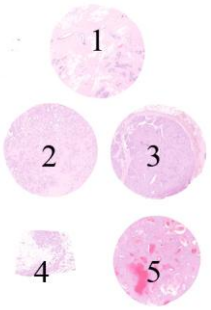
Assessment Run H3 2013

HER-2 ISH (BRISH or FISH)

Material

Table 1. Circulated material for the NordiQC HER-2 ISH assessment, run H3

	HER-2 IHC*	**Dual - SISH****	**FISH*****
	IHC score	**HER-2 gene / chromosome 17 ratio**	**HER-2 gene / chromosome 17 ratio**
1. Breast ductal carcinoma	0	0.8 - 1.1	1.1 - 1.3
2. Breast ductal carcinoma	3+	2.5 - 4.9	4.3 - 7.7
3. Breast ductal carcinoma	1+	1.2 - 1.5	1.3 - 1.7
4. Breast ductal carcinoma	2+	1.4 - 1.8	1.4 – 1.9
5. Breast ductal 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 reference labs.

All tissues were fixed for 24 - 48 h. in 10 % neutral buffered formalin according to the ASCO/CAP guidelines for the tissue preparation of breast tissue.

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 gene/chromosome 17 ratios in all five tissues.

A staining was assessed as good, if the HER-2 gene/chromosome 17 ratios could be evaluated in all five tissues, but the interpretation was slightly compromised (e.g. excessive retrieval, weak or excessive counterstaining).

A staining was assessed as borderline if one of the tissues could not be evaluated properly 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 evaluated properly.

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 HER-2 gene / chromosome 17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyze scoring concensus.

Concensus scores from the NordiQC FISH reference laboratories

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

Results BRISH

In total 132 laboratories participated in this assessment. 102 laboratories performed BRISH and of these 83 (81 %) 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**	57	Ventana	34	11	10	2	79 %	82 %
INFORM™ HER-2 Dual ISH **780-4332+780-4331**	11	Ventana	4	4	3	0	73 %	80%
DuoCISH pharmDx™ **SK109**	10	Dako	6	3	1	0	90 %	90 %
Zyto*Dot*® 2C **C-3022**	3	ZytoVision	2	1	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	12	Ventana	8	3	1	0	92 %	100 %
Zyto*Dot*® **C-3003**	6	ZytoVision	2	2	1	1	67 %	- %
SPOT-Light® **84-0150**	2	Invitrogen	0	2	0	0	-	-
"In-house"	1		0	1	0	0	-	-
Total	102		56	27	16	3	-	-
Proportion			55 %	26 %	16 %	3 %	81 %	

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

Comments

In this assessment, sufficient demonstration and evaluation of the HER-2 gene amplification status in all tissues in the multi block could be obtained by the different BRISH systems.
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 during the staining process.

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 of 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 Chr. 17 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, complete false negative staining or excessive background staining (e.g. due to silver precipitates) was seen. The remaining insufficient results were characterized by impaired morphology. This pattern was typically caused by excessive retrieval hampering the interpretation as the nuclei were almost totally digested.

For the **DuoCISH™ system SK109** (Dako), protocol settings with optimal results were based on HIER for 10-20 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 Chr. 17 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 90 % of the submitted protocols (9 of 10 laboratories). The insufficient result was characterized by a too weak staining of the HER-2 signals in both neoplastic cells and normal stromal cells in combination with 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** (ZytoVision) optimal results were obtained with proteolysis in pepsin for 4 min at room temp. or 3 min at 30°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.

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 100 % of the submitted protocols (9 of 9).

For the **Zyto*Dot*® CISH system C-3003**, ZytoVision, optimal results were obtained with proteolysis in pepsin for 1½ - 2 min at room temp, HIER in EDTA for 10-15 min at 98 -100°C, hybridization at 37°C for 14-20 hours and visualization with the ZytoVision detection kit C-3003. Using these protocol settings sufficient result were seen in 75 % of the submitted protocols (3 of 4).

HER-2 interpretation and scoring consensus:
Scoring sheets were completed by 118 of the 132 participating laboratories. These evaluations were compared to the HER2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in Fig. 2. No significant difference was seen between the participants using FISH or BRISH regarding the concordance rate and evaluation.

Table 3. NordiQC FISH amplification data

	NordiQC FISH HER-2 gene / chromosome 17 ratio	NordiQC HER-2 amplification status
1. Breast ductal carcinoma	1.1 - 1.3	Non-amplified
2. Breast ductal carcinoma	4.3 - 7.7	Amplified
3. Breast ductal carcinoma	1.3 - 1.7	Non-amplified
4. Breast ductal carcinoma	1.4 – 1.9	Non-amplified / Equivocal
5. Breast ductal carcinoma	1.3 – 1.5	Non-amplified

In general, high consensus was observed between participants and NordiQC regarding HER-2 amplification status in the breast carcinomas tissue cores no.1, 2 and 5.
Slightly lower consensus percentage rate was seen in the breast carcinomas tissue core no. 4 and in particular tissue core no. 3. This can be related to several parameters as the technical performance of the ISH assay and/or the interpretation.
The overall consensus percentage rate in core 3 was 73 %, but varied markedly between laboratories using BRISH and laboratories using FISH, Fig 1. None of the 30 laboratories using FISH interpreted the carcinoma core 3 as being amplified, while 12 out of the 90 laboratories using BRISH interpreted the carcinoma as amplified.

Fig. 1.

Graphic illustrations showing the HER-2 amplification ratios 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.

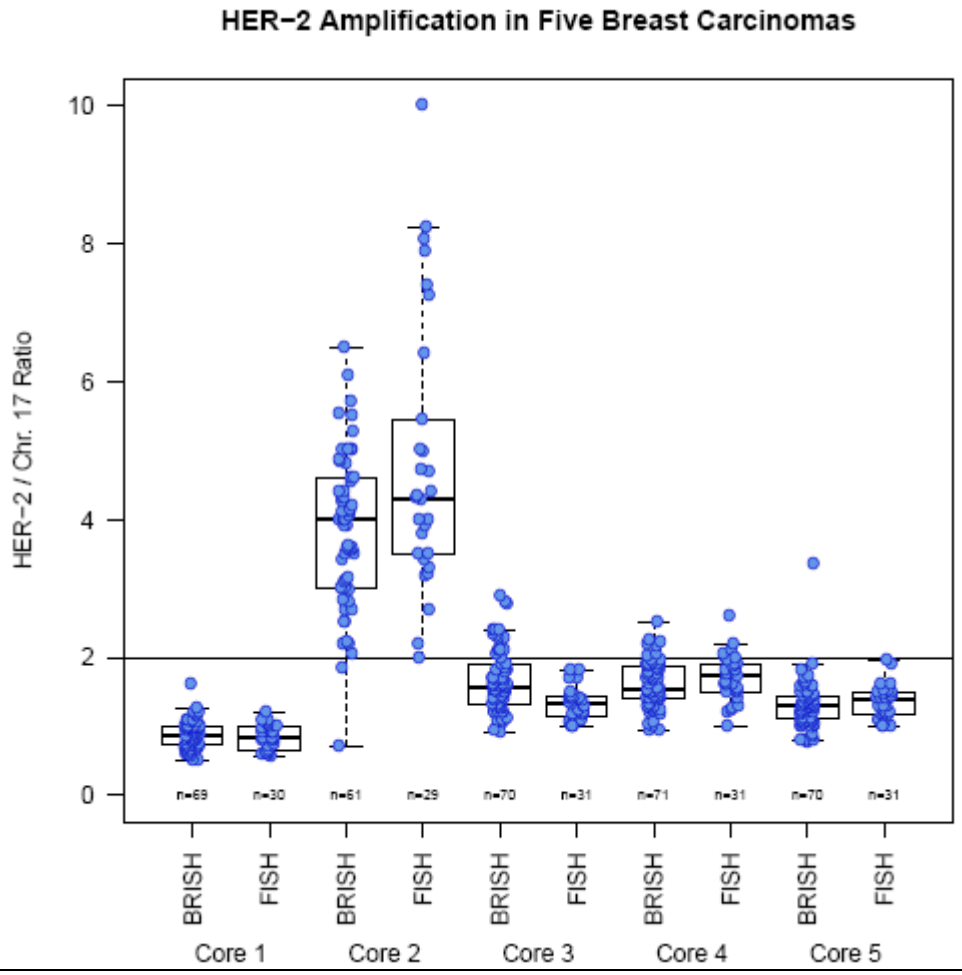
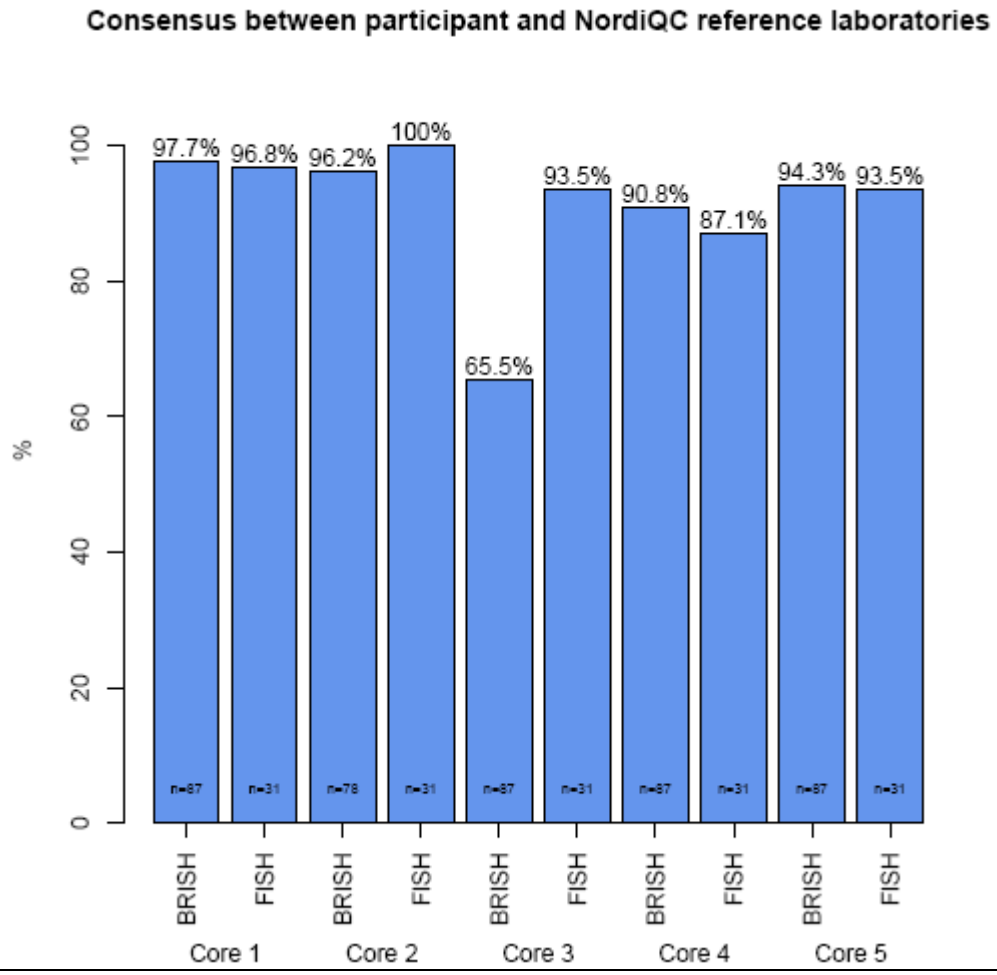


Fig. 2.

Graphic illustrations showing consensus between participants and NordiQC reference laboratories regarding the interpretation of the amplification status in the five carcinomas.



This was the 9th NordiQC assessment of HER-2 BRISH. Relatively consistent pass rate has been observed in the last four assessments.

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

	Run C1	Run C2	Run B9	Run B10	Run B11	Run B12	Run H1	Run H2	Run H3
Participants n=	17	34	53	57	65	71	84	91	102
Sufficient results	88 %	68 %	72 %	72 %	63 %	83 %	81 %	76 %	81 %

Conclusion

In this assessment, 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 produceoptimal demonstration.
Retrieval settings – HIER and proteolysis - must be carefully balanced to provide efficient sensitivity and preserved morphology. Attention must also be paid to interpretation in order to obtain correct HER-2 amplification status.

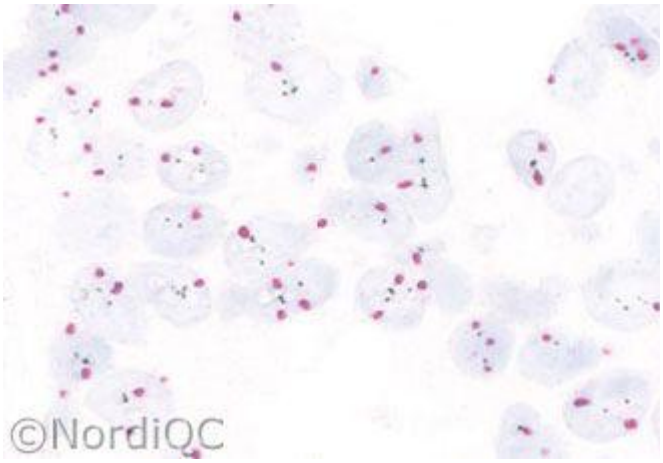


Fig 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained black and chr. 17 red.

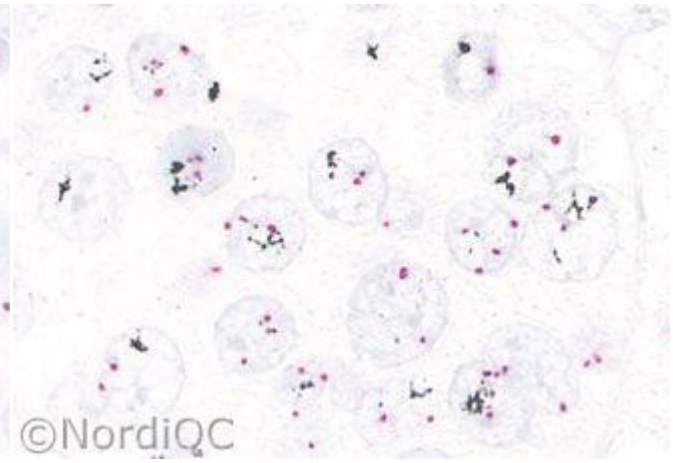


Fig 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 7.7*.
The HER-2 genes are stained black and chr. 17 red. Some of the Her-2 genes are located in clusters.

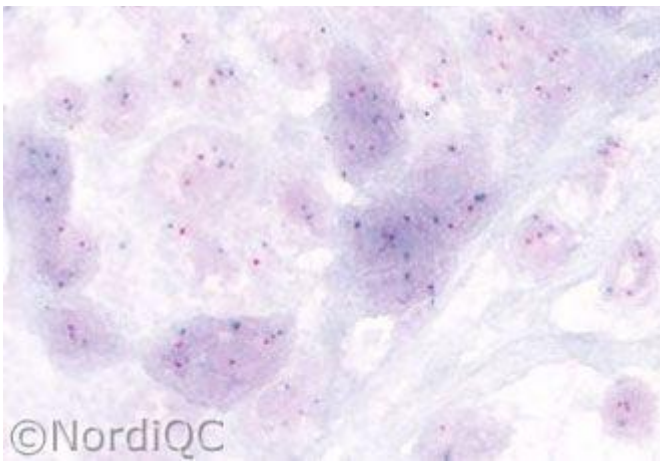


Fig 2a
Optimal demonstration for the HER-2 gene status using the DuoCISH™, Dako of the breast carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained red and chr. 17 blue.

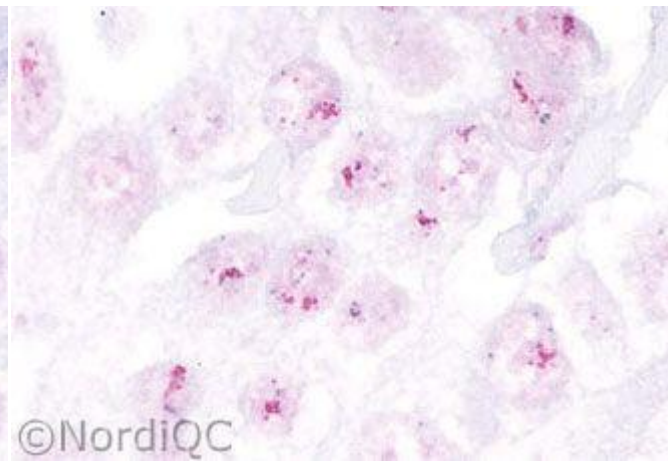


Fig 2b
Optimal demonstration of the HER-2 gene status using the DuoCISH™, Dako of the breast carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 7.7*.
The HER-2 genes are stained red and chr. 17 blue. Some of the Her-2 genes are located in clusters.

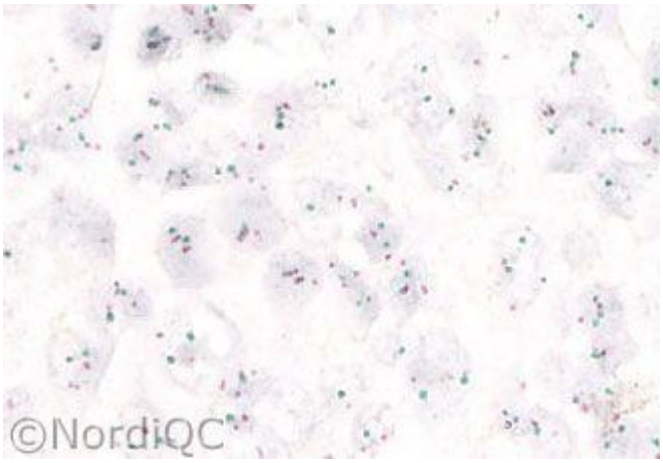


Fig 3a
Optimal demonstration for the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained green and chr. 17 red.

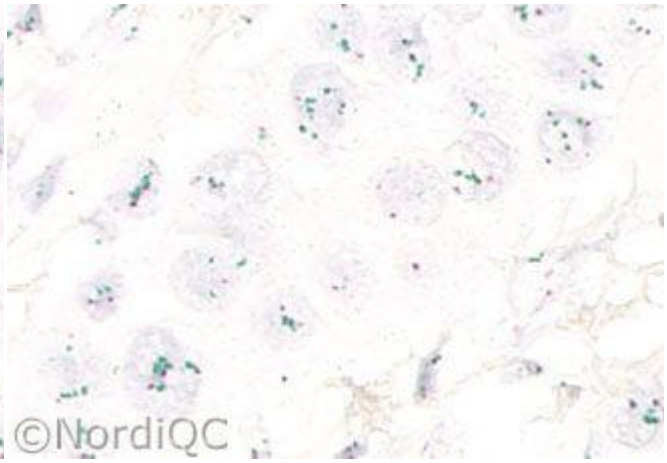


Fig 3b
Optimal demonstration of the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 4 without or equivocal gene amplification: HER-2/chr. 17 ratio 1.4 – 1.9*.
The HER-2 genes are stained green and chr. 17 red.

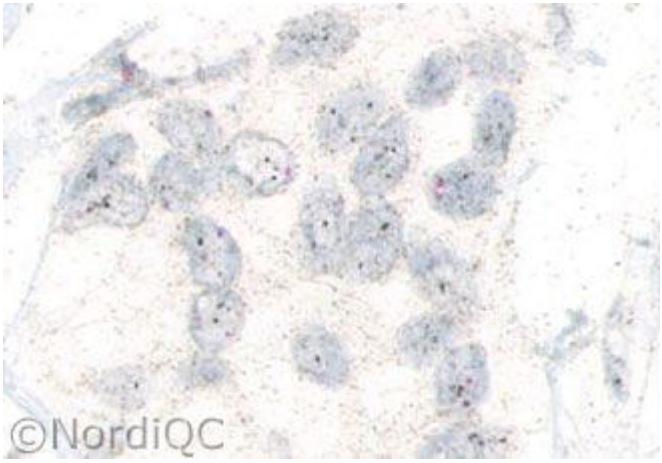


Fig 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 5 without HER-2 gene amplification: HER-2/chr. 17 ratio 1.3 – 1.5*. Due to silver precipitates both outside the cells and within in nuclei, the HER2 gene status can not be interpreted. This aberrant reaction most likely was caused by either a technical problem during the staining or prolonged time in the silver development step (Silver C).

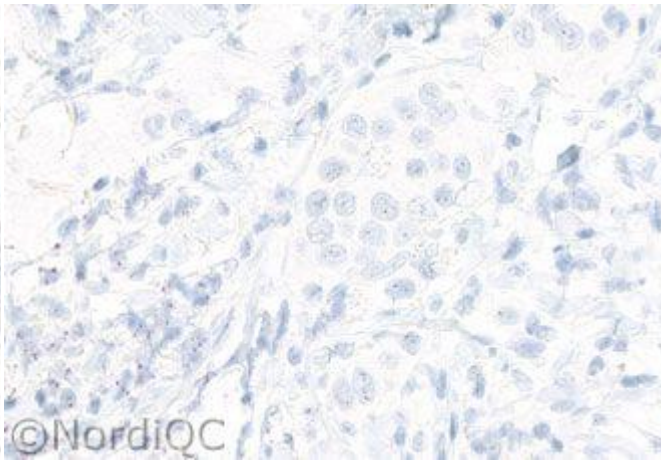


Fig 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 4 without or equivocal gene amplification: HER-2/chr. 17 ratio 1.4 – 1.9*.
The vast majority of the neoplastic cells are negative and only in scattered cells in the left corner HER-2 and chr. 17 signals can be identified. This aberrant reaction most likely was caused by a technical problem during the staining.
Small negative areas were accepted.

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

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