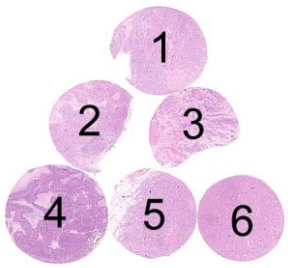
Assessment Pilot Run C2 2009

HER-2 CISH/SISH

The slide to be stained for CISH/SISH HER-2 comprised six breast ductal carcinomas (same block as used for pilot run C1) showing HER-2 gene/chromosome 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. Breast ductal carcinoma	1.4	1.0	1.2
2. Breast ductal carcinoma	1.5	1.2	1.4
3. Breast ductal carcinoma	1.4	1.1	1.4
4. Breast ductal carcinoma	2.7	2.9	2.7
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 SISH kit, Ventana (average of data from two reference labs.)
*** HER2 FISH pharmDX™ Kit, Dako (average of data from three tests perfomed in reference labs.).
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 over fixation.

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

34 laboratories participated in this assessment. 23 (68 %) achieved a sufficient mark. The results 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]**
Dual SISH	14	Ventana	6	4	3	1	71 %
DuoCISH	6	Dako	2	3	1	0	83 %
Zyto*Dot* 2 Colours	3	ZytoVision	0	2	1	0	-
One colour HER-2 systems							
Zyto*Dot*	5	ZytoVision	0	2	0	3	40 %
HER-2 SISH	3	Ventana	0	2	0	1	-
SPOT-Light	2	Zymed	0	1	1	0	-
"In-house" BAC	1		1	0	0	0	-
Total	34		9	14	6	5	-
Proportion			27 %	41 %	18 %	15 %	68 %

1) Proportion of sufficient stains

Comments
In this assessment and in accordance with the pilot run C1, both the Dual SISH system, Ventana and the DuoCISH, Dako could be used to obtain an optimal demonstration and evaluation of the HER-2 gene amplification status in all the tissues included in the multi block.
The most robust protocol for the Dual SISH system, Ventana was in brief based upon HIER in CCrb for 40 – 48 min and P3, 12 min. 6 hours hybridization for the SISH probe and 2 – 3 hours for the Chr. 17 probe.
For the DuoCISH system, Dako, the main protocol settings were based on HIER for 10 min in the pre-treatment buffer at 95°C and 2 – 3 min. in Pepsin at 37°C (both reagents included in the FISH pharmDX kit K5331, Dako).
The insufficient results were typically associated with too weak or completely negative signals in both the neoplastic cells and in the normal stromal cells. Excessive retrieval, most likely a harsh proteolysis, impaired the morphology, thus complicating the interpretation.

The laboratories were requested to send in their own interpretation on the stained sections. As regards amplification vs. non-amplification 27 out of the 34 laboratories interpreted and classified all 6 tumours correctly and in concordance to the HER-2 gene / chromosome 17 status generated in the reference laboratories. In the remaining 7 cases, 6 laboratories classified the low amplified tumour, tissue specimen no. 4 as non-amplified and 1 laboratory classified the non-amplified tumour, tissue specimen no. 2 as amplified.

This was the 2' assessment of HER-2 CISH / SISH in NordiQC. As seen in table 2 a decrease in the pass rate and proportion of sufficient results was seen compared to the first assessment.

Table 2. **Proportion of sufficient results for HER-2 CISH/SISH in the two NordiQC runs performed**

	Run C1 2009	Run C2 2009
Participants, n=	17	34
Sufficient results	88 %	68 %

Conclusion
In this assessment the commercially available two-colour HER-2 systems Dual SISH, Ventana, and DuoCISH, Dako, were the most robust methods for the determination of the HER-2 gene status. Also an in-house established method could be used to obtain an optimal result. For an optimal performance the retrieval settings – HIER + proteolysis - must be carefully balanced to provide an efficient sensitivity and preserved morphology.

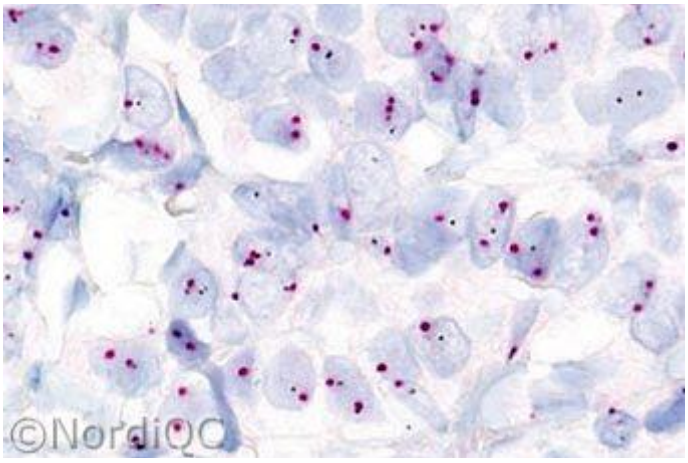


Fig. 1a
Optimal staining for HER-2 gene using the Dual SISH kit, Ventana of the breast ductal carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.2*. The HER-2 genes are stained black, while chr. 17 is stained red.

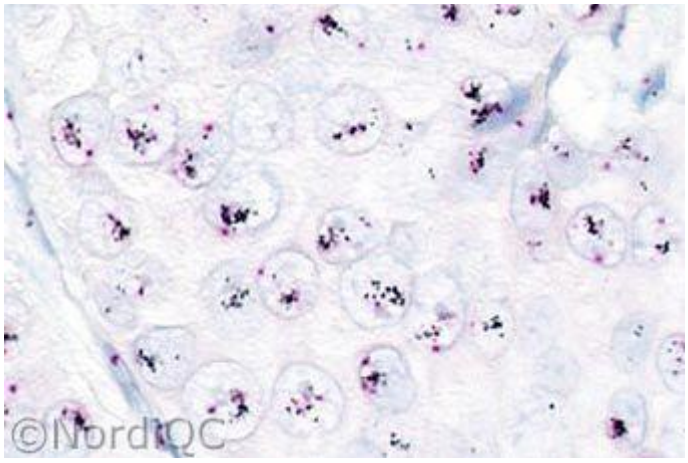


Fig. 1b
Optimal staining for HER-2 gene status using the Dual SISH kit, Ventana of the breast ductal carcinoma no. 6 with gene amplification: HER-2/chr. 17 ratio > 6.0*. The HER-2 genes are stained black and located in clusters, while chr. 17 is stained red.

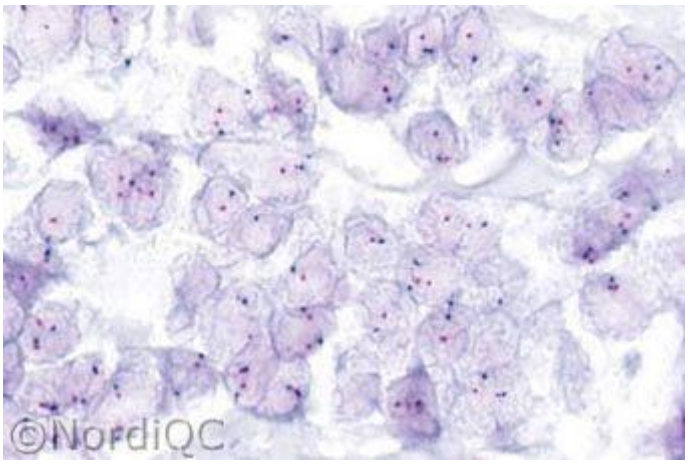


Fig. 2a
Optimal staining for HER-2 gene status using the DuoCISH kit, Dako of the breast ductal carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.2*. The HER-2 genes are stained red, while chr. 17 is stained blue.

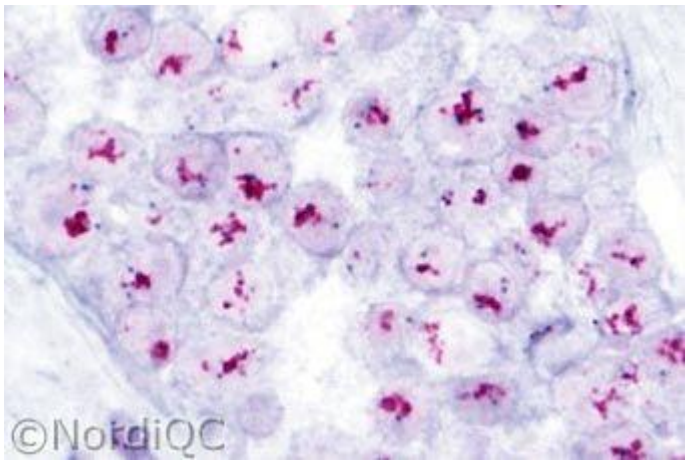


Fig. 2b
Optimal staining for HER-2 gene status using the DuoCISH kit, Dako of the breast ductal carcinoma no. 6 with gene amplification: HER-2/chr. 17 ratio > 6.0*. The HER-2 genes are stained red and located in clusters, while chr. 17 is stained blue.

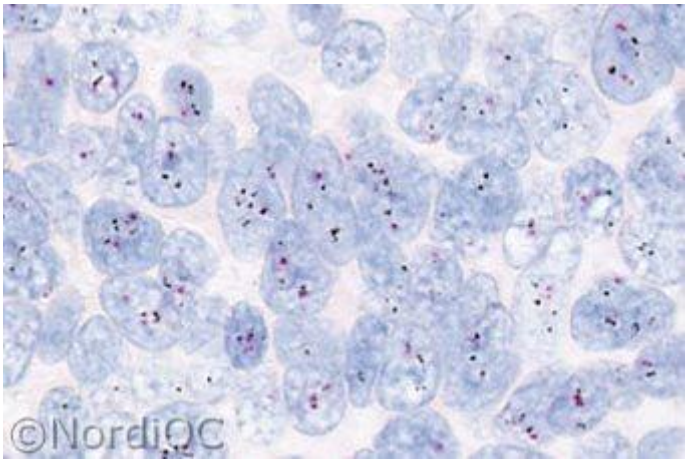


Fig. 3a
Optimal staining for HER-2 gene status using the Dual SISH kit, Ventana of the breast ductal carcinoma no. 4 with a low level of gene amplification: HER-2/chr. 17 ratio 2.7*. The HER-2 genes are stained black, while the chr. 17 is stained red.

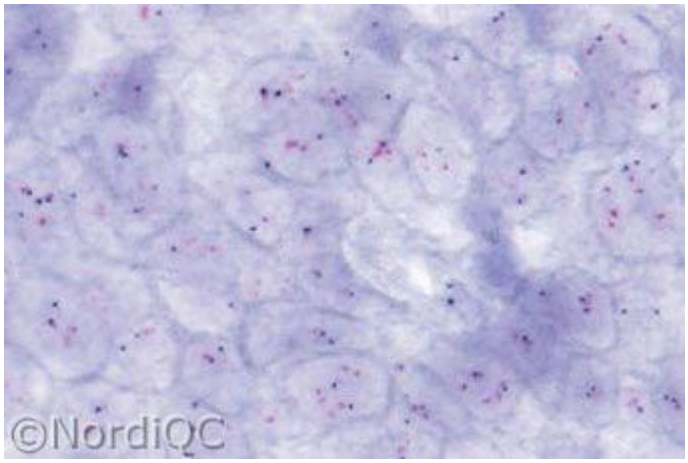


Fig. 3b
Optimal staining for HER-2 gene status using the Dual CISH kit, Dako of the breast ductal carcinoma no. 4 with a low level of gene amplification: HER-2/chr. 17 ratio 2.7*. The HER-2 genes are stained red, while chr. 17 is stained blue.

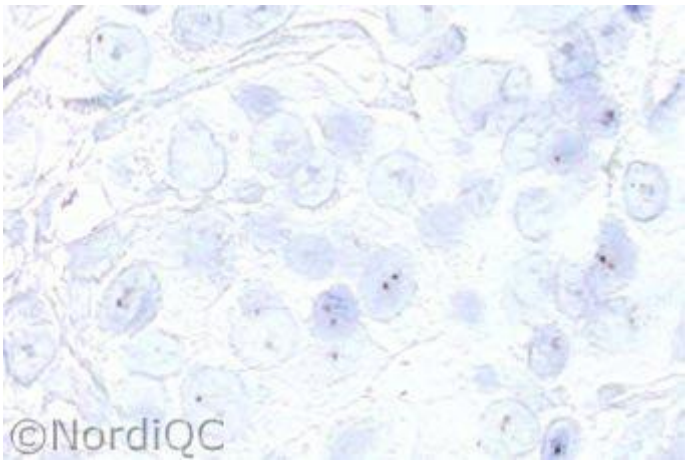


Fig. 4a
Insufficient staining for HER-2 gene status using the single colour SISH kit, Ventana of the breast ductal carcinoma no. 1 without gene amplification: HER-2/chr. 17 ratio 1.2*. Only scattered cells show a positive staining for HER-2 and the morphology of the nuclei is heavily impaired due to excessive pre-treatment. Also compare with Fig. 4b – same protocol.

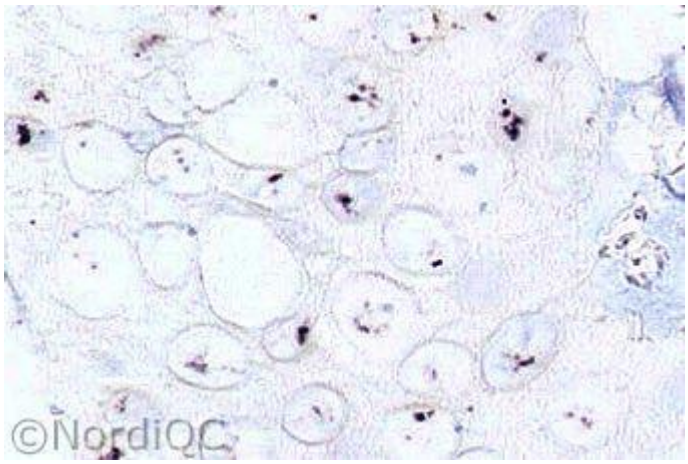


Fig. 4b
Insufficient staining for HER-2 gene status using the single colour SISH kit, Ventana the breast ductal carcinoma no. 6 with gene amplification: HER-2/chr.17 ratio > 6.0*. The morphology of the nuclei is heavily impaired due to excessive pre-treatment. Also compare with Fig. 1b, same tumour and same SISH method for the HER-2 genes.

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

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