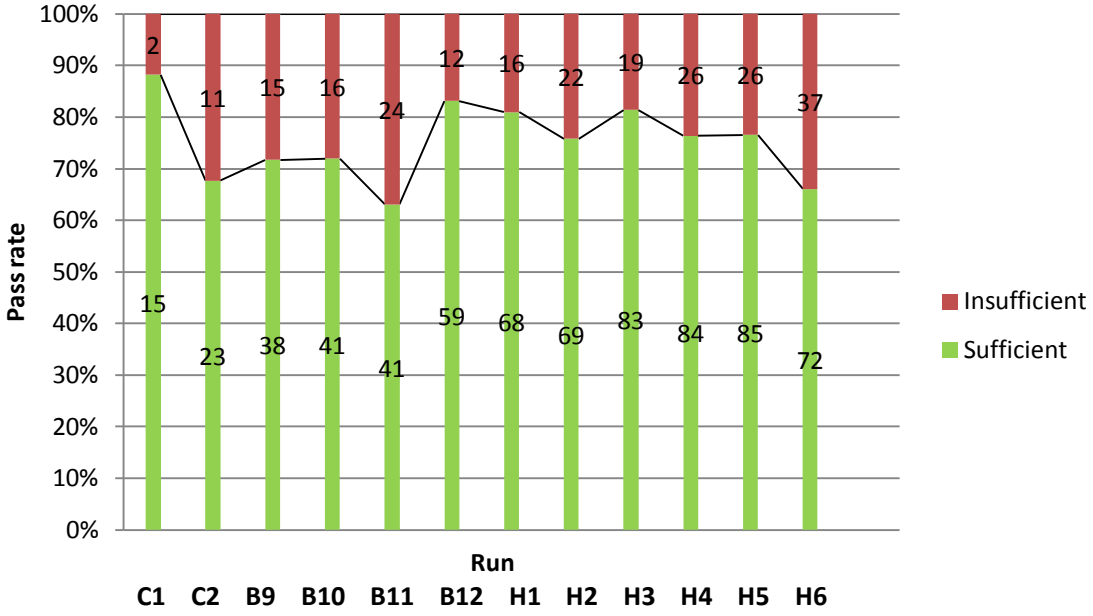
For the **Zyto*Dot*® 2C system C-3022, C-3032** (ZytoVision) optimal results were obtained with proteolysis in pepsin for 4-5 min. (room temperature or at 30-37°C), HIER in EDTA for 15 min. at 98°C, hybridization at 37°C for 16-20 hours and visualization with the ZytoVision detection kit C-3022 or C-3044. Using these protocol settings, optimal results were seen in 4 of 4 laboratories.

One-colour HER-2 systems

For the **Zyto*Dot*® CISH system C-3003**, ZytoVision, optimal results were obtained with proteolysis in pepsin for 4 min. at room temperature, HIER in EDTA for 15 min. at 96°C, 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 2 of 2 laboratories.

Performance history
This was the 12th assessment of HER-2 BRISH in NordiQC and in this run a slightly reduced pass was observed compared to the relatively consistent levels obtained in the latest runs. Data is shown in Table 3. No explanation for the reduced pass rate in this run could be identified. The material circulated in run H6 was completely identical to run H5 and same scoring criteria have been applied in both runs.

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**

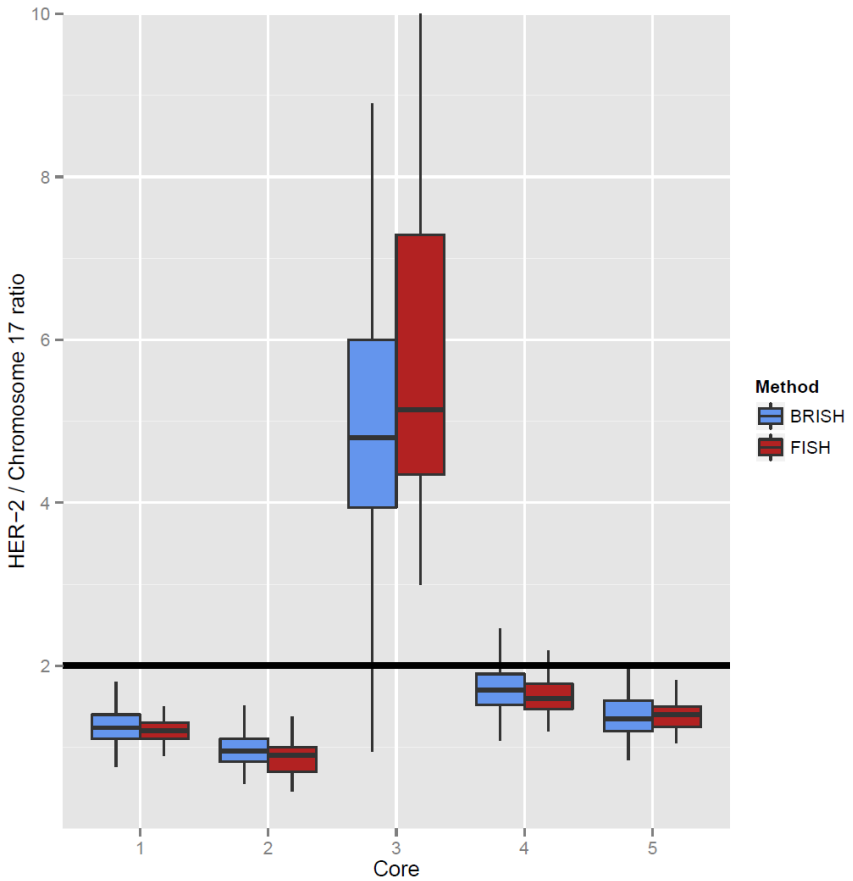
	NordiQC FISH HER2/chr17 ratio	**NordiQC HER-2 amplification status**
1. Breast ductal carcinoma	1,0 – 1,2	Non-amplified
2. Breast ductal carcinoma	1,0 – 1,3	Non-amplified
3. Breast ductal carcinoma	6,8 – 7,6	Amplified
4. Breast ductal carcinoma	1,5 – 2,0*	Non-amplified / Equivocal
5. Breast ductal carcinoma	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 134 of the 146 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. Almost same level of 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 94% (32 of 34 laboratories) compared to 89% for the laboratories using BRISH (89 of 100 laboratories).

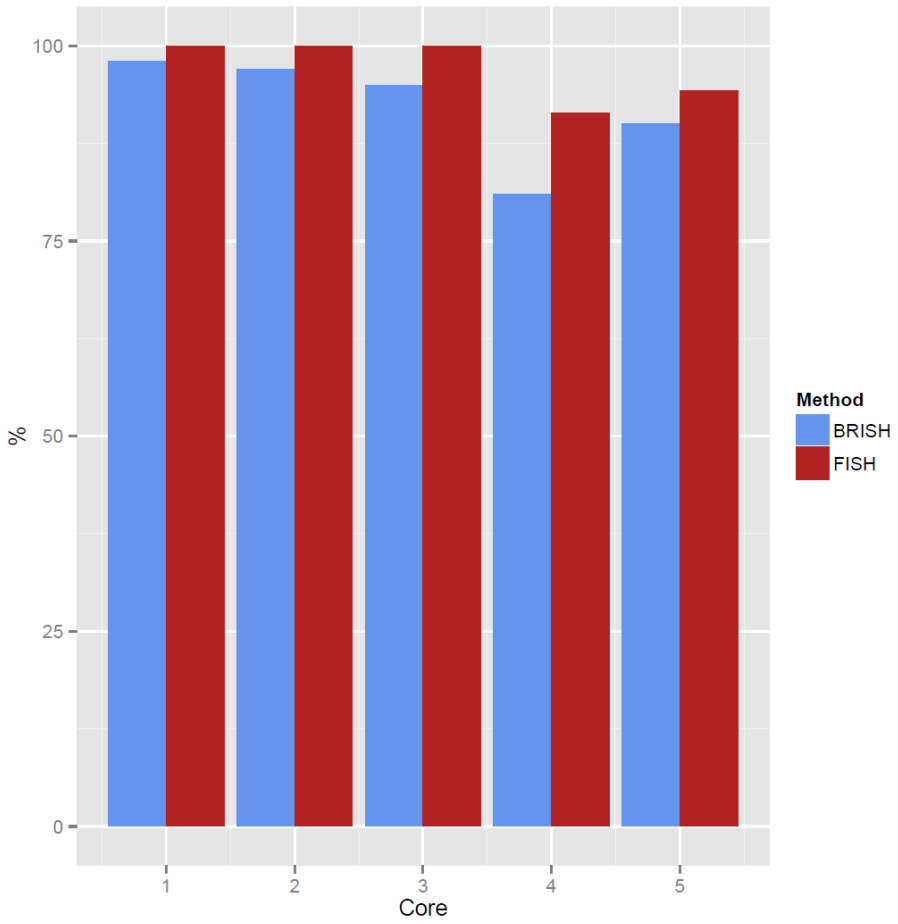
In general for both BRISH and FISH, high consensus rates were observed between participants and NordiQC regarding the HER-2 amplification status in the breast carcinomas tissue cores no. 1, 2 and 3. The interpretation of HER-2 amplification status was more challenging for tissue core no. 4. This tumour 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.
The overall interpretation of amplification ratios and consensus rates of the participants are shown in Figs. 1 and 2.

Fig. 1



NordiQC HER-2 ISH run H6: participants interpretation of amplification status

Fig. 2



NordiQC HER-2 ISH run H6: consensus between participants and NordiQC

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 system 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 a high proportion of technical insufficient results were seen, indicating other issues are influencing the quality of the BRISH assays. E.g. the capability of present instrumentation technique to provide a reproducible performance of the protocols.
Attention must be paid to interpretation in order to obtain correct HER-2 amplification status.

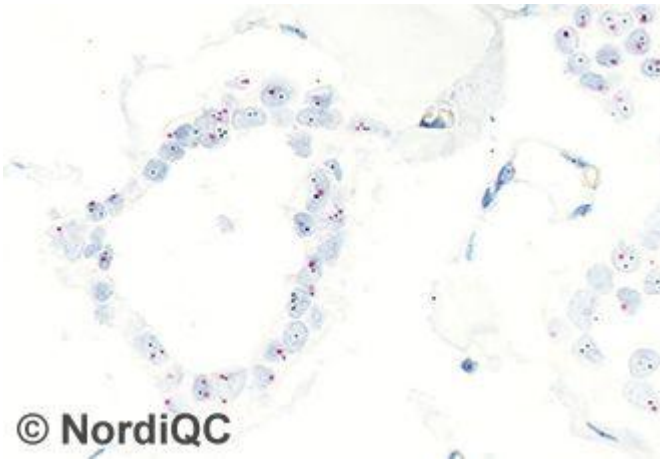


Fig. 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 1 without gene amplification: HER-2/chr17 ratio 1.0 – 1.2*.
The HER-2 genes are stained black and chr17 red.
The signals are distinctively demonstrated.

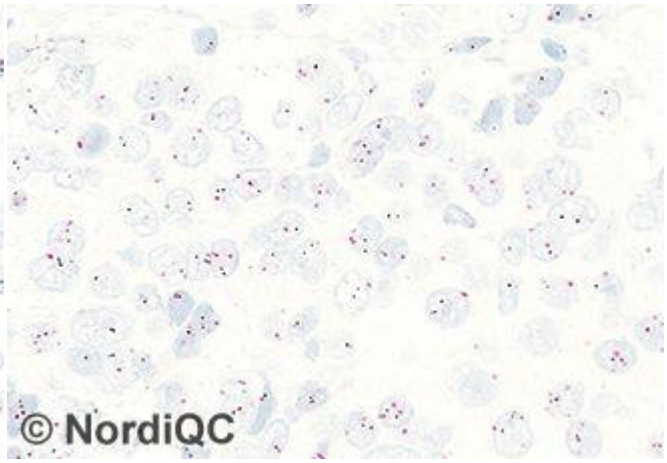


Fig. 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 2 without gene amplification: HER-2/chr17 ratio 1.0 – 1.3.
The HER-2 genes are stained black and chr17 red.
The signals are distinctively demonstrated.

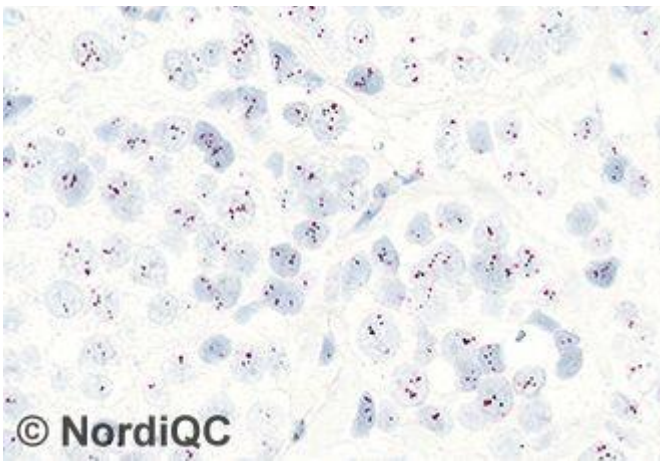


Fig. 2a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 4 showing HER-2/chr17 ratio of 1.5 – 2.0.
The HER-2 genes are stained black and chr17 red.
Few cells show low level of gene amplification but the majority with normal ratio. The tumour was by the NordiQC reference laboratories and by the vast majority of participants scored as non-amplified.

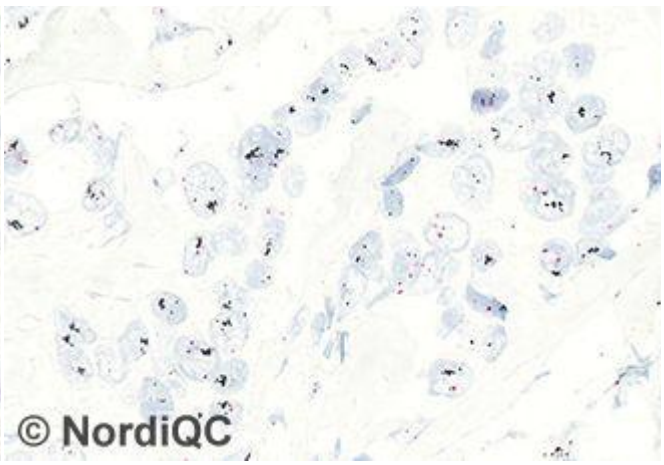


Fig. 2b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 3 with gene amplification: HER-2/chr17 ratio 6.8 – 7.6.
The HER-2 genes are stained black and chr17 red.
Some of the Her-2 genes are located in clusters.

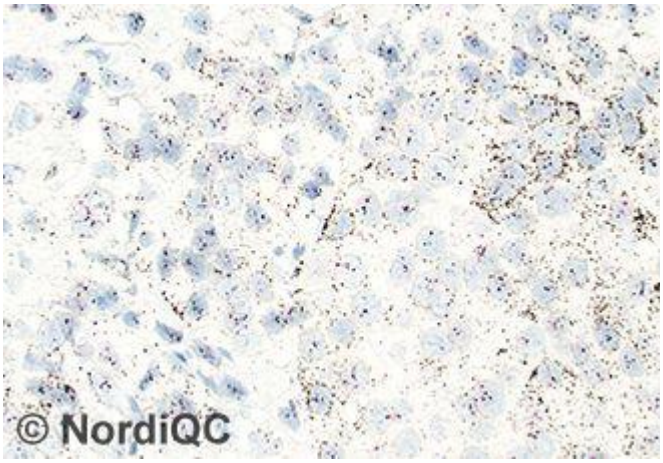


Fig. 3a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 2 without HER-2 gene amplification: HER-2/chr17 ratio 1.0 – 1.3.
Due to extensive silver precipitates the HER2 gene status cannot be interpreted. This aberrant 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.

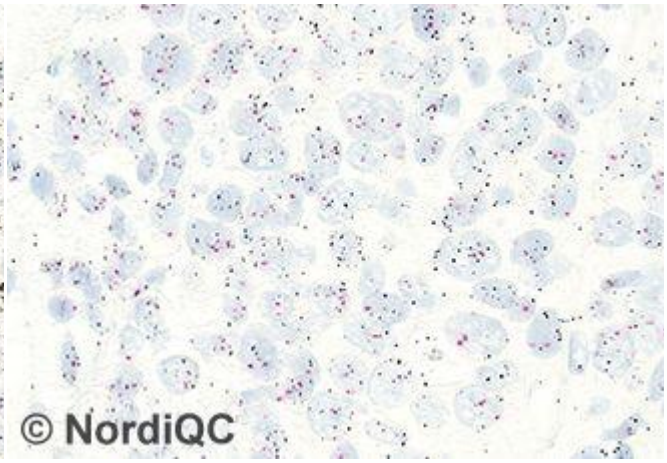


Fig. 3b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 2 without HER-2 gene amplification: HER-2/chr17 ratio 1.0 – 1.3.
Due to silver precipitates both outside the cells and within the nuclei, the HER2 gene status cannot reliably be interpreted. Note the large precipitates outside the tissue mimicking the HER-2 signals within the cells.
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 sufficient and evaluated the tumour as being amplified.

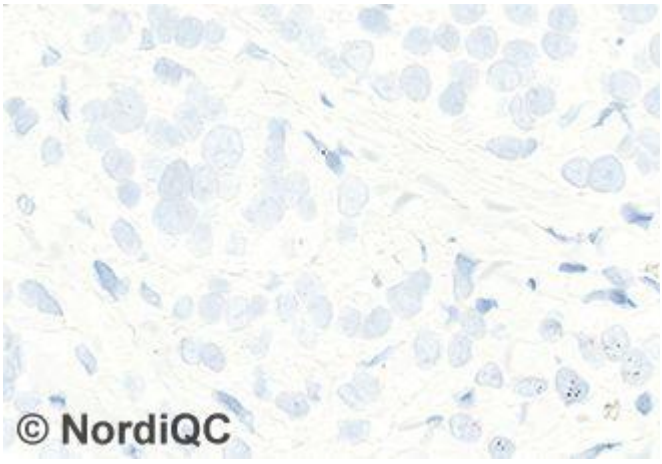


Fig. 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 5 without gene amplification: HER-2/chr17 ratio 1.3 – 1.5.
The vast majority of the neoplastic cells are negative and only in scattered cells HER-2 and chr17 signals can be identified. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument. 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.

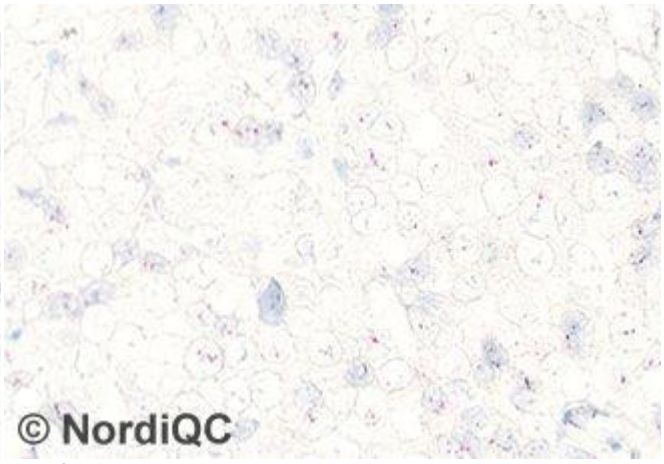


Fig. 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422/780-4422, Ventana of the breast carcinoma no. 3 without gene amplification: HER-2/chr17 ratio 1.3 – 1.5.
Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.
Proteolytic pre-treatment must be adjusted to the formalin fixation time and conditions used for the material tested.
The laboratory reported the result as technically insufficient and new test required with optimized pre-treatment settings.

* Reference: HER2 FISH pharmDX™ Kit, Dako & HER2 FISH, Zytovision (range of data from three reference labs.).

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