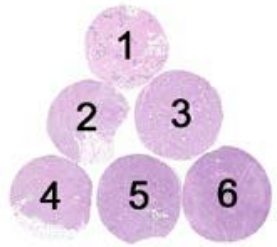
Assessment Run B10 2010

HER-2 BRISH

The material circulated for HER-2 BRISH (Brightfield In Situ Hybridization) assessment run 10 was identical to the material used in run B9, 2010 and comprised normal breast tissue & 5 breast ductal carcinomas showing HER-2 gene/chromosome 17 (HER-2/chr17) ratios as follows:

	HER-2/chr17 ratio		
	Duo - CISH*	**Dual - SISH****	**FISH*****
1. Normal breast tissue[1]	1.1	1.0	1.1
2. Breast ductal carcinoma	> 6.0	> 6.0	> 6.0
3. Breast ductal carcinoma	1.4	1.3	1.3
4. Breast ductal carcinoma	2.5	2.6	2.3
5. Breast ductal carcinoma	2.2	2.7	2.3
6. Breast ductal carcinoma	2.4	2.8	2.6



* HER-2 DuoCISH™ kit, Dako (data from one ref. lab.)
** HER-2 Dual SISH kit, Ventana (average of data from two ref. labs.),
*** HER2 FISH pharmDX™ Kit, Dako (average of data from three tests perfomed in one ref. lab.).
All carcinomas were fixed for 24 h in 10 % neutral buffered formalin (NBF), except for the carcinoma in tissue core nos. 5 and 6, the same tumour fixed for 48 and 72 h. respectively.

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 breast ductal carcinomas no. 2, 4, 5 and 6 corresponding an amplified status
- Staining with preserved morphological details and a minimal background reaction.

A staining was assessed as good, if the above mentioned criteria were fulfilled for the normal breast tissue (no. 1) and the three ductal carcinomas fixed for 24 h (no. 2-4), but not fulfilled for the carcinoma no. 5 and 6 fixed for 48 and 72 h. respectively in NBF. Even though carcinoma no. 6 was overfixed according to the ASCO/CAP guidelines, it was from a technical perspective important to see if the labs could carry out a successful CISH/SISH procedure also for this tumour in spite of prolonged fixation. The tumour had successfully been evaluated by FISH for HER-2 in the NordiQC reference laboratories.
A staining was assessed as borderline if one of the other carcinomas could not be properly evaluated due to a too weak signal or a low signal-to-noise ratio.
A staining was assessed as poor in case that more of the other carcinomas could not be properly evaluated.

Results

57 laboratories participated in this assessment. 41 (72 %) achieved a sufficient mark. The results are summarized in Table 1.

Table 1. **Abs and assessment marks for CISH/SISH HER-2, run B10**

Two colour HER-2 systems	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.[1]**
INFORM™ HER-2 Dual SISH	27	Ventana	10	11	3	3	77 %
DuoCISH™	11	Dako	3	4	3	1	64 %
ZytoDot® 2 C	2	ZytoVision	0	2	0	0	-
One colour HER-2 systems							
SPOT-Light®	4	Invitrogen	0	3	1	0	-
ZytoDot ®	7	ZytoVision	0	3	1	3	43 %

INFORM™ HER-2 SISH	4	Ventana	2	1	0	1	-
"In-house"	2		1	1	0	0	-
Total	57		16	25	8	8	-
Proportion			28 %	44 %	14 %	14 %	72 %

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

Comments

In this assessment and in accordance with the previous run B9 for HER-2 BRISH, the two most widely BRISH systems used, the INFORM™ Dual SISH system, Ventana, and the DuoCISH™, Dako could both be used to obtain an optimal demonstration of the HER-2 gene amplification status in the tissues included in the multitissue block. In this assessment also the single colour HER-2 SISH system, Ventana could be used to obtain an optimal demonstration of HER-2 BRISH.

The most widely used protocol for the INFORM™ Dual SISH system, Ventana giving an optimal demonstration for HER-2 BRISH was in brief based upon HIER in Cell Conditioning reaction buffer (CCrb) for 40 – 48 min. at 90°C and proteolysis in P3 for 8 - 12 min. 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.

For the DuoCISH™ system, Dako, the main protocol settings were based on HIER for 10 min in the pre-treatment buffer at 95 - 99°C and proteolysis for 2 min. in Pepsin at 37°C (both reagents included in the FISH pharmDX kit K5331, Dako). The HER-2 and the Chromosome 17 probe (K5331, Dako) was applied for 14 – 20 hours at 45°C and visualized by the DuoCISH™ kit SK108, Dako.

The prevalent feature of an insufficient result was typically a general too weak or completely negative reaction of the HER-2 signals in both the neoplastic cells and in the normal stromal cells. This feature can be related to many factors as e.g. insufficient retrieval or too low sensitivity of the detection system applied. For the INFORM™ Dual SISH, Ventana the insufficient retrieval most commonly was noticed as short HIER in CCrb for 20 – 28 min or HIER in CC1. In a few laboratories an insufficient result was obtained despite optimal protocol settings was applied. At present NordiQC can not identify any reason for this.

For the DuoCISH™ system, Dako the insufficient retrieval seemed to be related to insufficient proteolysis in Pepsin – too short time and/or reduced enzymatic capacity of the applied Pepsin. Pepsin is a relative fragile enzyme and rapidly deteriorates if stored at room temperature. Pepsin should always be stored at 2 - 6°C and kept on ice when taken out of the refrigerator to secure optimal storage conditions.

In a few laboratories it was also observed that the insufficient result mainly was related to the demonstration of the HER-2 signals, whereas the chr17 signals were distinctively demonstrated. 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.

It was also seen that excessive retrieval, most likely due to a too harsh proteolysis in Pepsin, impaired the morphology as the nuclei were almost totally digested, complicating the identification of the BRISH signals. This observation was not related to any specific system.

As discussed in run B9, the breast ductal carcinoma no. 5 and 6, fixed for 48 and 72 hours respectively, was much more challenging in order to perform an optimal BRISH. This is most likely due to the prolonged fixation in NBF compared to the 24 hours fixation time for the 4 other tissues included in the multitissue block. However, an interpretation was possible for the 16 laboratories assessed as optimal. For unexplained reasons the tumour tissue no. 5 fixed for 48 h. was much more difficult to stain than the same tumour no. 6 fixed for 72 h.

The laboratories were requested to send in their own interpretation on the stained sections. As regards amplification vs. non-amplification 31 out of the 39 laboratories (80 %) assessed as optimal or good and returning their own interpretation of the tumours, interpreted and classified the tumours in concordance to the HER-2/chr17 statuses generated in the reference laboratories. 10 out of the 31 laboratories interpreted the low level amplified tumour no. 4 (ratio of 2.3 - 2.6) as equivocal, which was accepted, as the tumour according to general accepted guidelines would have been re-evaluated. However, potentially the tumour could erroneously be classified as non-amplified.

In the remaining 8 cases, 5 laboratories classified the low amplified tumour, tissue specimen no. 4 as non-amplified and 3 laboratories classified the non-amplified tumour, tissue specimen no. 3 as amplified.

In total 31 out of the 54 laboratories (57%) participating in this assessment B10 and returning the slip obtained a sufficient mark (good or optimal) and interpreted the result in concordance to the reference HER-2 /chr17 statuses.

This was the fourth assessment of HER-2 BRISH in NordiQC. The pass rate was the same as in the previous run (Table 2).

Table 2. **Proportion of sufficient results for HER-2 BRISH in four NordiQC runs**

	Run C1 2009	Run C2 2009	Run B9 2010	Run B10 2010
Participants, n=	17	34	53	57
Sufficient results	88 %	68 %	72 %	72 %

Conclusion

In this assessment an optimal demonstration of HER-2 BRISH could be obtained by the two-colour HER-2 systems INFORM™ HER-2 Dual SISH, Ventana and DuoCISH™, Dako. Also the single-colour HER-2 system, INFORM™ HER-2 Dual SISH, Ventana 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. Only 57 % of the laboratories obtained a sufficient result together with a correct interpretation.

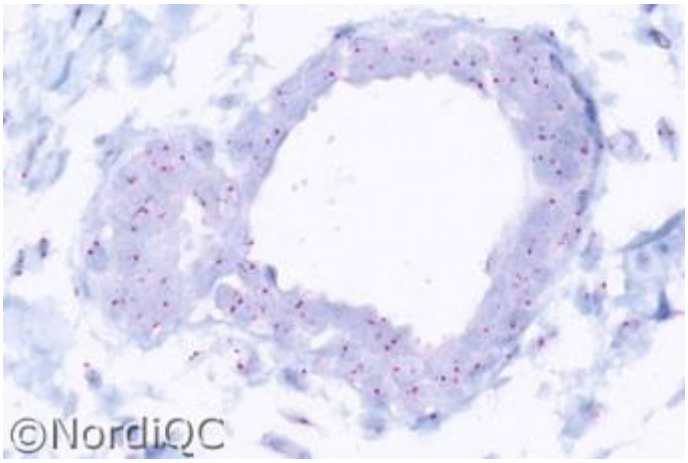


Fig. 1a
Optimal staining for HER-2 gene status using the INFORM™ Dual SISH kit, Ventana, of the normal breast tissue no. 1 without gene amplification: HER-2/chr17 ratio 1.1‡. The HER-2 gene is stained black, while chr17 is stained red.

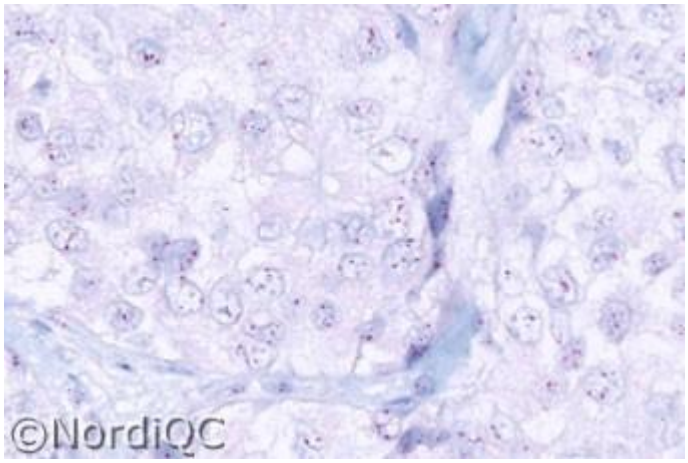


Fig. 1b
Optimal staining for HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 3 without gene amplification: HER-2/chr17 ratio 1.3*‡. The HER-2 gene is stained black, while chr17 is stained red.

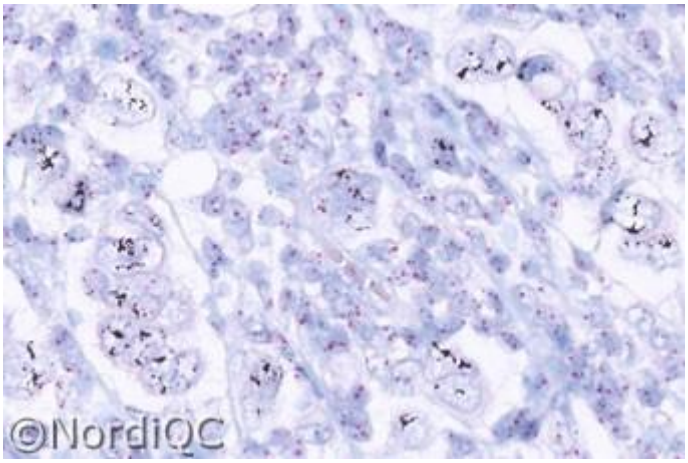


Fig. 2a
Optimal staining for HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr17 ratio > 6.0‡. The HER-2 gene is stained black and located in clusters, while chr17 is stained red.

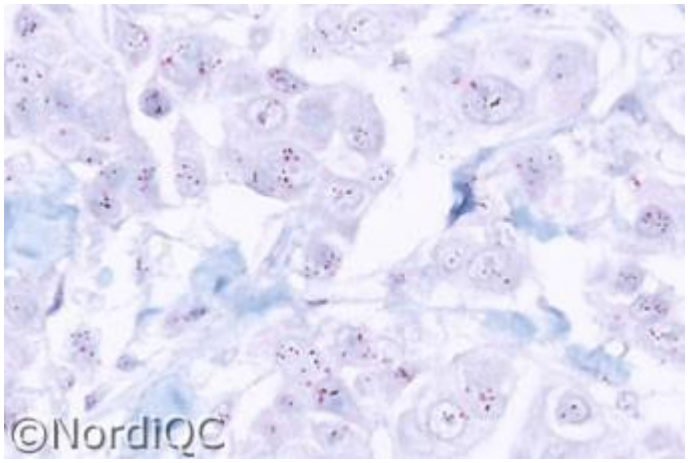


Fig. 2b
Optimal staining for HER-2 gene status using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification: HER-2/chr17 ratio 2.3‡. The HER-2 gene is stained black (increased number & small clusters), while chr17 is stained red.

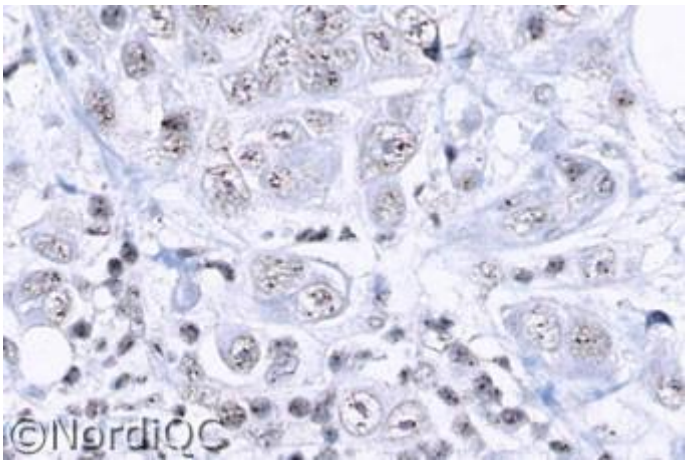


Fig. 3a
Insufficient staining for HER-2 gene using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification. Due to excessive silver deposits it is impossible reliably to identify and count the HER-2 and chr17 signals. A combination of excessive cell conditioning / proteolysis, and insufficient stringency washing may be the cause for this reaction pattern.

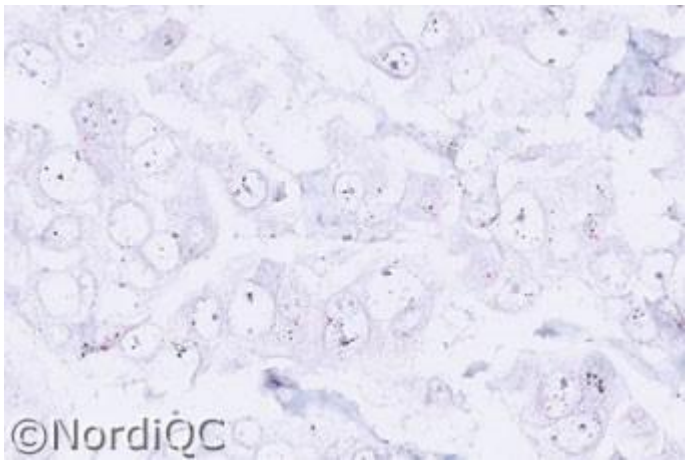


Fig. 3b
Insufficient staining for HER-2 gene using the INFORM™ Dual SISH kit, Ventana of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification. Due to excessive proteolytic pre-treatment the nuclear morphology is severely impaired complicating the interpretation.

‡ Reference: HER2 FISH pharmDX™ Kit, Dako: Average of data from three tests performed in reference labs.

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