Assessment Run B12 2011
BRISH HER-2

Material

The material circulated for the BRISH (Bright field In Situ Hybridization) HER-2 assessment run B12 was identical to run B11 and comprised one normal breast tissue and five breast ductal carcinomas showing the HER-2 gene/chromosome 17 (chr17) ratios as follows:

	HER-2 IHC*	Dual - SISH**	FISH***
	IHC score	HER-2 gene/ chr17 ratio	HER-2 gene/ chr17 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+	1.8 - 2.1	2.1 - 2.4
5. Breast ductal carcinoma	2+	1.9 - 2.1	2.0 - 2.4
6. Breast ductal carcinoma	2+	1.6 - 2.0	1.7 - 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 (range of data from two reference labs.).

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

Criteria for assessing a BRISH HER-2 analysis as optimal included:

- Staining of the normal breast tissue and the ductal carcinoma no. 3 corresponding a non-amplified status.
- Staining of the breast ductal carcinoma no. 2 corresponding an (highly) amplified status.
- Staining of the breast ductal carcinoma no. 4 & 5 corresponding an equivocal or low amplified status.
- Staining with preserved morphological details and a minimal background reaction.

§ The breast ductal carcinoma no. 6 was only evaluated regarding the ability to demonstrate the HER-2 signals in both the normal and neoplastic cells, whereas the interpretation was not included, because the tumour revealed a range from non-amplified to low amplified in the reference laboratories.

A staining was assessed as good, if the above mentioned criteria were fulfilled, but the interpretation was slightly compromised, e.g., due to a weak or excessive counterstaining, excessive retrieval or similar.

A staining was assessed as borderline if one of the tissue cores could not be properly evaluated due to a too weak signal or too low signal-to-noise ratio.

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

Results

71 laboratories participated in this assessment. 59 (83 %) achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Systems and assessment marks for BRISH HER-2, run B12**

Two colour HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **780-4332+780-4331 800-4422**	40	Ventana	13	19	6	2	80 %	86 %
DuoCISH™ **SK109**	7	Dako	4	2	0	1	83 %	100 %
DuoCISH™ **SK108 + K5331**	5	Dako	3	2	0	0	100 %	100 %

ZytoDot® 2C **C-3032**	1	ZytoVision	0	1	0	0	-	-
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	8	Ventana	5	3	0	0	100 %	100 %
ZytoDot ® **C-3001**	3	ZytoVision	0	1	1	1	-	-
ZytoDot ® **C-3003**	3	ZytoVision	2	0	0	1	-	-
SPOT-Light® **84-0150**	3	Invitrogen	1	2	0	0	-	-
"In-house"	1		1	0	0	0	-	-
Total	71		29	30	7	5	-	-
Proportion			41 %	42 %	10 %	7 %	83 %	

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 multitissue block could be obtained by all the different 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 breast tissue. Even though same fixation and tissue processing conditions were identical for the 6 included tissues, it was observed that the breast ductal carcinoma no. 4 was more challenging than the other tissues regarding the protocol settings. For this tumour the ability to demonstrate the HER-2 signals was highly influenced by the pre-treatment conditions such as excessive retrieval typically impaired the morphology (the nuclei were almost totally digested), 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 typically based upon HIER in Cell Conditioning 2 (CC2) for 28 min. at 86-90°C and proteolysis in P3 for 8 - 12 min at 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 was seen in 86 % of the submitted stains (13 out of 15). 2 laboratories used a protocol with optimal settings, but for unexplained reasons a completely false negative staining was seen, or an excessive background e.g. due to silver precipitates appeared. The remaining insufficient results were typically characterized by an impaired morphology and/or weak demonstration of the HER2 signals. 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. This was typically seen when using CC2 for > 28 min combined with proteolysis in P3 for 16 - 20 min.

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 - 97°C and proteolysis for 2-3 min. in Pepsin at 37°C (both reagents included in the HER2 CISH pharmDX kit SK109). The HER-2 and the Chr. 17 probe was 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 100 % of the submitted protocols (6 out of 6). In the insufficient result a false negative reaction for both the HER-2 and chr17 signals in both the neoplastic cells and in the normal stromal cells was seen and most likely related to insufficient proteolysis in Pepsin, as this was performed for only 3 min. at room temperature. According to the data sheet for SK109, Dako, the Pepsin reagent can be used at room temperature, but the incubation time should be approximately 8 min.

One-colour HER-2 systems
For the **INFORM™ HER-2 SISH**, Ventana, an optimal demonstration for HER-2 BRISH was typically based upon HIER in CC2 or Reaction buffer (RB) for 24-36 min. at 86-96°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 (6 out of 6).

For the **ZytoDot® CISH system C-3003**, ZytoVision, an optimal result was obtained by using proteolysis in Pepsin for 3 min at room temp., HIER in EDTA for 15-20 min. at 98°C, hybridization at 37°C for 14-16 hours and visualized by the ZytoVision detection kit C-3003. The protocols were applied according to the recommendations given in the package insert from ZytoVision.

For the **SPOT-Light® CISH system 84-0150**, Invitrogen, an optimal result was obtained by using proteolysis in Pepsin for 5 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. The protocols were applied according to the recommendations given in the package insert from Invitrogen.

The laboratories were requested to send in their own interpretation on the stained sections, which was completed by 55 out of the 59 laboratories obtaining a sufficient mark (optimal or good). 11 out of these (20 %) interpreted and classified all the tissues/tumours in concordance to the HER-2 gene / chr17 statuses generated in the reference laboratories.
The discrepancies between the interpretations between the laboratories and the NordiQC data were mainly related to the breast ductal carcinomas no. 4, 5 and 6, which typically were interpreted as non-amplified (in this context it has to be mentioned that the tumour no. 6 was excluded due to non-conclusive data from the reference laboratories).
The 3 breast ductal carcinomas no. 4 - 6 were all very challenging regarding the interpretation as all 3 showed either an equivocal or low level of amplification, which most likely explains the low consensus rate. In comparison a consensus rate of 89 % (49 out of 55 laboratories) was seen between the participants and NordiQC regarding the interpretation in the normal breast tissue no. 1, the breast ductal carcinomas no. 2 with a high level of amplification and the non-amplified breast carcinoma no. 3.

This was the 6th NordiQC HER-2 BRISH assessment. As seen in table 2, a significantly higher pass rate was seen in this run compared to the previous assessment run B11 (based on the same material circulated).

Table : Proportion of sufficient results for HER-2 BRISH in the six NordiQC runs performed

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

In the previous assessment of HER-2 BRISH, a total of 24 laboratories obtaining an insufficient result were given specific recommendations how to improve their protocol - typically to reduce the pre-treatment. 18 laboratories submitted a new stain for the current run. 12 followed the recommendations given of which10 improved their result to good or optimal (83 %). 6 laboratories did not follow the recommendations, and 2 of these (33 %) obtained a sufficient staining in the current run.

Conclusion

In this assessment an optimal demonstration of HER-2 using 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, ZytoDot ®, 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 a high sensitivity without hampering the morphology. Attention must also be addressed to the interpretation as only 20 % of the laboratories gave a correct interpretation in concordance with the NordiQC reference data.

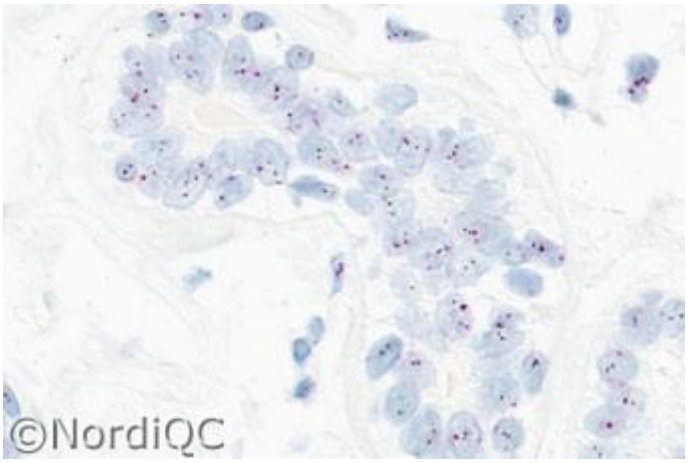

Fig. 1a
Optimal staining for HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained black and chr. 17 red.

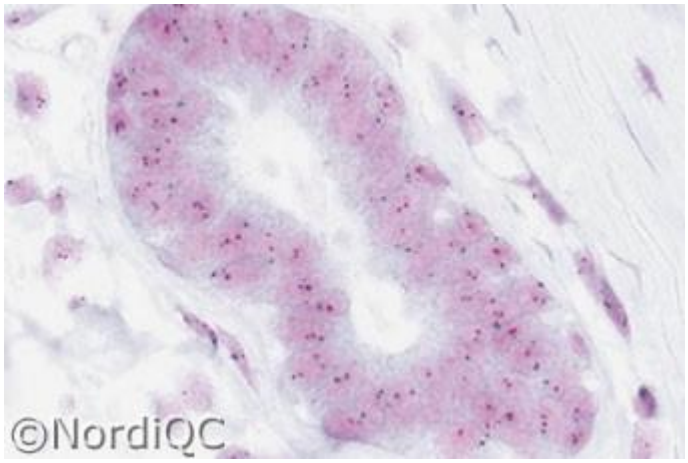

Fig. 1b
Optimal staining for HER-2 gene status using the DuoCISH™, Dako of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1 - 1.3*.
The HER-2 genes are stained red and chr. 17 blue.

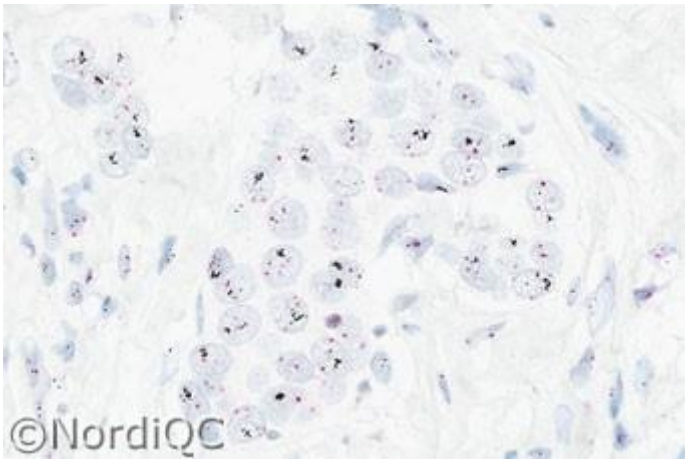

Fig. 2a
Optimal staining for HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 5.5*.
The HER-2 genes are stained black and chr. 17 red. The Her-2 genes are located in cluster.

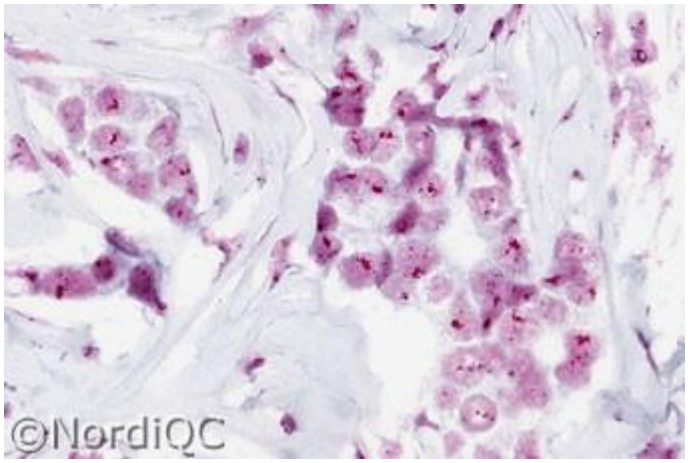

Fig. 2b
Optimal staining for HER-2 gene status using the DuoCISH™, Dako of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio 4.3 – 5.5*.
The HER-2 genes are stained red and chr. 17 blue. The Her-2 genes are located in cluster.

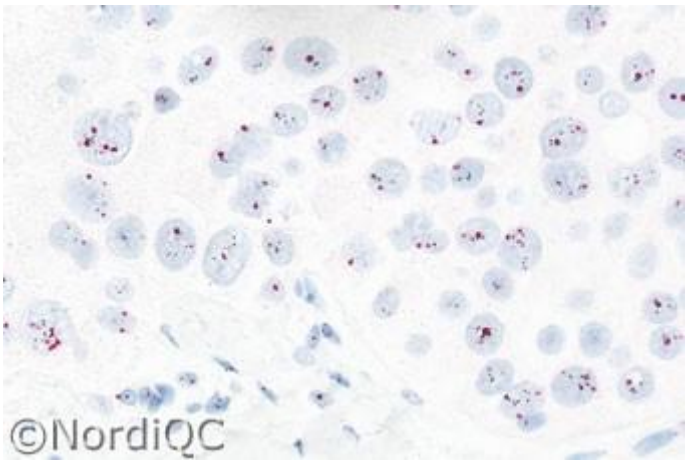

Fig. 3a
Optimal staining for HER-2 gene status using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 5 with a low level of HER-2 gene amplification: HER-2/chr. 17 ratio 2.0 – 2.4*.
The HER-2 genes are stained black and chr. 17 red.

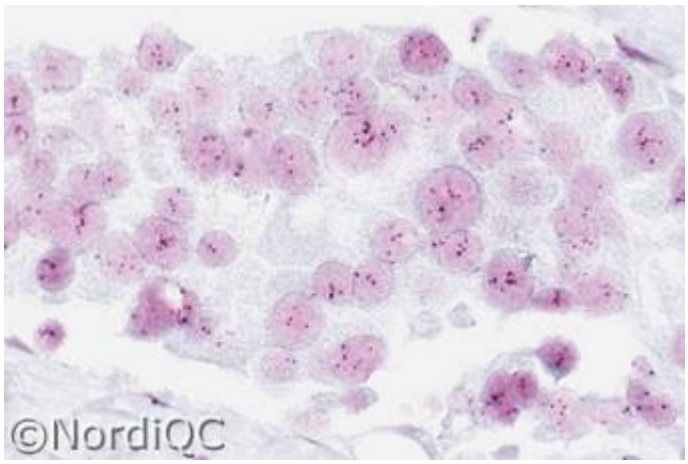

Fig. 3b
Optimal staining for HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 5 with a low level of HER-2 gene amplification: HER-2/chr. 17 ratio 2.0 – 2.4*.
The HER-2 genes are stained red and chr. 17 blue.

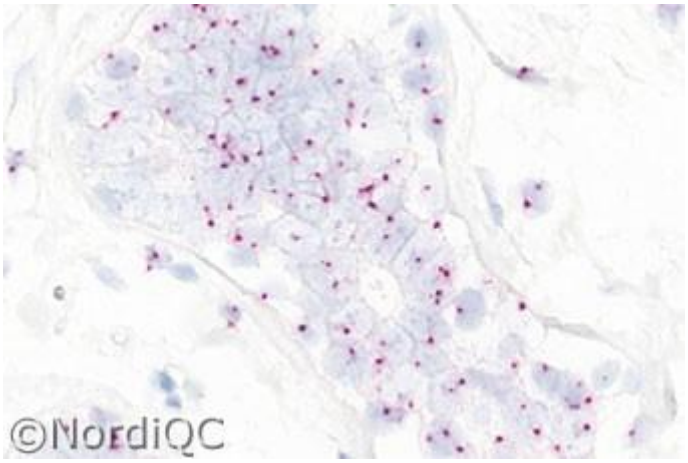

Fig. 4a
Insufficient staining for HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the normal breast tissue no. 1 without gene amplification. Only scattered HER-2 signals can be identified, and only the chr. 17 signals can be easily be interpreted. This aberrant reaction may be caused by inadequate protocol settings for the HER-2 gene demonstration as inappropriate pre-treatment or a technical problem during the staining.

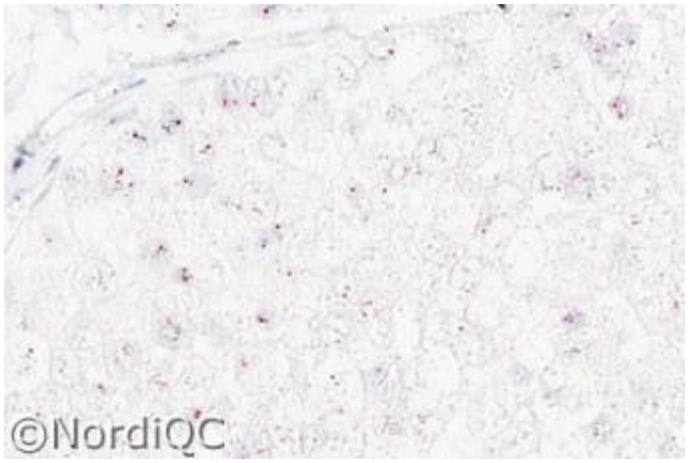

Fig. 4b
Insufficient staining for HER-2 gene using the INFORM™ Dual ISH kit, Ventana of the breast ductal carcinoma no. 3 without HER-2 gene amplification.
Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.

* Reference: HER-2 FISH pharmDX™ Kit, Dako (range of ratio from two tests performed in two reference laboratoriess).

SN/MV/LE 6-12-2011