# HER-2 ISH (BRISH or FISH)
Assessment run H4 2013

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:

Table 1. **Material overview for the NordiQC HER-2 ISH assessment, run H4**

	HER-2 IHC*	Dual - SISH**	FISH***
	IHC score	HER-2/chr17 ratio	HER-2/chr17 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
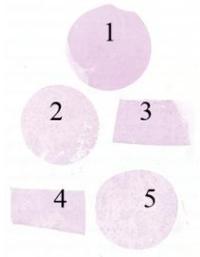
* PATHWAY® (Ventana), data from two reference labs.
** Inform HER-2 Dual ISH kit (Ventana), range of data from two reference labs.
*** HER2 FISH pharmDX™ Kit (Dako) and HER2 FISH (Zytovision), range of data from three reference labs.

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 were 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 excessive retrieval, weak or excessive counterstaining or focal negative areas.
A 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 or a low signal-to-noise ratio due to excessive background staining.
A 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 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 HER-2/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, 3 & 5: non-amplified
- Breast ductal carcinoma no. 2: (highly) amplified
- Breast ductal carcinomas no. 4: non-amplified or equivocal

The ASCO/CAP guidelines, 2007 were applied for the interpretation of the HER-2 status
Unamplified: HER-2 gene/chromosome 17 ratio < 1.8 using a dual probe assay or an average < 4 HER-2 gene copies per cell/nucleus
Equivocal: HER-2 gene/chromosome 17 ratio of 1.8 – 2.2 using a dual probe assay or an average of 4 - 6 HER-2 gene copies per cell/nucleus
Amplified: HER-2 gene/chromosome 17 ratio > 2.2 using a dual probe assay or an average > 6 HER-2 copies per cell/nucleus

Results BRISH, technical assessment
In total 147 laboratories participated in this assessment. 110 laboratories performed BRISH and of these 84 (76 %) 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**	73	Ventana	45	11	10	7	77 %	79 %
INFORM™ HER-2 Dual ISH **780-4332+780-4331**	3	Ventana	1	1	1	0	-	-
DuoCISH pharmDx™ **SK109**	15	Dako	6	5	2	2	73 %	89 %
Zyto*Dot*® 2C **C-3022**	4	ZytoVision	4	0	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	9	Ventana	5	1	2	1	80 %	83 %
Zyto*Dot*® **C-3003**	5	ZytoVision	2	2	1	0	80 %	100 %
"In-house"	1		1	0	0	0	-	-
Total	110		64	20	16	10	76 %	-
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 multi block could be obtained by all BRISH systems.
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 future NordiQC modules, assessment criteria will be additionally based on the 2013 ASCO/CAP HER2 recommendations where a new ISH assay must be performed if more than 25 % of the signals/cells can be interpreted due to artefacts. In these cases the staining will be rated as insufficient.

Optimal protocol settings: Two-colour HER-2 systems
For the **INFORM™ Dual IS 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 subsequently 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 79 % of the submitted protocols (49 of 62). 13 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 the insufficient results could be related to the protocol applied, reagents, the platform being either BenchMark XT, GX or Ultra or other general parameters used by the participants.
The remaining insufficient results were characterized by impaired morphology hampering the interpretation. This pattern was typically caused by excessive retrieval and as a consequence the nuclei were almost totally digested and virtually no counterstaining was seen complicating the interpretation.

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 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 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 89 % of the submitted protocols (8 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** (ZytoVision) optimal results were obtained with proteolysis in pepsin for 2-4 min. (room temperature or 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. Using these protocol settings sufficient results were seen in 100 % of the submitted protocols (4 of 4).

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 83 % of the submitted protocols (5 of 6).

For the **Zyto*Dot*® CISH system C-3003** (ZytoVision), optimal results were obtained with proteolysis in pepsin for 1½ - 3 min. at room temp., HIER in EDTA for 10-20 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 results were seen in 100 % of the submitted protocols (4 of 4).

HER-2 interpretation and scoring consensus:
Scoring sheets were completed by 128 of the 147 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 84 % (27 of 32 laboratories) compared to 71 % for the laboratories using BRISH (54 of 76 laboratories). In this comparison only laboratories with a technically sufficient result were included.

Table 3. **NordiQC FISH amplification data**

	NordiQC FISH HER-2/chr17 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 for both BRISH and FISH, a high consensus rate was observed between participants and NordiQC regarding the HER-2 amplification status in the breast carcinomas tissue cores no. 1, 2 and 5.
In the breast carcinoma no 3, scattered cells showed a slightly increased number of HER2 signals typically in the range of 3-4 signals per cell, but also a corresponding increased number of chr17 signals. The NordiQC reference laboratories observed ratios of 1.3 – 1.7 and thus determined breast carcinoma no. 3 as non-amplified. The overall consensus percentage rate in core 3 was 82 % - a small difference was seen between laboratories using BRISH and laboratories using FISH, see Figs. 1 and 2.
In the breast carcinoma no. 4 scattered tumour cells showed low level of amplification but the overall estimation of the HER-2 amplification was determined to be < 1.9 in the NordiQC reference laboratories and subsequently categorized as non-amplified or equivocal using the ASCO/CAP guidelines, 2007. The vast majority (88%) of the participating laboratories were in agreement.

Conclusion
In this assessment, technically 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 an optimal demonstration.
Retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient sensitivity and preserved morphology.
Attention must also 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.

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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.


HER−2 Amplification in Five Breast Carcinomas
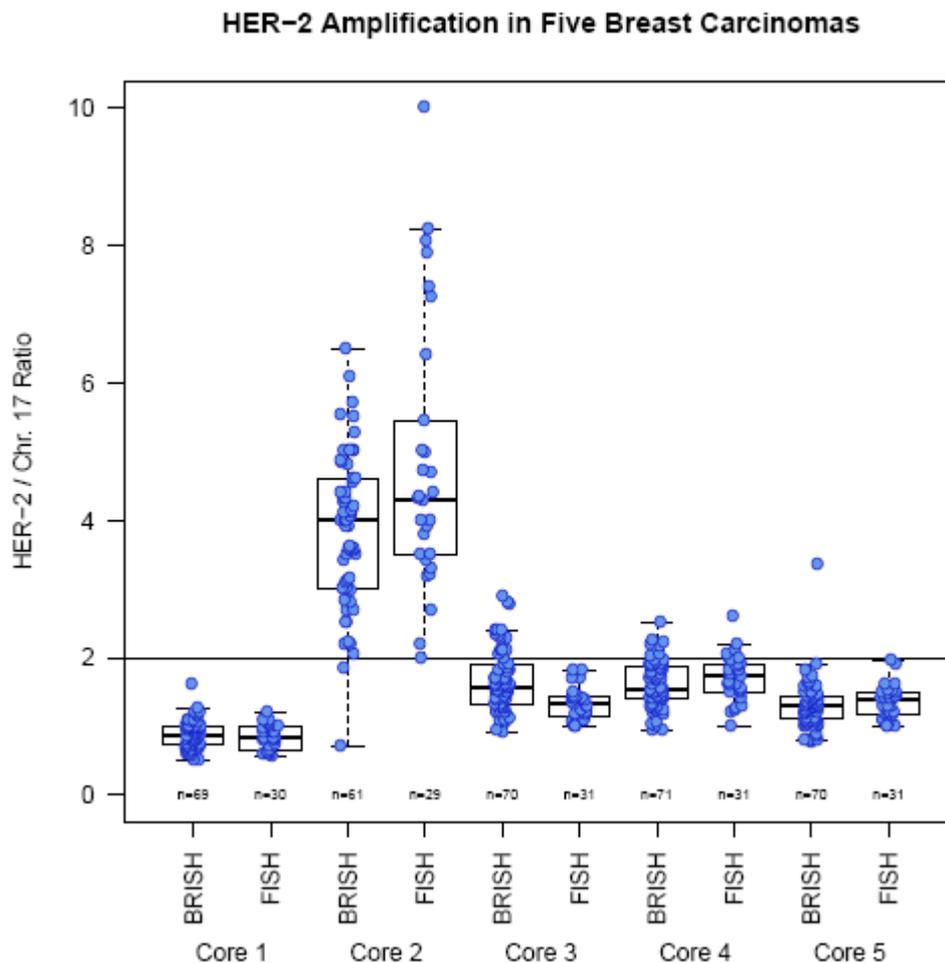
Fig. 2.

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

Consensus between participants and NordiQC reference laboratories
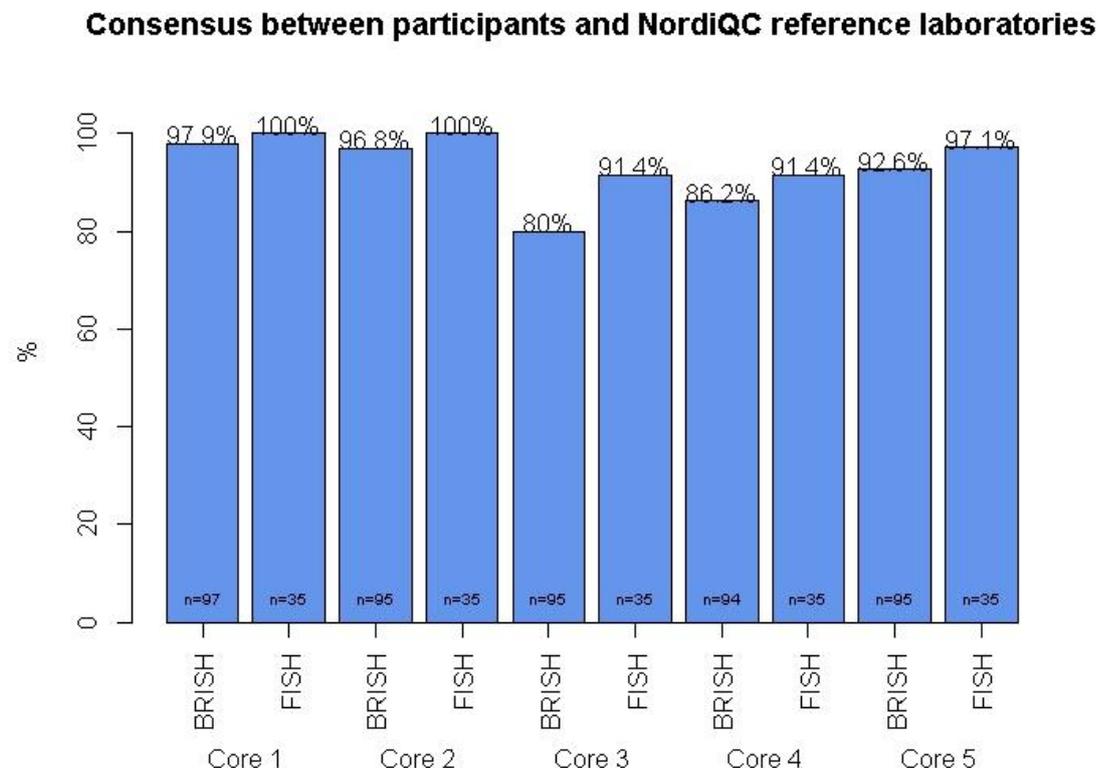
Table 4: **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**
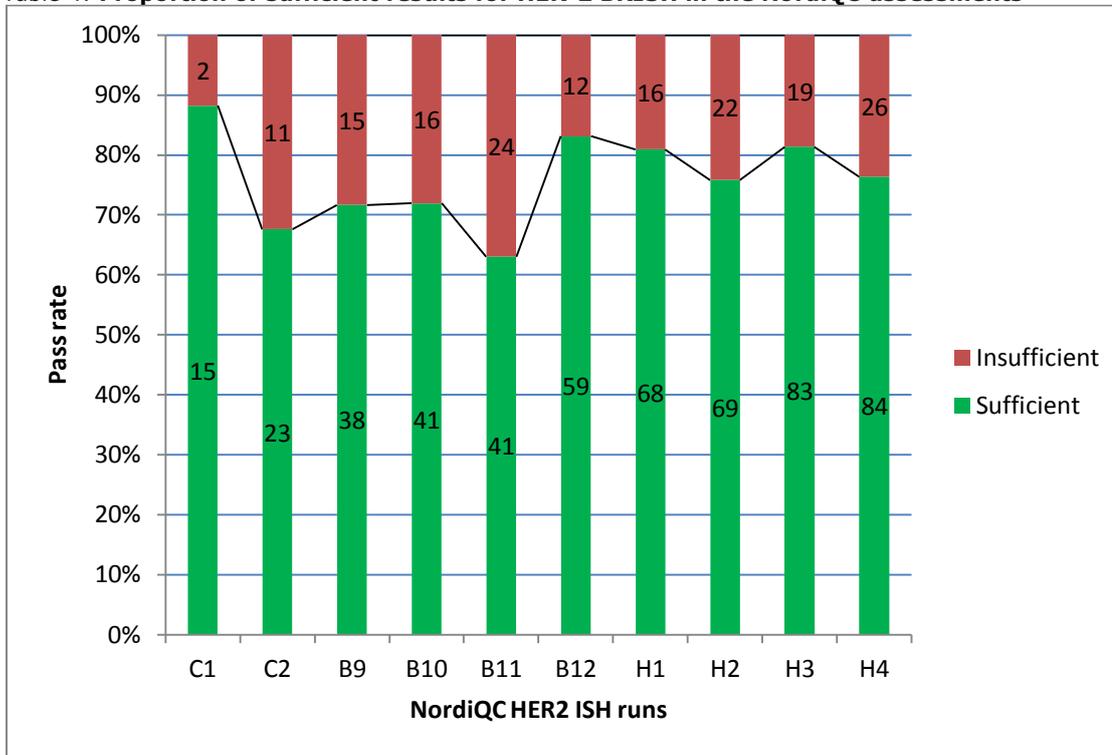
Figures

* Reference: HER2 FISH pharmDX™ Kit, Dako & HER2 FISH, Zytovision (range of data from three reference labs.).
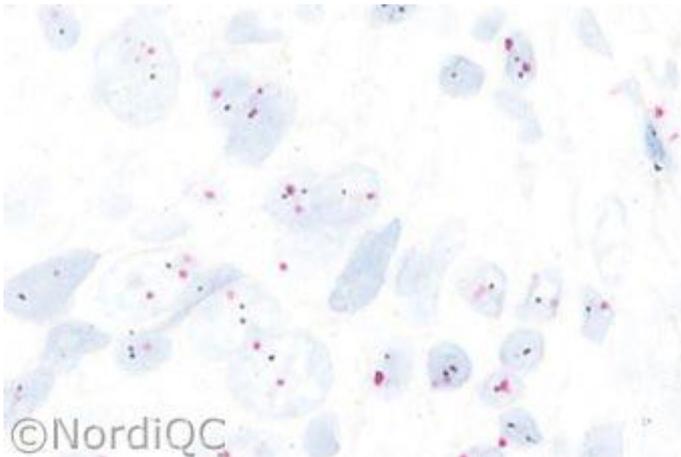
Fig 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 1 without gene amplification: HER-2/chr 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained black and chr17 red.
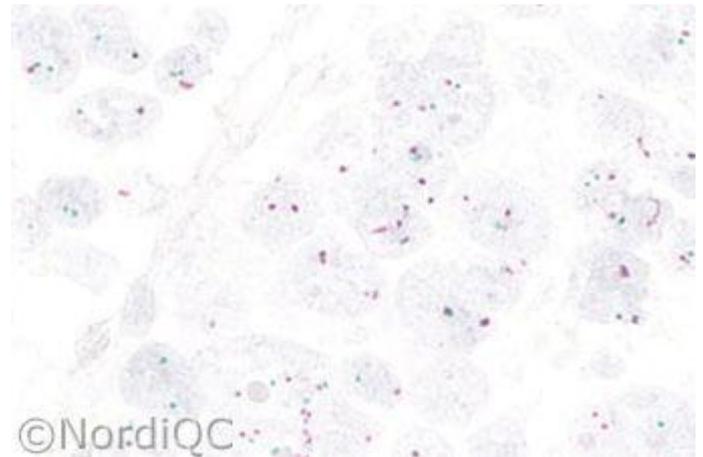
Fig 1b
Optimal demonstration for the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 1 without gene amplification: HER-2/chr 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained green and chr17 red.
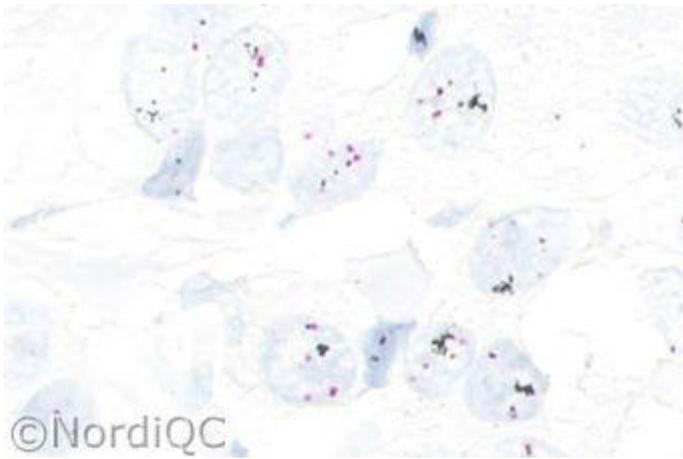
Fig 2a.
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 2 with gene amplification: HER-2/chr 17 ratio 4.3 – 7.7*.
The HER-2 genes are stained black and chr17 red. Some of the Her-2 genes are located in clusters.
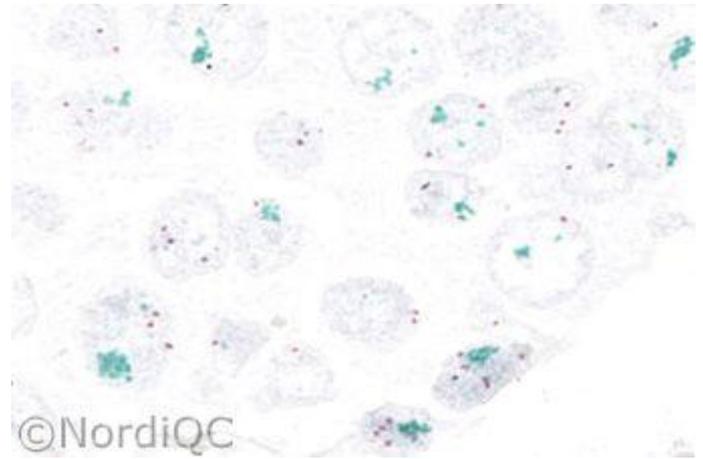

Fig 2b.
Optimal demonstration of the HER-2 gene status using the ZytoDot® 2C, Zytovision of the breast carcinoma no. 2 with gene amplification: HER-2/chr 17 ratio 4.3 – 7.7*.
The HER-2 genes are stained green and chr 17red. Some of the Her-2 genes are located in clusters.
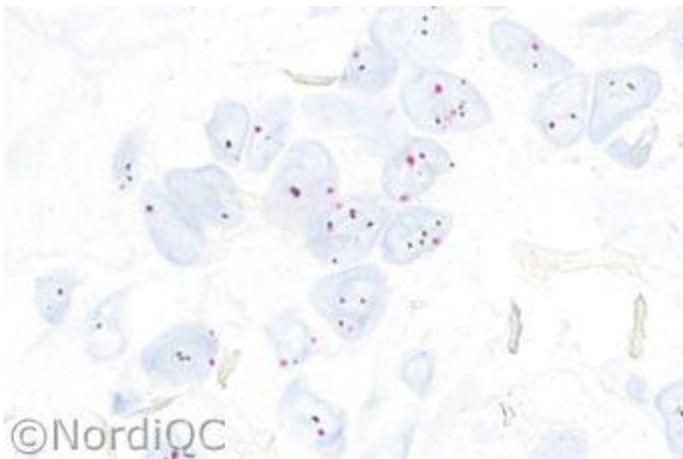
Fig 3a.
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 3 without gene amplification: HER-2/chr17 ratio 1.3 - 1.7*. The HER-2 genes are stained black and chr 17 red.
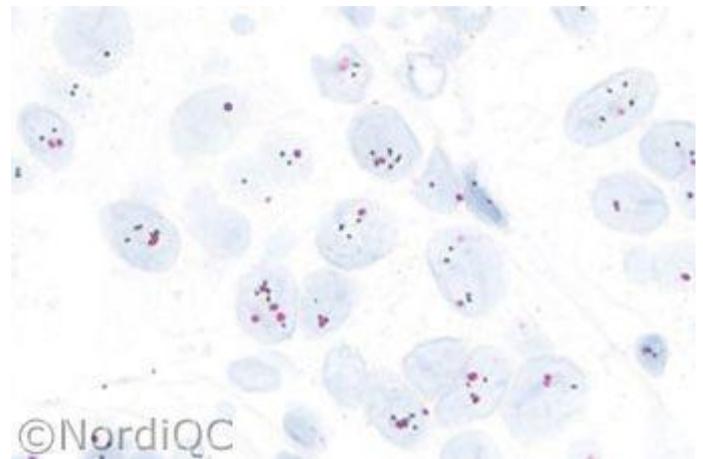

Fig 3b.
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 4 without gene amplification: HER-2/chr17 ratio 1.4 - 1.9*. Single cells show a low level of amplification, but the overall status was interpreted as non-amplified or equivocal by the NordiQC reference labs and most participants.
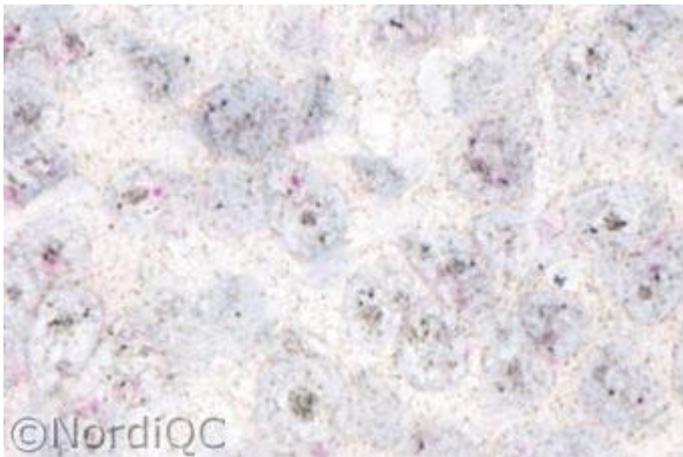

Fig 4a.
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 1 without HER-2 gene amplification: HER-2/chr17 ratio 1.1 – 1.3*. Due to silver precipitates both outside the cells and within in the nuclei, the HER2 gene status can not be interpreted. This aberrant reaction was most likely caused by a technical problem with the BenchMark.
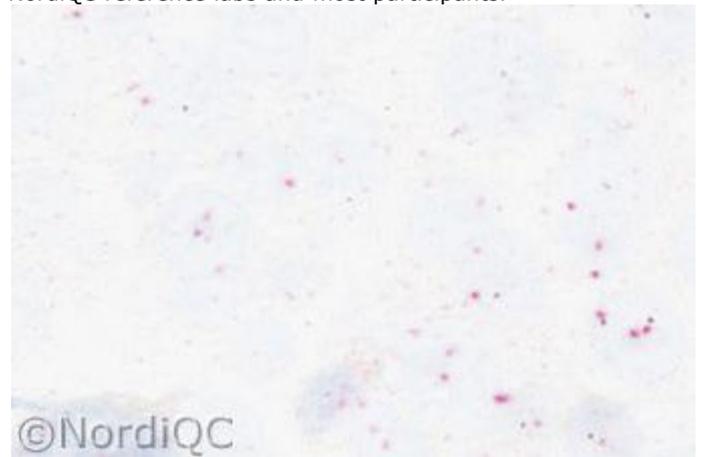

Fig 4b.
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast carcinoma no. 4 without or equivocal gene amplification: HER-2/chr17 ratio 1.4 – 1.9*. The vast majority of the neoplastic cells are negative and only in scattered cells in the right corner HER-2 and chr17 signals can be identified, most likely due a technical problem with the BenchMark.