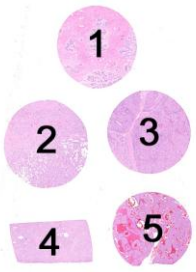
Assessment Run H1 2012

HER-2 ISH (BRISH or FISH)

Material

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

	HER-2 IHC*	**Dual - SISH****	**FISH*****
	IHC score	**HER-2 gene / chromosome 17 ratio**	**HER-2 gene / chromosome 17 ratio**
1. Normal breast tissue	0	1.1 - 1.2	1.1 - 1.3
2. Breast ductal carcinoma	3+	3.5 - 4.0	4.3 - 5.5
3. Breast ductal carcinoma	1+	1.2 - 1.4	1.3 - 1.5
4. Breast ductal carcinoma	2+	1.5 - 1.8	1.8 - 2.2
5. Breast ductal carcinoma	2+	1.3 - 1.6	1.5 - 1.9



* 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 two 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 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 to e.g. due to a weak or excessive counterstaining, excessive retrieval or similar.
A staining was assessed as borderline if one of the tissue could not be properly evaluated due to a too weak signal 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 gene / chromosome 17 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 an (highly) amplified status
- Evaluation of the breast ductal carcinoma no. 4 corresponding an equivocal or low amplified status.
- Evaluation of the breast ductal carcinoma no. 5 corresponding a non-amplified or equivocal status.

Results BRISH

In total 105 laboratories participated in this assessment. 84 laboratories performed BRISH and out of these 68 (81 %) 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	Borderline	Poor	Suff.[1]	Suff. OPS[2]

INFORM™ HER-2 Dual ISH **780-4332+780-4331 800-4422, 780-4422**	57	Ventana	32	12	7	6	77 %	76 %
DuoCISH pharmDx™ **SK109**	9	Dako	3	3	2	1	67 %	75 %
DuoCISH™ **SK108 + K5331**	3	Dako	3	0	0	0	-	-
Unknown	1	Unknown	0	1	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	7	Ventana	6	1	0	0	100 %	100 %
Zyto*Dot*® **C-3003**	4	ZytoVision	4	0	0	0	-	-
SPOT-Light® **84-0150**	2	Invitrogen	2	0	0	0	-	-
"In-house"	1		0	1	0	0	-	-
Total	84		50	18	9	7	-	-
Proportion			60 %	21 %	11 %	8 %	81 %	

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 in all the tissues included in the multi block could be obtained by all the different BRISH systems used by the laboratories. All the 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. However the breast ductal carcinoma, tissue no. 4 was slightly more challenging than the other tissues regarding the protocol settings as this tumour was found to be less robust regarding the pre-treatment conditions and excessive retrieval typically impaired the morphology and thus complicating the identification of the BRISH signals. This pattern was seen for all systems used.

Optimal protocol settings

Two-colour HER-2 systems
For the **INFORM™ Dual ISH system**, Ventana, an optimal demonstration for HER-2 BRISH was in brief typically based upon 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 a sufficient result was seen in 76 % of the submitted protocols (35 out of 46). 11 laboratories used a protocol with optimal settings, but for unexplained reasons a complete false negative staining reaction 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, the main protocol settings giving an optimal result were based on HIER for 10 min in the pre-treatment buffer at 95 - 98°C and proteolysis in Pepsin for 2-3 min. at 37°C or 7 - 10 min. at room temp. (both reagents included in the HER2 CISH pharmDX kit SK109). The HER-2 and the Chr. 17 probes were applied for 14 – 20 hours at 45°C and visualized by the DuoCISH™ kit SK109, Dako.
Using these protocol settings a sufficient result was seen in 75 % of the submitted protocols (6 out of 8). In the insufficient results typically a too weak or false negative staining for the HER-2 signals in both the neoplastic cells and in the normal stromal cells was seen. This observation might be related to a too low sensitivity of the reagents used for the immunohistochemical demonstration of the HER-2 genes. In this context it has to be stressed that it is of utmost importance that the Red chromogene used for the visualization of the HER-2 genes in the DuoCISH™ system is prepared immediately before use and that Pepsin is stored at appropriate conditions in order to maintain the proteolytic capacity.

One-colour HER-2 systems
For the **INFORM™ HER-2 SISH**, Ventana, an optimal result typically was based upon HIER in CC2 or Reaction buffer (RB) for 24-32 min. at 90-95°C and proteolysis in P3 for 4 - 8 min 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 (7 out of 7).

For the **Zyto*Dot*® CISH system C-3003**, ZytoVision, an optimal result typically was obtained by using proteolysis in Pepsin for 3 min at room temp, HIER in EDTA for 10-15 min. at 98°C, hybridization at 37°C for 14-16 hours and visualized by the ZytoVision detection kit C-3003.

For the **SPOT-Light® CISH system 84-0150**, Invitrogen, an optimal result was obtained by using proteolysis in Pepsin for 5-7 min. at room temp, HIER in Tris-EDTA for 15 min. at 98°C, hybridization at 37°C for 14 hours and visualized by Invitrogen detection kit 84-0150.

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, which was completed by 98 out of the 105 laboratories participating in this run. The participants evaluations were compared to the HER2 FISH amplification status generated by the NordiQC reference laboratories. The results are 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 gene / chromosome 17 ratio	**HER2 amplification status**	**Consensus evaluation**
1. Normal breast tissue	1.1 - 1.3	Non-amplified	99 %
2. Breast ductal carcinoma	4.3 - 5.5	Amplified	100 %
3. Breast ductal carcinoma	1.3 - 1.5	Non-amplified	71 %
4. Breast ductal carcinoma	1.8 - 2.2	Equivocal / Low-amplified	43 %
5. Breast ductal carcinoma	1.5 - 1.9	Non-amplified / Equivocal	90 %

A high consensus rate (99-100%) between the participants and NordiQC regarding the determination of the HER2 amplification status was seen for the normal breast tissue no.1 and the highly amplified breast carcinoma, tissue no. 2, whereas a moderately reduced consensus rate (71–90%) was seen in the breast carcinomas. tissues no. 3 and 5, respectively. For the breast carcinoma, tissue no. 4 with a HER-2 gene / chromosome 17 ratio in the range of 1.8 – 2.2 and thus to be categorized as equivocal or low-amplified a low consensus rate of 43 % was seen.
This underlines the need both to focus on the technical set-up for the ISH procedure but also to establish more standardized tools as e.g. image analysis to support and harmonize the interpretation of the ISH results.

This was the 7' assessment of HER-2 BRISH7ISH in NordiQC and as seen in table 3, and a similar pass rate was seen in this run compared to the previous assessment run B12.

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

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

Conclusion
In this assessment an optimal demonstration of HER-2 BRISH could be obtained by the two commercially available two-colour HER-2 systems INFORM™ HER-2 Dual ISH, Ventana and DuoCISH™,Dako.
Also the single-colour HER-2 systems, INFORM™ HER-2 SISH, Ventana, Zyto*Dot*®, ZytoVision and SPOT-Light®, Invitrogen could be used to obtain an optimal demonstration.
For an optimal performance the retrieval settings – HIER + proteolysis - must be carefully balanced to provide an efficient sensitivity and preserved morphology. Attention must also be addressed to the interpretation as a high inter-observer variation was seen.

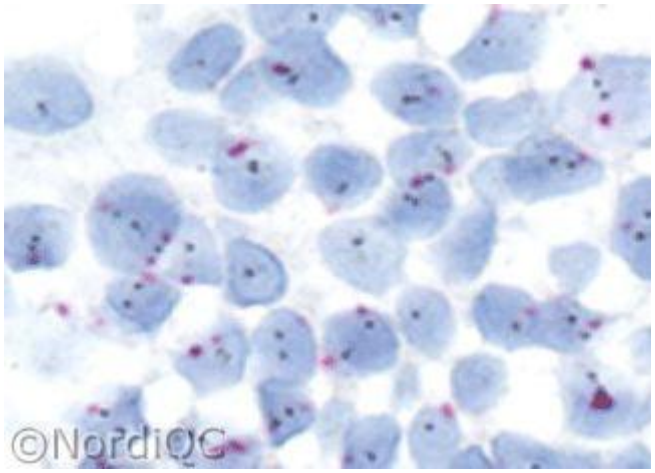


Fig 1a
Optimal staining for the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained black and chr. 17 red.

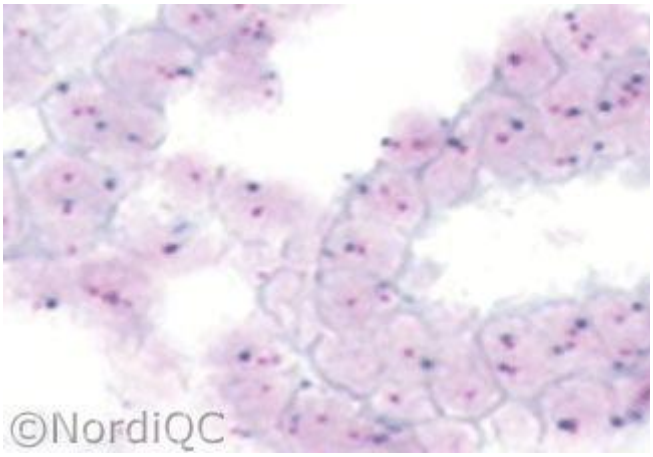


Fig 1b
Optimal staining for the HER-2 gene status using the DuoCISH™, Dako of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained red and chr. 17 blue.

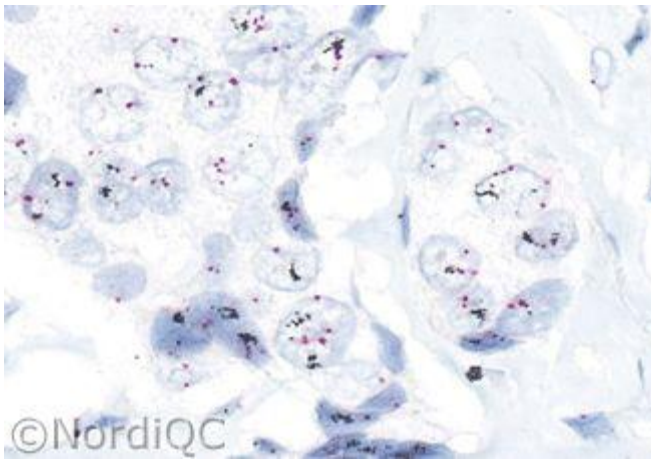


Fig 2a
Optimal staining for the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 5.5*.
The HER-2 genes are stained black and chr. 17 red. Some of the Her-2 genes are located in clusters.

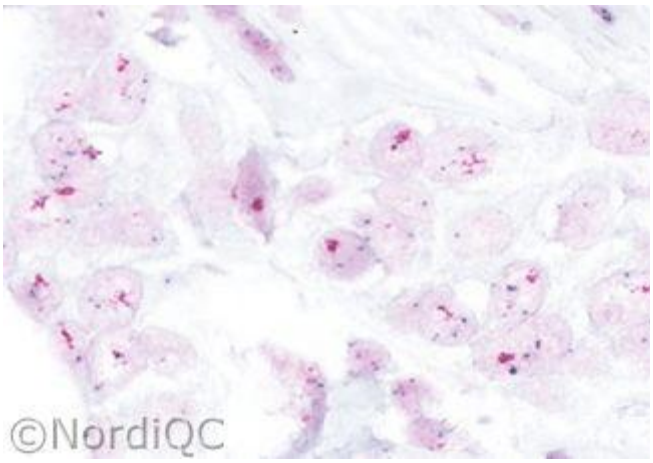


Fig 2b
Optimal staining for the HER-2 gene status using the DuoCISH™, Dako of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 5.5*.
The HER-2 genes are stained red and chr. 17 blue. Some of the Her-2 genes are located in clusters.

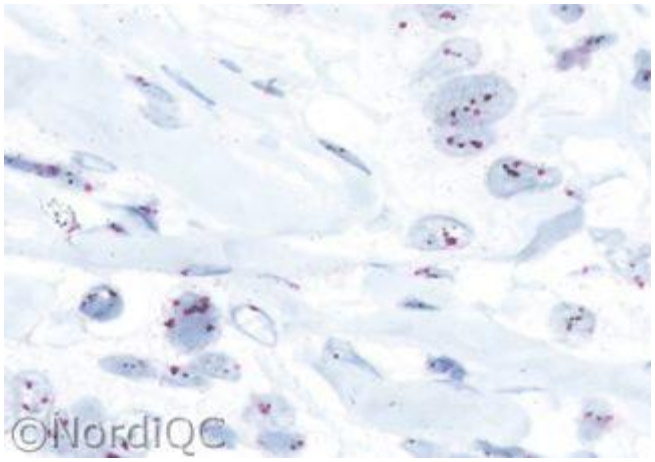


Fig 3a
Optimal staining for the HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal or low level of HER-2 gene amplification: HER-2/chr. 17 ratio 1.8 – 2.2*.
The HER-2 genes are stained black and chr. 17 red.

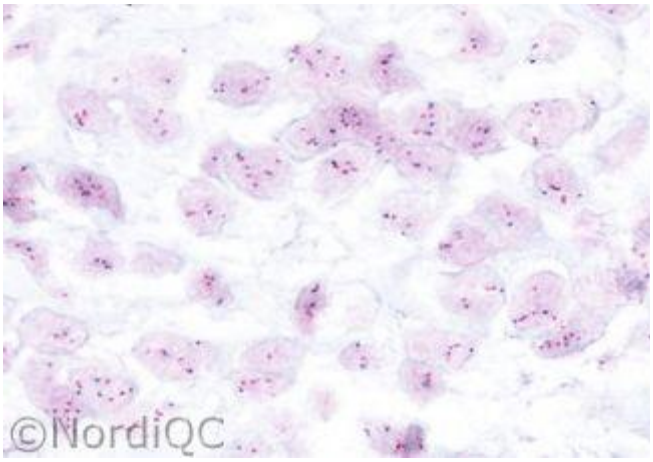


Fig 3b
Optimal staining for the HER-2 gene status using the DuoCISH™, Dako of the breast ductal carcinoma no. 4 with an equivocal or low level of HER-2 gene amplification: HER-2/chr. 17 ratio 1.8 – 2.2*.
The HER-2 genes are stained red and chr. 17 blue.

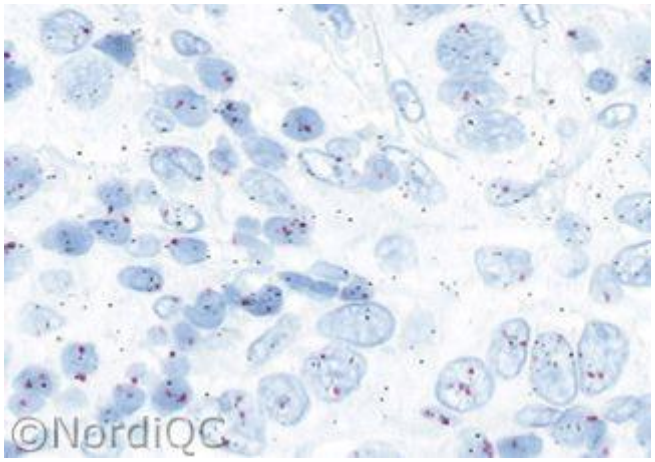


Fig 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal or low level of HER-2 gene amplification: HER-2/chr. 17 ratio 1.8 – 2.2*. Due to silver precipitates both outside the cells and within in nuclei, the HER2 gene status can not be interpreted. This aberrant reaction most likely was caused by a technical problem during the staining.

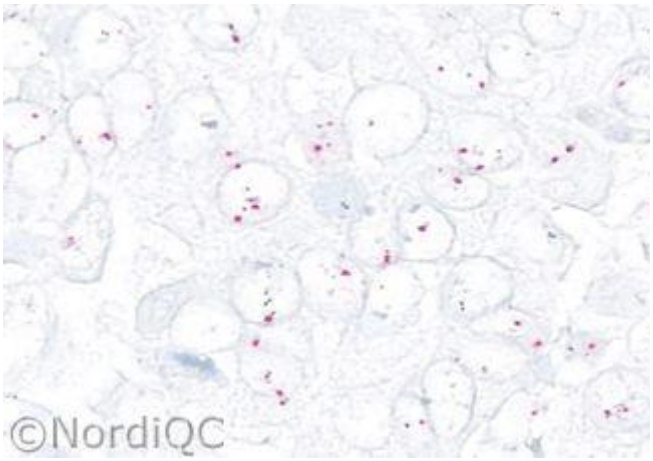


Fig 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 4 with an equivocal or low level of HER-2 gene amplification: HER-2/chr. 17 ratio 1.8 – 2.2*. Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.

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

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