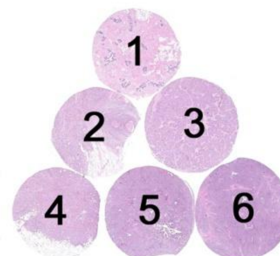
Assessment Run B9 2010

HER-2 BRISH

The slide to be stained for HER-2 BRISH (Bright field in situ hybridization)comprised normal breast tissue and breast ductal carcinomas showing HER-2 gene/chromosome (chr) 17 ratios as follows:

	Duo - CISH*	Dual - SISH**	FISH***
	HER-2 gene/ chr 17 ratio	HER-2 gene/ chr 17 ratio	HER-2 gene/ chr 17 ratio
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 reference lab.),
** INFORM™ HER-2 Dual SISH kit, Ventana (average of data from two reference labs.),
*** HER2 FISH pharmDX™ Kit, Dako (average of data from two reference labs.).
1 Tissue erroneously stated as breast ductal carcinoma in accompany letter.



All carcinomas were fixed for 24 h in 10 % neutral buffered formalin (NBF), except for the carcinoma no. 5 and no. 6, which were fixed for 48 and 72 h., respectively – same tumour.

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

- Staining of the normal breast tissue and 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. in NBF. It could be argued that this tumour should be excluded from the assessment, as the tissue was not processed similar to the other tissues and for one of them not according to the recommended ASCO/CAP guidelines (fixation time of 6 – 48 h in NBF). However, from a technical perspective it was valuable to see if some laboratories could carry out a successful BRISH procedure also for this tumour in spite of prolonged fixation. The tumours had previously been successfully evaluated by FISH for HER-2 in two NordiQC reference laboratories.

A staining was assessed as borderline if one of the other tissues (no. 1-4) 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 two or more of the other tissues (no. 1-4) could not be properly evaluated.

Results

53 laboratories participated in this assessment. 38 (72 %) achieved a sufficient mark. The systems and marks are summarized in Table 1.

Table 1. **Systems and assessment marks for CISH/SISH HER-2**

Two colour HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]
INFORM™ HER-2 Dual SISH	23	Ventana	7	11	3	2	78 %
DuoCISH™	13	Dako	6	3	3	1	82 %
ZytoDot® 2 C	3	ZytoVision	0	1	2	0	-
One colour HER-2							

systems							
SPOT-Light®	5	Invitrogen	0	3	0	2	-
ZytoDot®	4	ZytoVision	0	3	1	0	-
INFORM™ HER-2 SISH	3	Ventana	2	0	0	1	-
“In-house”	2		0	2	0	0	-
Total	53		15	23	9	6	-
Proportion			28 %	44 %	17 %	11 %	72 %

1) Proportion of sufficient stains.

Comments

In this assessment and in accordance with the pilot runs for HER-2 BRISH, both the Dual SISH system, Ventana and the DuoCISH™, Dako could be used to obtain an optimal demonstration of the HER-2 gene amplification status in the tissues included in the multitissue block.

The most successful protocol for the Dual SISH system, Ventana, was in brief based upon HIER in 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 chr. 17 probe (K5331, Dako) was applied for 14 – 20 hours at 45˚C and visualized by the DuoCISH™ kit SK108, Dako.

The insufficient results were typically due to either generally too weak or completely negative signals in both the neoplastic cells and in the normal stromal cells. This feature most likely was due to insufficient epitope retrieval. For the INFORM™ Dual SISH, Ventana, the insufficient retrieval most commonly was seen as a too short HIER in CCrb for 20 – 28 min or HIER in CC1. For the DuoCISH™ system, Dako and the SPOT-Light®, Invitrogen, 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.
It was also seen that excessive retrieval, most likely due to a too harsh proteolysis, impaired the morphology as the nuclei were almost totally digested, thus complicating the identification and interpretation of the BRISH signals.

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 15 laboratories assessed as optimal. For unexplained reasons the tumour tissue fixed for 48 h caused more difficulties than the tissue fixed for 72 h. Interestingly, four laboratories performed FISH on the material and all four laboratories correctly interpreted both tumours as amplified. Three used HER-2 FISH from Vysis, PathVision and one HER-2 FISH from Kreatech.

The laboratories were requested to send in their own interpretation on the stained sections. As regards amplification vs. non-amplification 26 out of the 35 laboratories assessed as optimal or good and returning their own interpretation of the tumours, classified the tumours correctly and in concordance to the HER-2 gene / chr 17 status generated in the reference laboratories. In the remaining 9 cases, 7 laboratories classified the low amplified tumour, tissue specimen no. 4 as non-amplified and 2 laboratories classified the non-amplified tumour, tissue specimen no. 3 as amplified.

This was the third assessment of HER-2 BRISH in NordiQC. The pass rates (proportion of sufficient results) are shown in Table 2.

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

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

Conclusion

In this assessment the commercially available two-colour HER-2 systems INFORM™ HER-2 Dual SISH, Ventana, and DuoCISH™, Dako, were the most successful methods for the determination of the HER-2 gene status. For an optimal performance the retrieval settings (HIER + proteolysis) must be carefully balanced to provide an efficient sensitivity and preserved morphology.

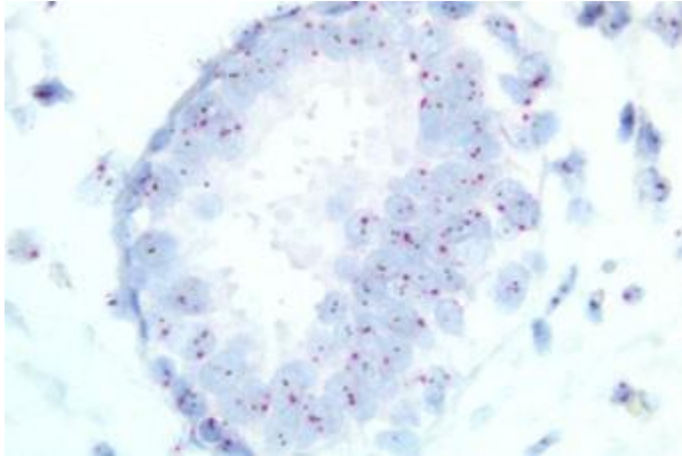


Fig. 1a
Optimal staining for HER-2 gene using the Dual SISH kit, Ventana, of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1*. The HER-2 gene is stained black, while chr. 17 is stained red.

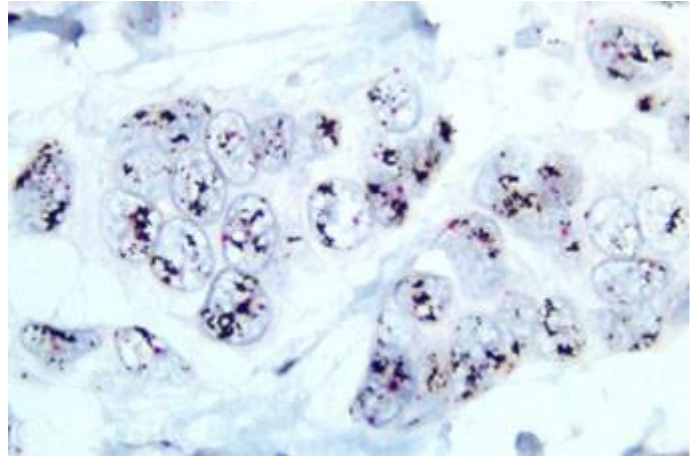


Fig. 1b
Optimal staining for HER-2 gene using the Dual SISH kit, Ventana of the breast ductal carcinoma no. 2 with gene amplification: HER-2/chr. 17 ratio > 6.0*. The HER-2 gene is stained black and located in clusters, while chr. 17 is stained red.

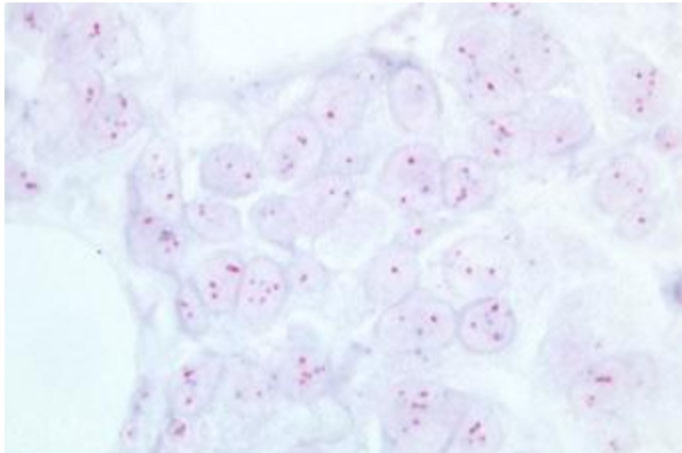


Fig. 2a
Optimal staining for HER-2 gene using the DuoCISH kit, Dako of the breast ductal carcinoma no. 4 with a low level of HER-2 gene amplification: HER-2/chr. 17 ratio 2.3*. The HER-2 gene is stained red, while chr. 17 is stained blue.

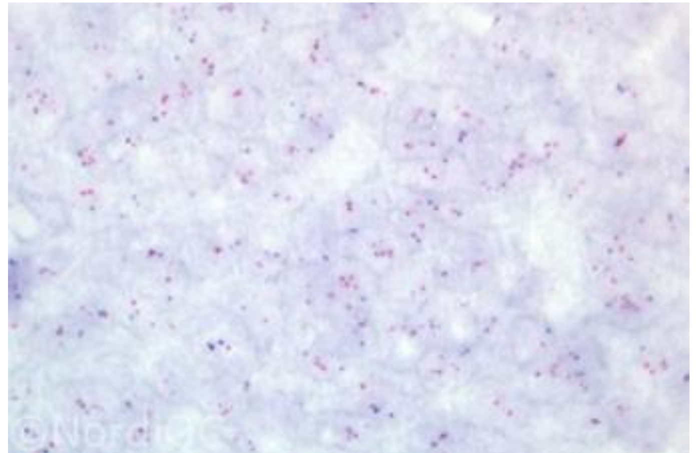


Fig. 2b
Optimal staining for HER-2 gene using the DuoCISH kit, Dako of the breast ductal carcinoma no. 6 (fixed for 72 h in NBF) with a low level of HER-2 gene amplification: HER-2/chr. 17 ratio 2.6*. The HER-2 gene is stained red, while chr. 17 is stained blue. It is difficult to see the individual cell borders but still possible to assess a gene amplification ratio.

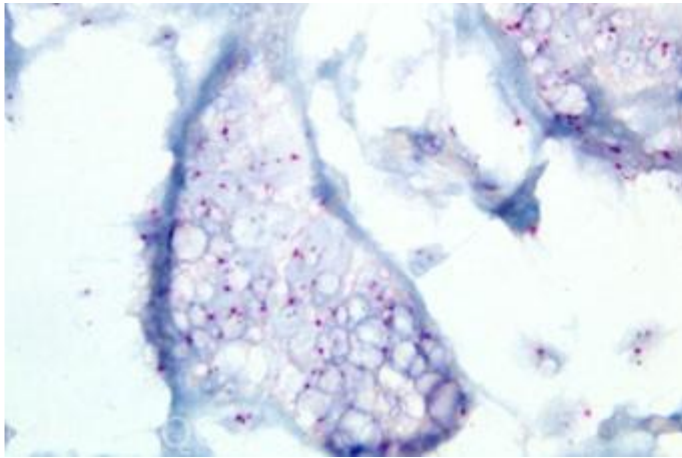


Fig. 3a
Insufficient staining for HER-2 gene using the Dual SISH kit, Ventana of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1*. Due to excessive nuclear "bubbling" and excessive retrieval it is impossible reliably to identify and count the HER-2 and chr. 17 signals. A combination of inadequate deparaffination and excessive cell conditioning / proteolysis may be the cause for this reaction pattern.

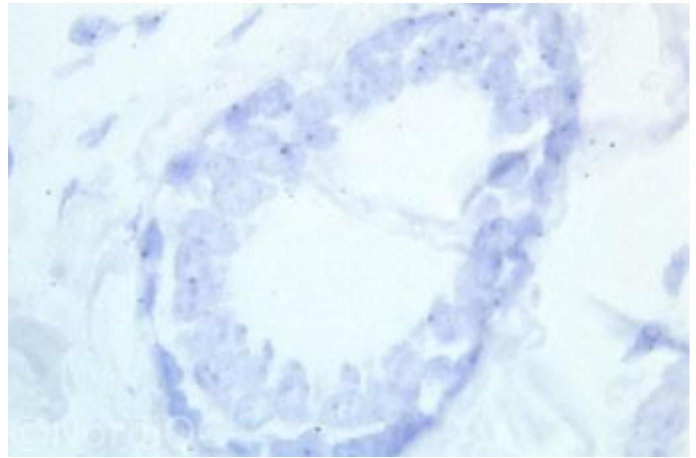


Fig. 3b
Insufficient staining for HER-2 gene using the DuoCISH kit, Dako of the normal breast tissue no. 1 without gene amplification: HER-2/chr. 17 ratio 1.1*. No HER-2 signals can be identified, as only scattered chr. 17 signals can be seen. This aberrant reaction may be caused by insufficient retrieval e.g. too short HIER and/or too short proteolysis with Pepsin (or Pepsin with reduced activity, due to storage at room temp.).

*Reference: HER2 FISH pharmDX™ Kit, Dako (average of data from two tests perfomed in reference labs.).

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