Assessment Run H2 2012

HER-2 ISH

Material

The material circulated for the HER-2 ISH assessment run comprised one normal breast tissue and four breast ductal carcinomas showing the HER-2 gene/chromosome 17 (HER-2/chr17) ratios as follows:

	HER-2 IHC*	Dual - SISH**	FISH***
	IHC score	HER-2/chr17 ratio	HER-2/chr17 ratio
1. Normal breast tissue	0	1.1 - 1.2	1.1 - 1.3
2. Breast ductal carcinoma	3+	3.5 - 4.0	3.0 - 3.6
3. Breast ductal carcinoma	1+	1.2 - 1.4	1.3 - 1.5
4. Breast ductal carcinoma	2+	1.5 - 1.8	1.5 - 1.9
5. Breast ductal carcinoma	2+	1.3 - 1.6	1.5 - 2.2

*PATHWAY®, Ventana (data from one reference lab.),
**Inform HER-2 Dual SISH kit, Ventana (range of data from two reference labs.), ***HER2 FISH pharmDX™ Kit, Dako & HER2 FISH, ZytoVision (range of data from three reference labs.).

All tissues were fixed for 24 - 48 h. in 10 % neutral buffered formalin (NBF).

HER-2 BRISH, Technical assessment

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

A staining was assessed as good, if the HER-2/chr17 ratios could be evaluated in all five tissues, but the interpretation was slightly compromised e.g. due to a weak or excessive counterstaining, or excessive retrieval.

A staining was assessed as borderline if one of the tissues could not be properly evaluated e.g. due to weak signals or a low signal-to-noise ratio.

A staining was assessed as poor if two or more of the tissue cores could not be properly evaluated.

HER-2 BRISH and FISH interpretation

For both BRISH and FISH the participating laboratories were asked to submit a scoring sheet with the interpretation of the HER-2/chr17 ratio for all the five tissues. The NordiQC FISH data was used as reference in order to evaluate the scoring consensus between the participating laboratories and NordiQC. A concordant interpretation of the five tissues was seen if the five tissues were evaluated as listed below.

- Evaluation of the normal breast tissue and the ductal carcinoma no. 3 corresponding a non-amplified status.
- Evaluation of the breast ductal carcinoma no. 2 corresponding a (highly) amplified status
- Evaluation of the breast ductal carcinomas no. 4 & 5 corresponding a non-amplified or equivocal status.

Results BRISH

In total 118 laboratories participated in this assessment. 91 laboratories performed BRISH and out of these 69 (76 %) achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Systems and assessment marks for BRISH HER-2.**

Two colour HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422, 780-4422**	50	Ventana	25	12	6	7	74 %	78 %
INFORM™ HER-2 Dual ISH **780-4332+780-4331**	14	Ventana	5	5	2	2	71 %	80 %
DuoCISH pharmDx™ **SK109**	12	Dako	5	5	2	0	83 %	100 %
ZytoDot® 2C **C-3022**	2	ZytoVision	1	0	1	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	7	Ventana	6	0	1	0	86 %	100 %
ZytoDot® **C-3003**	3	ZytoVision	1	1	1	0	-	-
SPOT-Light® **84-0150**	2	Invitrogen	0	2	0	0	-	-
"In-house"	1		0	1	0	0	-	-
Total	91		43	26	13	9	-	-
Proportion			47 %	29 %	14 %	10 %	76 %	

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

Comments
In this assessment a sufficient demonstration and evaluation of the HER-2 gene amplification status could be obtained by all the different BRISH systems used by the laboratories. All included tissues were fixed in 10 % neutral buffered formalin for 24-48 hours according to the ASCO/CAP guidelines for the tissue preparation of breast tissue.
Minor focal staining artefacts were accepted if they did not compromise the overall interpretation in each of the 5 individual tissue cores. Artefacts were silver precipitates, excessive background or negative areas caused by drying out due the staining process etc.

Optimal protocol settings:Two-colour HER-2 systems
For the **INFORM™ Dual ISH systems 800-4422, 780-4422, 780-4332+780-4331** (Ventana), an optimal demonstration for HER-2 BRISH was typically based on HIER in Cell Conditioning 2 (CC2) for 24-32 min. at 86-90°C and 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 a sufficient result (optimal or good) was seen in 78 % of the submitted protocols (39 of 50). 11 laboratories used a protocol with optimal settings, but for unexplained reasons a complete false negative staining or a staining result with an excessive background staining (e.g. due to silver precipitates) was seen. The remaining insufficient results were characterized by an impaired morphology. This pattern was typically caused by excessive retrieval hampering the interpretation as the nuclei were almost totally digested complicating the identification and interpretation of the BRISH signals.

For the **DuoCISH™ system SK109** (Dako), main protocol settings giving an optimal result were based on HIER for 10 min in pre-treatment buffer at 95 - 98°C and 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 a sufficient result was seen in 100 % of the submitted protocols (10 of 10 laboratories). Insufficient results using the DuoCISH™ kit, typically characterized by a too weak or false negative staining in both neoplastic cells and normal stromal cells, seemed to be caused by omission of or too insufficient proteolysis.

For the **ZytoDot® 2C system C-3022** (ZytoVision), an optimal result was obtained by using proteolysis in pepsin for 4 min at room temp, HIER in EDTA for 15 min. at 98°C, hybridization at 37°C for 20 hours and visualization with the ZytoVision detection kit C-3022.

One-colour HER-2 systems
For the **INFORM™ HER-2 SISH 780-4332** (Ventana), an optimal result typically was based on HIER in CC2 or Reaction buffer (RB) for 24-32 min. at 90-95°C and proteolysis in P3 for 4 - 12min at 37°C. The HER-2 SISH probe was typically applied for 6 hours at 50-52°C. Using these protocol settings a sufficient result was seen in 100 % of the submitted protocols (6 of 6).

For the **ZytoDot® CISH system C-3003** (ZytoVision) an optimal result was obtained by using proteolysis in pepsin for 2 min at room temp, 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.

HER-2 interpretation and scoring consensus
Both the laboratories performing BRISH and FISH were requested to send in their own interpretation on the stained sections. This was completed by 112 of the 118 participating laboratories. These evaluations were compared to the HER2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in table 2. No significant difference was seen between the participants using FISH or BRISH regarding the concordance rate and evaluation.

Table 2. **Interpretation and scoring consensus between the participants and the NordiQC FISH data**

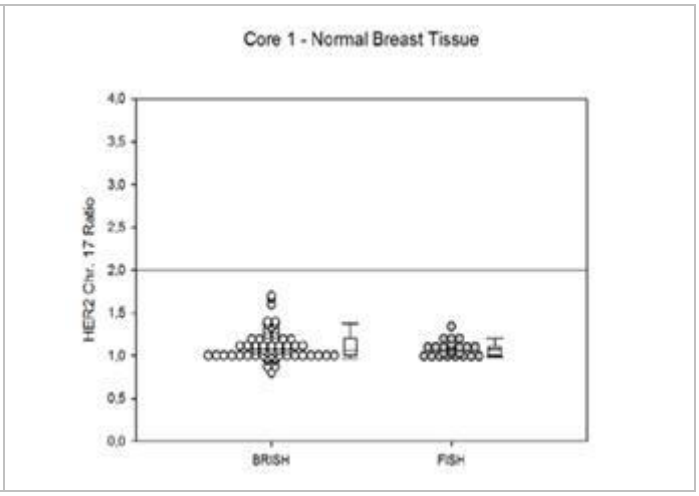
	FISH HER-2/chr17 ratio	HER2 amplification status	Consensus evaluation
1. Normal breast tissue	1.1 - 1.3	Non-amplified	100 %
2. Breast ductal carcinoma	3.0 - 3.6	Amplified	97 %
3. Breast ductal carcinoma	1.3 - 1.5	Non-amplified	85 %
4. Breast ductal carcinoma	1.5 - 1.9	Non-amplified/Equivocal	91 %
5. Breast ductal carcinoma	1.5 - 2.2	Non-amplified/Equivocal	94 %

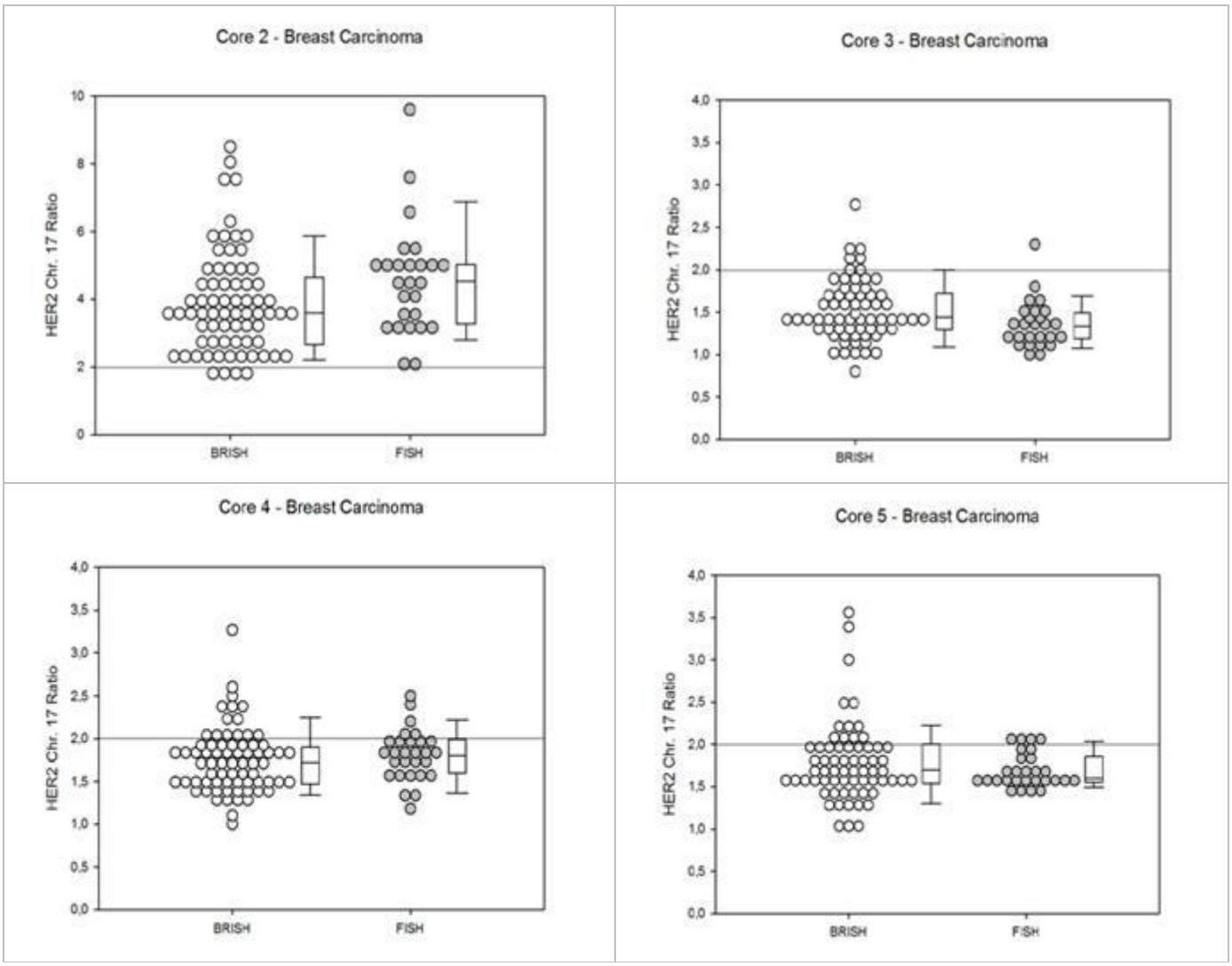
In general a high consensus rate was seen between the participants and NordiQC regarding the determination of the HER-2 amplification status, particular in the normal breast tissue core no.1 and the highly amplified breast carcinoma, tissue core no. 2 (97 and 100 %, respectively). A slightly lower consensus percentage was seen in the other breast carcinomas (tissue cores no. 3, 4 & 5) and can be related to several parameters in both the technical performance of the ISH assay and the interpretation method applied.
The consensus percentage in core 3 was 90 % (54 of 60) for the laboratories obtaining a sufficient result based on BRISH, compared to 65 % (15 of 23) for the laboratories obtaining an insufficient result. An inferior BRISH assay e.g. due to weak signals, precipitates and similar might affect the interpretation stressing the importance to optimize the ISH assays.
Focus must also be directed to establish supplementary tools as image analysis to support and harmonize the interpretation of the ISH results.

Fig. 1.
Graphic illustrations showing the HER-2 amplifications in the five tissue cores as assessed by the laboratories.

Each circle represents the HER-2/chr17 ratio given by a laboratory. The box shows the 25/75 percentile and the line within the box the median value. Whiskers show the 10/90 percentile.

In each graph, left columns represent laboratories performing BRISH, right columns laboratories performing FISH.





This was the eight assessment of HER-2 BRISH in NordiQC and, as seen in table 3, a slightly lower pass rate was seen in the current run compared to the previous.

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

	Run C1 2009	**Run C2 2009**	**Run B9 2010**	**Run B10 2010**	**Run B11 2011**	**Run B12 2011**	**Run H1 2012**	**Run H2 2012**
Participants, n=	17	34	53	57	65	71	84	91
Sufficient results	88 %	68 %	72 %	72 %	63 %	83 %	81 %	76 %

Conclusion

In this assessment an 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). Also the single-colour HER-2 systems, INFORM™ HER-2 SISH (Ventana) and ZytoDot® (ZytoVision) could be used to obtain an optimal demonstration.

For an optimal performance the retrieval settings – HIER and proteolysis - must be carefully balanced to provide efficient sensitivity and preserved morphology. Attention must also be payed to interpretation in order to obtain correct HER-2 amplification status.

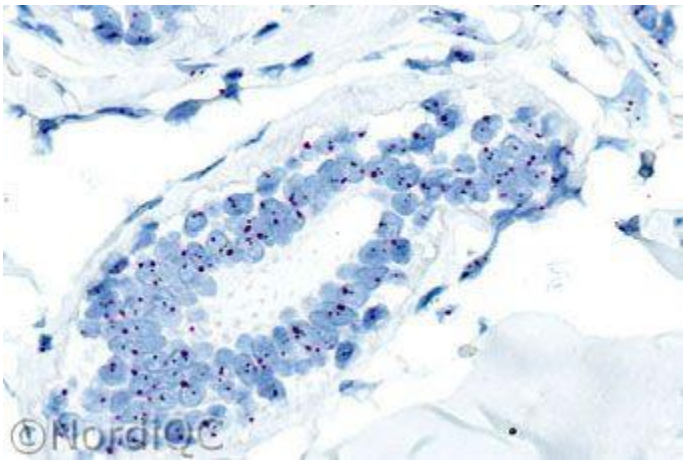


Fig 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the normal breast tissue no. 1 without gene amplification: HER-2/chr17 ratio 1.1 - 1.3*. The HER-2 genes are stained black and chr17 red.

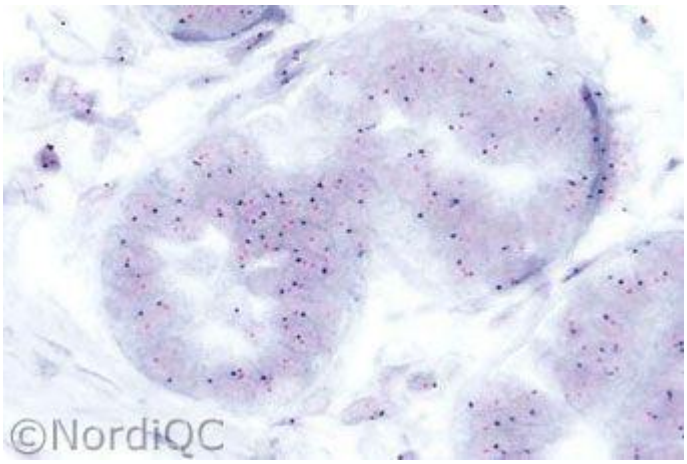


Fig 1b
Optimal demonstration of the HER-2 gene status using the DuoCISH™, Dako of the normal breast tissue no. 1 without gene amplification: HER-2/chr17 ratio 1.1 - 1.3*.
The HER-2 genes are stained red and chr17 blue.

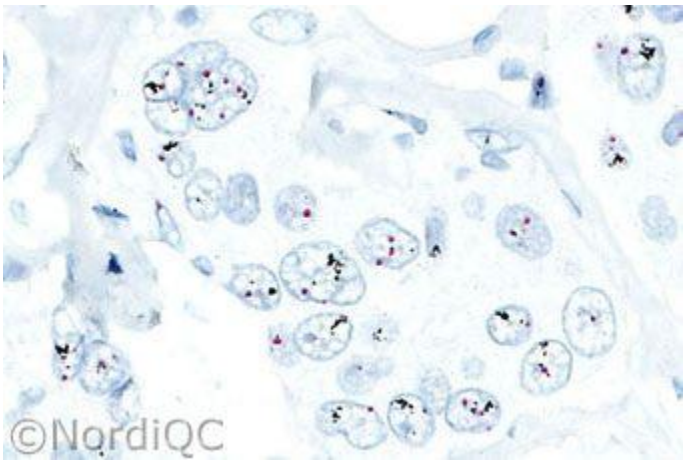


Fig 2a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr17 ratio 3.0 – 3.6*. The HER-2 genes are stained black and chr17 red. Some of the Her-2 genes are located in clusters.

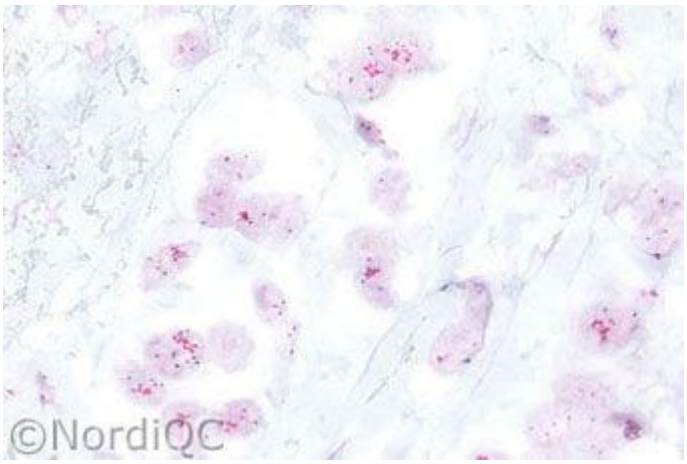


Fig 2b
Optimal staining for the HER-2 gene status using the DuoCISH™, Dako of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr17 ratio 3.0 – 3.6*. The HER-2 genes are stained red and chr17 blue. Some of the Her-2 genes are located in clusters.

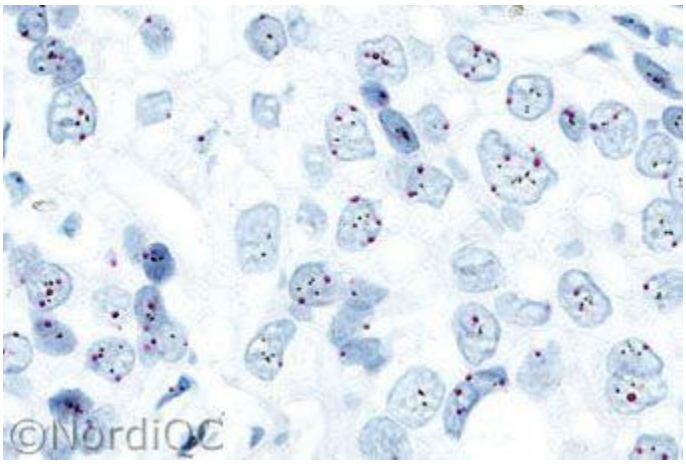


Fig 3a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 3 without gene amplification: HER-2/chr17 ratio 1.3 – 1.5*. Note more cells show chr17 polysomia. The HER-2 genes are stained black and chr17 red.

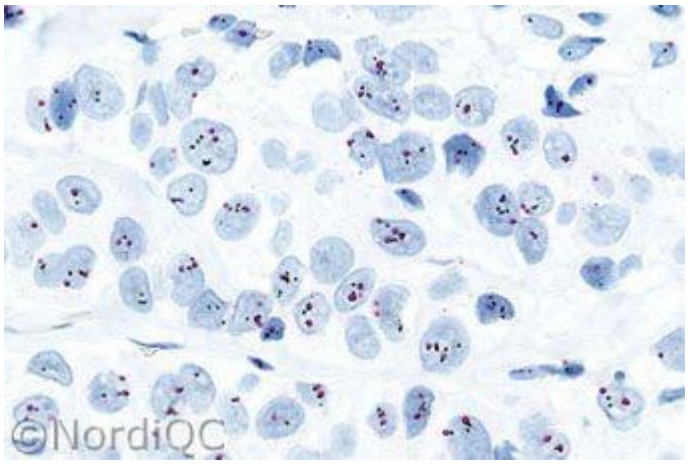


Fig 3b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal gene amplification: HER-2/chr17 ratio 1.5 – 1.9*. The HER-2 genes are stained black and chr17 red.

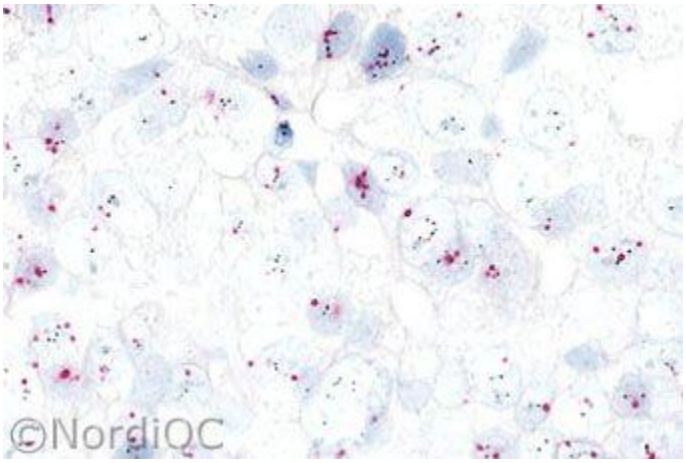


Fig 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal level of HER-2 gene amplification: HER-2/chr17 ratio 1.5 – 1.9*. Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.

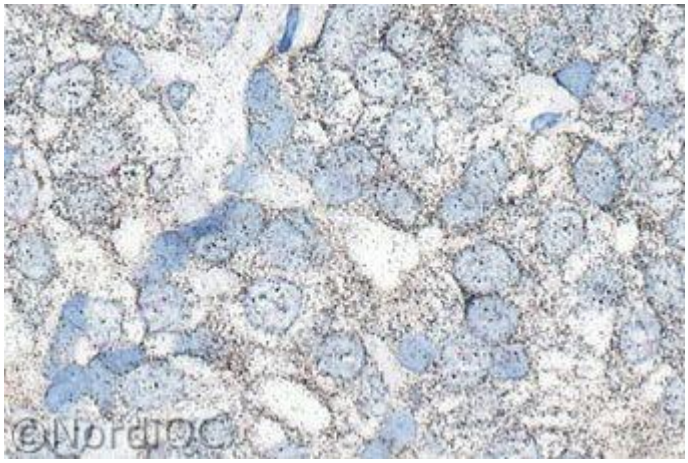


Fig 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal level of HER-2 gene amplification: HER-2/chr17 ratio 1.5 – 1.9*. Due to silver precipitates both outside the cells and within in nuclei, the HER2 gene status can not be interpreted. This aberrant reaction most likely was caused by a technical problem during the staining.

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

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