Assessment Run B4 2007

HER-2

The slide to be stained for HER-2 comprised:

	IHC*	FISH*
	HER-2 Score (0, 1+, 2+, 3+)	**HER-2 gene / chromosome 17 ratio**
1. Breast ductal carcinoma	3+	Clusters > 5
2. Breast ductal carcinoma	3+	3.2
3. Breast ductal carcinoma	1+	1.3
4. Breast ductal carcinoma	1+	1.5
5. Breast ductal carcinoma	0	1.1

*****Verified by 4 NordiQC reference laboratories

The stains were primarily assessed with respect to the capability of the laboratories to identify and determine the level of the HER-2 protein expression in the specimens corresponding to the gene status.
The specimens were scored using the following set of criteria:

Score 0	No staining is observed or cell membrane staining is observed in less than 10% of the tumour cells.
Score 1+	A faint perceptible membrane staining can be detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane.
Score 2+	A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells.
Score 3+	A strong complete membrane staining is observed in more than 10% of the tumour cells.

Criteria for assessing a HER-2 staining as optimal included:

- A clear and unequivocal immunohistochemical staining marked as score 3+ in the two breast ductal carcinomas no. 1 and 2.
- A clear and unequivocal immunohistochemical staining marked as score 1+ in the breast ductal carcinoma no 3 and 4.
- A clear and unequivocal immunohistochemical staining marked as score 0 in the breast ductal carcinoma no 5.
- Negative reaction in normal breast glandular epithelial cells.
- No or only weak cytoplasmic reaction that did not affect the interpretation of the true membranous HER-2 reaction.

A staining was assessed as good, if one of the tumours no. 1 or 2 showed a 2+ reaction, or if one of the tumours no. 3 or 4 showed a 2+ reaction. (An equivocal 2+ IHC staining should always be analyzed by FISH according to the ASCO guidelines and the national guidelines in Denmark, Norway and Sweden).

A staining was assessed as borderline if a 2+ reaction appeared and the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, hampering the interpretation.

A staining was assessed as poor in case of false negativity (a 3+ tumour showing a 1+/0 reaction) or false positivity (a 0/1+ tumour showing a 3+ reaction). Also a staining was assessed as poor if the signal-to-noise ratio was very low, i.e., because of a strong cytoplasmic reaction.

91 laboratories participated in the assessment with an IHC HER-2 staining (3 also with CISH and 1 with SISH) and 2 laboratories participated only with FISH. 38 laboratories achieved an optimal staining (42 %), 40 good (44 %), 6 borderline (7 %) and 7 (8 %) poor staining.

The table shows the systems/Abs used and the scores given.

	Score			
	Optimal	**Good**	**Borderline**	**Poor**
FDA approved systems:				
HercepTest K5204, K5206, K5207, SK001 (Dako, n=41)	24	16	1	0
PATHWAY rmAb clone **4B5** (Ventana=24)	11	12	0	1
Abs in in-house systems:				
pAb clone **A0485** (Dako, n=14)	2	8	3	1
rmAb clone **SP3** (NeoMarkers, n=5)	1	1	0	3
mAb clone **3B5** (NeoMarkers, n=2)	0	2	0	0
mAb clone **CB11** (Novocastra, n=3)	0	0	2	1
mAb clone **CB11** (NeoMarkers, n=1)	0	1	0	0
mAb clone **e2-4001+3B5** (NeoMarkers, n=1)	0	0	0	1

HercepTest (Dako): 24 out of 41 (58%) obtained an optimal mark. In all cases the procedure was performed according to the instructions from the company. 98 % obtained a sufficient result (optimal or good).

PATHWAY rmAb clone **4B5 (RTU)** (Ventana): 11 out of 24 (46%) obtained an optimal mark. The optimal protocols were all based on HIER in either Cell Conditioning1 (Benchmark, Ventana, 10 out of 21) or EDTA/EGTA pH 8 (1 out of 2). Using CC1 as HIER buffer 21 out of 21 laboratories (100%) obtained a sufficient result.

Grouped together, using one of these two FDA approved systems, 97% (63 out of 65) obtained a sufficient result.

A0485: 2 out of 14 (14%) obtained an optimal mark. These were based on HIER using Citrate pH 6 or Cell Conditioning1 (Benchmark, Ventana). The pAb A0485 was diluted in the range of 1:800-900. Using these settings 10 out of 14 (71 %) obtained a sufficient staining.

SP3: 1 out of 5 (20%) obtained an optimal mark. The protocol resulting in an optimal staining was based on HIER using Citrate pH 6 and a dilution of 1:60.

Grouped together, using one of the six in-house systems, 15 out of 26 (58%) obtained a sufficient result.

For the in-house systems, the most frequent causes of insufficient stains were (often in combination):
- Less successful primary Ab
- Wrong calibration of the primary Ab
- Excessive retrieval

The prevalent feature of an insufficient staining was a too weak or false negative reaction in the breast carcinoma with a moderate gene amplification (ratio 3.2), or a false positive reaction in one of the breast carcinomas without gene amplification as well as in the normal breast glandular epithelium.

The laboratories were requested to send in their own scores on the multitissue section. For 40 out of 84 laboratories (48 %) returning the slip, the scores on the multi-tissue sections were in concordance with those given by the NordiQC assessors.

The proportion of sufficient HER-2 stains was 86% compared to 74% in the previous Run B3, 2007. As seen in the previous assessments virtually all the laboratories adhered strictly to the protocol settings described in the FDA approved HER-2 systems, which was the main reason for the high overall pass rate. Laboratories using in-house (home made) systems also improved their performance, as the proportion of sufficient stains increased from 33% in Run B3 to 58% in the current run. Still, this is low compared to the FDA approved systems, and NordiQC cannot recommend in-house systems to be used for HER-2.

However, only 24/40 (60%) and 11/23 (48%) of the sufficient protocols with HercepTest and Pathway, respectively, were assessed as optimal - enabling the IHC result to be used directly to give an accurate evaluation of the HER-2 status. In the rest, an equivocal 2+ IHC reaction was obtained and an ISH method

should be performed to give the precise information of the HER-2 status.
With an in-house system only 20% of the sufficient stains could directly be used to evaluate the HER-2 status, whereas 80% should be sent to ISH.

Two labs had performed FISH and reported a correct score. Four labs submitted CISH/SISH stained slides. Three of these were marked sufficient, one was insufficient.

Conclusion
The two FDA approved HER-2 systems HercepTest (Dako) and the PATHWAY rmAb clone 4B5 (Ventana) were also in this assessment the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression. Training in scoring is still highly warranted and image analysis assisted scoring has to be taken in consideration to improve and facilitate the interpretation.

Figs. 1a and 1b. Optimal staining results (same protocol)

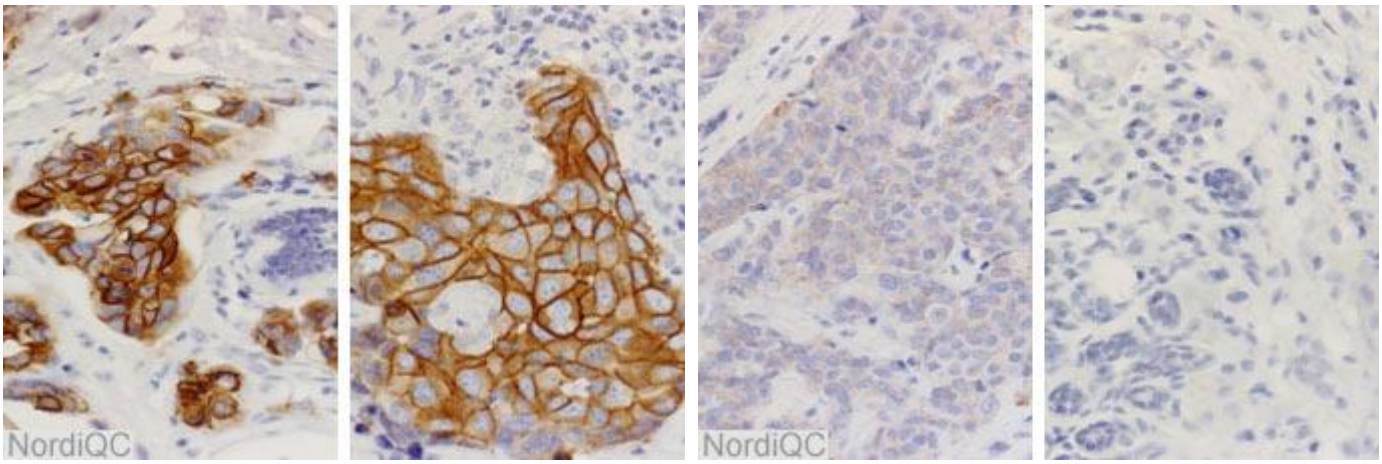

Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 1 with high level of gene amplification – "clusters". Most neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a moderate level of gene amplification. Most neoplastic cells show a strong and complete membranous staining corresponding to 3+.

Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.5. Most neoplastic cells show a faint membrane staining corresponding to 1+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.1. Both the normal epithelial cells and the neoplastic cells are negative corresponding to score 0.

Figs. 2a and 2b. Good staining results (same protocol)

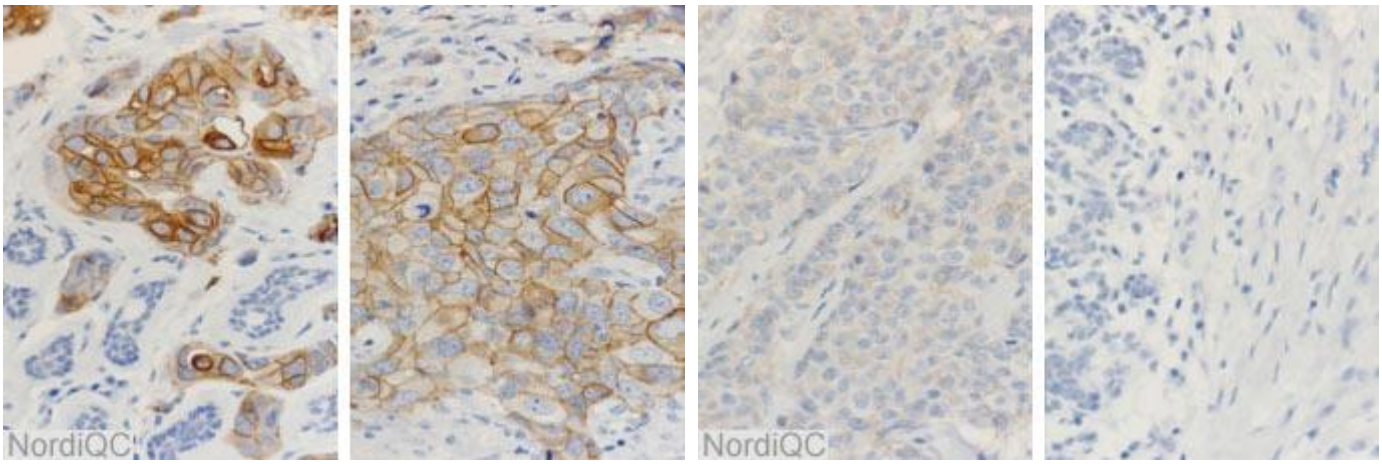

Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with high level of gene amplification – "clusters". Most neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a moderate level of gene amplification. Most neoplastic cells show a moderate and complete membranous staining corresponding to 2+.

Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 4 with no gene amplification and a HER-2 / Chromosome 17 ratio of 1.5. Most neoplastic cells show a faint membrane staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 5 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.1. Both the normal epithelial cells and the neoplastic cells are negative corresponding to score 0.

Figs. 3a and 3b. Poor staining results (false negative; same protocol)

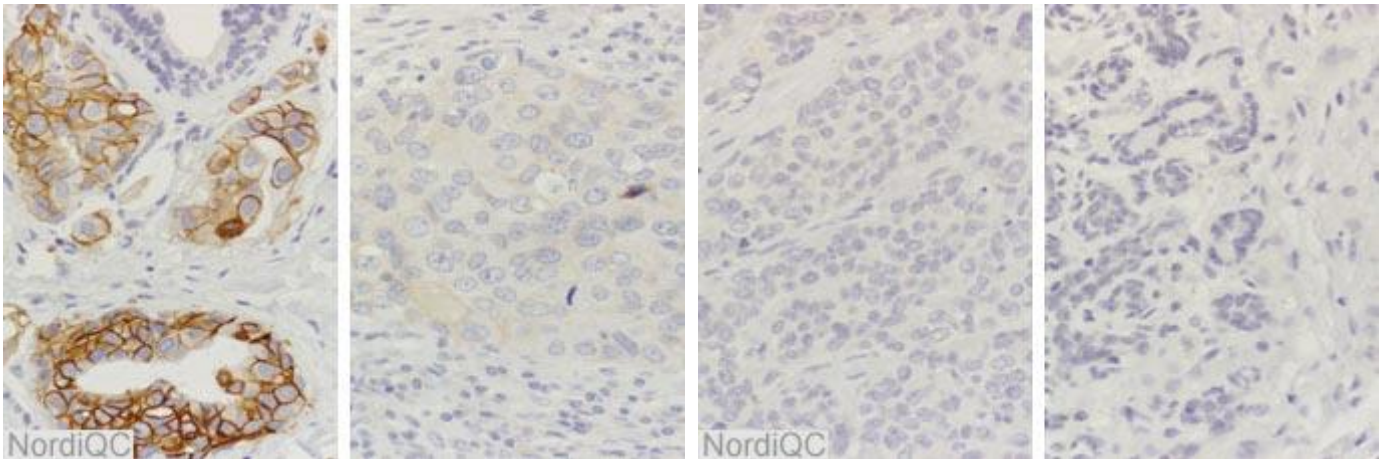

Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with high level of gene amplification – "clusters". Most neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a moderate level of gene amplification. The neoplastic cells only show a faint membrane staining corresponding to 1+.

Fig. 3b
Left: Staining for HER-2 of the breast ductal carcinoma no. 4 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.5. Most neoplastic cells show a faint membrane staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 5 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.1. Both the normal epithelial cells and the neoplastic cells are negative corresponding to score 0.

Figs. 4a and 4b. Poor staining results (very low signal-to-noise ratio; same protocol)

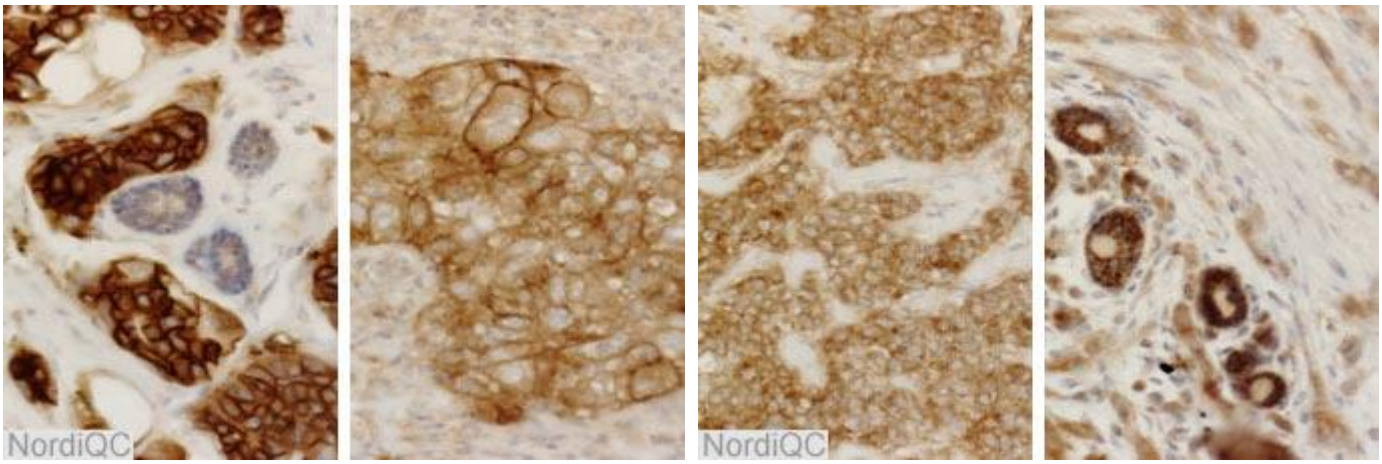

Fig. 4a
Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with high level of gene amplification – "clusters". Most neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a moderate level of gene amplification. Due to the excessive cytoplasmic reaction, determination of the HER-2 status is impossible.

Fig. 4b
Left: Staining for HER-2 of the breast ductal carcinoma no. 4 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.5. Due to excessive cytoplasmic reaction, determination of the HER-2 status is impossible.
Right: Staining for HER-2 of the breast ductal carcinoma no. 5 with no gene amplification and a HER-2/Chromosome 17 ratio of 1.1. The normal epithelial cells show a strong membrane reaction and the neoplastic cells a cytoplasmic reaction.

SN/HN/MV/LE 30-11-2007