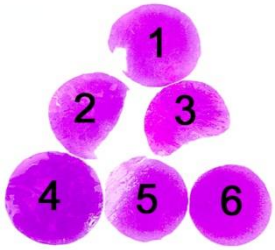
Assessment Pilot Run C1 2009

HER-2 CISH/SISH

The slide to be stained for CISH/SISH HER-2 comprised six breast ductal carcinomas showing HER-2 gene/chr(chr) 17 ratios as follows:

	DuoCISH*	Dual color SISH**	FISH***
	HER-2 gene/chr17 ratio	HER-2 gene/chr17 ratio	HER-2 gene/chr17 ratio
1. Breast ductal carcinoma	1.4	1.1	1.2
2. Breast ductal carcinoma	1.5	1.7	1.5
3. Breast ductal carcinoma	1.4	1.4	1.5
4. Breast ductal carcinoma	2.7	NA	2.6
5. Breast ductal carcinoma	> 6.0	> 6.0	> 6.0
6. Breast ductal carcinoma	> 6.0	> 6.0	> 6.0



* HER-2 DuoCISH™ kit (Dako)(data from one reference lab.)
** HER-2 Dual color SISH kit (Ventana)(data from one reference lab.)
*** HER2 FISH pharmDX™ Kit (Dako)(average of data from two reference labs.). NA: Not assessable.
All carcinomas were fixed for 24 h in 10 % neutral buffered formalin, except for carcinoma no. 4, which was fixed for 72 h.

Criteria for assessing a CISH/SISH HER-2 analysis as optimal included:

- Staining of breast ductal carcinomas no. 1, 2 and 3 corresponding a non-amplified status.
- Staining of breast ductal carcinomas no. 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 five carcinomas fixed for 24 h, but not for carcinoma no. 4 fixed for 72 h. It could be argued that this tumour should be excluded from the assessment, as the tissue was not processed according to the recommended ASCO/CAP guidelines of a fixation time of 6 – 48 h. However, from a technical perspective it was valuable to see if some laboratories could carry out a successful CISH/SISH procedure also for this tumour in spite of the extended fixation time comparable to the successful result obtained with FISH.

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

17 laboratories participated in this assessment. 14 (88 %) achieved a sufficient mark. The results are summarized in Table 1.

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

HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]
Dual SISH	7	Ventana	1	5	0	1	86 %
DuoCISH	6	Dako	4	1	1	0	84 %
CISH Spot-Light	4	Zymed	1	3	0	0	100 %
Total	17		6	9	1	1	-
Proportion			35 %	53 %	6 %	6 %	88 %

1) Proportion of sufficient stains

Comments

All three CISH/SISH HER-2 systems could be used to obtain an optimal result. DuoCISH (Dako) gave the highest proportion of optimal results. However, the number of stains is small and the FISH analysis was performed on the basis of the HER2 FISH pharmDX™ Kit, Dako, which is the basis for the DuoCISH (Dako) regarding pre-treatment and hybridization conditions, thus facilitating the performance of the DuoCISH system in this run. If the over fixed carcinoma was excluded, all 3 systems would have almost an identical proportion of optimal results. As it was observed, that all 3 systems could give an optimal result in all 6 tissues e.g. by adjusting the pre-treatment

steps and/or other protocol settings it is mandatory to have the specific protocol data from the laboratories in order to give information and recommendation how to optimize the technical set-up of the procedures. This information will be highly valuable for both the vendors, participants and NordiQC. In the coming runs of CISH/SISH HER-2 more focus will be given regarding the technical data of the protocols.

The two insufficient results were due to a generally too weak or completely negative signal in both the neoplastic cells and the normal stromal cells. It is not possible from the protocols submitted to identify a specific cause.

The laboratories were requested to send in their own scores on the stained sections. As regards amplification vs. non-amplification a discrepancy between the laboratory and NordiQC was revealed in only one case: The Dual SISH staining of the non-amplified tumour no. 2 (ratio 1.7) was interpreted by the laboratory as amplified with a ratio of 3.0.

Conclusion
All three CISH/SISH HER-2 systems used by the laboratories in this pilot run could be used to obtain an optimal result. The proportion of sufficient results was high (88 %).

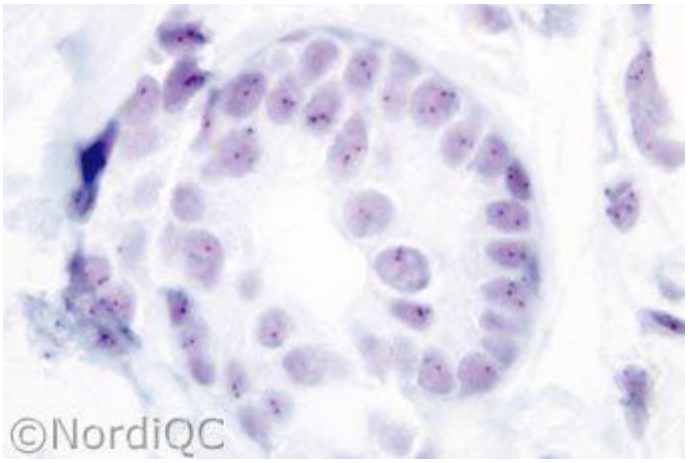


Fig. 1a
Optimal staining for HER-2 gene status using the DuoCISH kit, Dako of the breast ductal carcinoma no. 3 without gene amplification (HER-2/chr17 ratio 1.4). The HER-2 genes are stained red, while chr17 is stained blue.

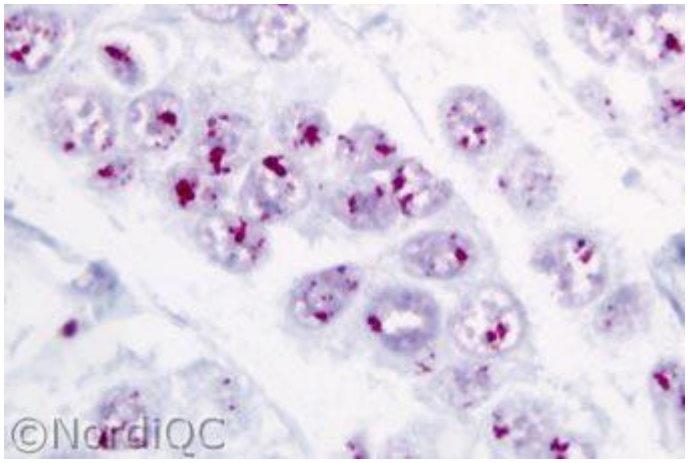


Fig. 1b
Optimal staining for HER-2 gene status using the DuoCISH kit, Dako of the breast ductal carcinoma no. 6 with gene amplification (HER-2/chr17 ratio > 6.0). The HER-2 genes are stained red and located in clusters, while chr17 is stained blue.

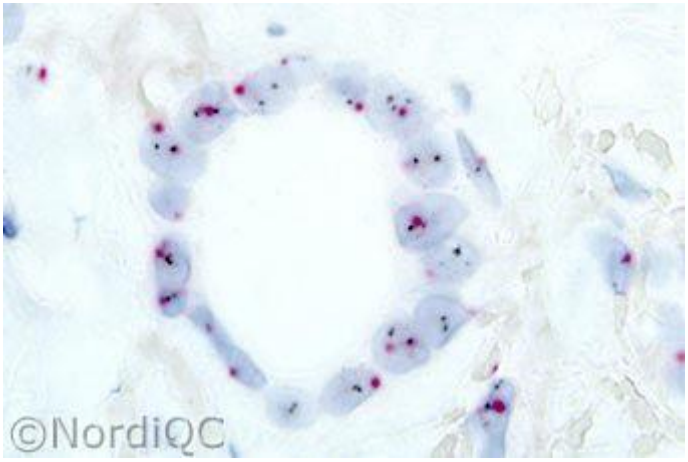


Fig. 2a
Optimal staining for HER-2 gene status using the Dual color SISH kit (Ventana) of the breast ductal carcinoma no. 3 without gene amplification (HER-2/chr17 ratio 1.4). The HER-2 genes are stained black, while chr17 is stained red.

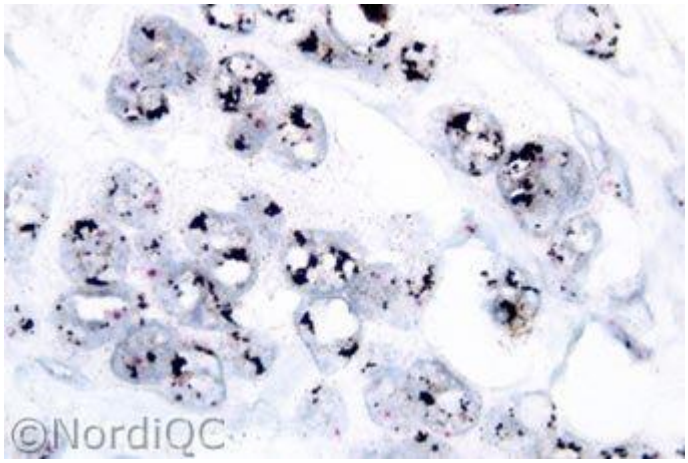


Fig. 2b
Optimal staining for HER-2 gene status using the Dual color SISH kit (Ventana) of the breast ductal carcinoma no. 6 with gene amplification (HER-2/chr17 ratio > 6.0). The HER-2 genes are stained black and located in clusters, while chr17 is stained red.

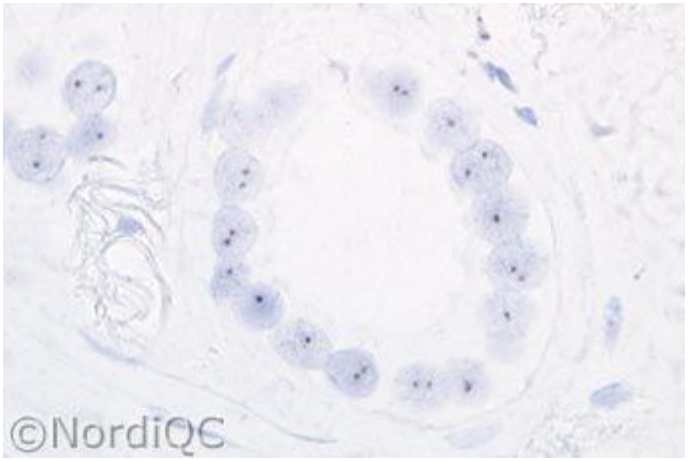


Fig. 3a
Optimal staining for HER-2 gene status using the CISH Spot-Light kit (Zymed) of the breast ductal carcinoma no. 3 without gene amplification (HER-2/chr17 ratio 1.4). The HER-2 genes are stained black.

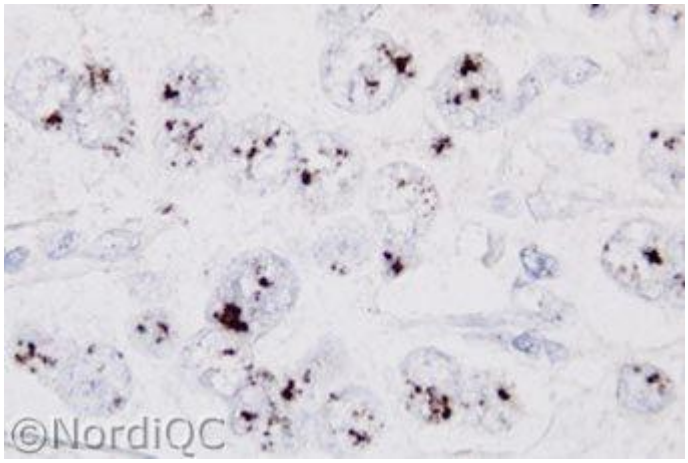


Fig. 3b
Optimal staining for HER-2 gene status using the CISH Spot-Light kit (Zymed) of the breast ductal carcinoma no. 6 with gene amplification (HER-2/chr17 ratio > 6.0). The HER-2 genes are stained black and located in large clusters.

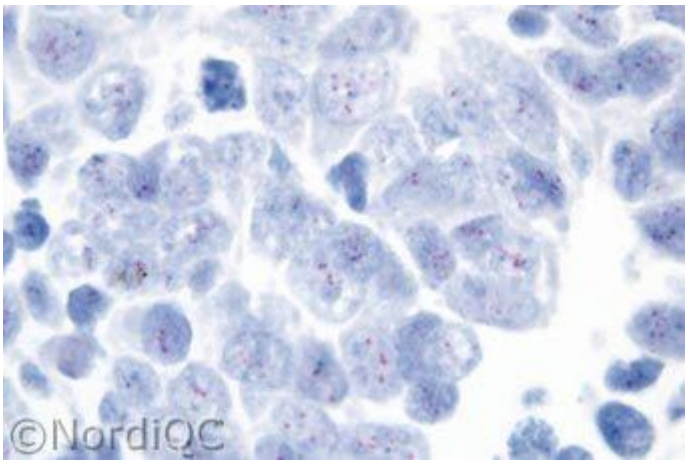


Fig. 4a
Optimal staining for HER-2 gene status using the DuoCISH kit (Dako) of the breast ductal carcinoma no. 4 with a low level of gene amplification (HER-2/chr17 ratio 2.6). The HER-2 genes are stained red, while chr17 is stained blue.

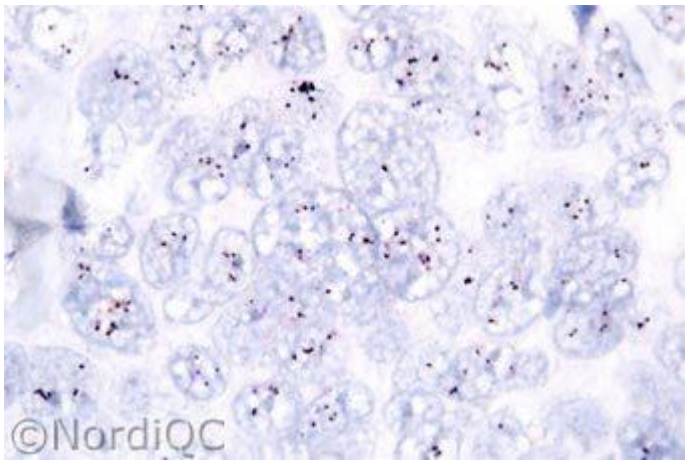


Fig. 4b
Staining for HER-2 gene status using the Dual color SISH kit (Ventana) of the breast ductal carcinoma no. 4 with a low level of gene amplification (HER-2/chr17 ratio 2.6). The staining is marked as good: The HER-2 genes are demonstrated, but only scattered cells show a weak staining for chr17. Thus, the ratio could not be estimated.

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