Assessment Run B2 2006

HER-2

The slide to be stained for HER-2 comprised:

1. Cell line JIMT-1 (Gene amplification confirmed by FISH & CISH)
2. Cell line MDA-453 (Gene amplification confirmed by FISH & CISH)
3. Cell line MCF-7 (Without gene amplification confirmed by FISH & CISH)
4. Cell line BT474 (Gene amplification confirmed by FISH & CISH)
5. Breast ductal carcinoma (Without gene amplification confirmed by FISH & CISH)
6. Breast ductal carcinoma (Without gene amplification confirmed by FISH & CISH)
7. Breast ductal carcinoma (Gene amplification confirmed by FISH & CISH)
8. Breast ductal carcinoma (Gene amplification confirmed by FISH & CISH)

The immunohistochemical staining was primarily evaluated and marked by NordiQC with respect to the capability of the laboratories to identify and determine the level of the HER-2 protein expression corresponding to the gene status of the above mentioned carcinoma specimens. Surprisingly, the staining reaction of the cell lines deviated in a number of cases more than in previous runs from the expected, when compared with the tissue specimens. Consequently, a suboptimal staining of the cell lines were not used to mark down stains with optimal or good results in the carcinomas. Also one of the carcinomas (specimen 6) revealed an unexpected variation in sufficient stains, however the membrane reaction were hardly complete in more then 10% of the tumour cells in any of the slides.

The immunohistochemical scoring system used:

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. 7 and 8.
- A clear and unequivocal immunohistochemical staining marked as score 1+ in the breast ductal carcinoma no 6.
- A clear and unequivocal immunohistochemical staining marked as score 0 in the breast ductal carcinoma no 5.
- Negative reaction in the normal breast glandular epithelial cells.
- No or only weak cytoplasmic staining that did not mask the membrane staining.
- Balanced counterstain that did not mask the membrane staining.

79 laboratories participated in the assessment. 40 laboratories achieved an optimal staining (51 %), 19 good (24 %), 13 borderline (16 %) and 7 (9 %) 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=44)	33	6	4	1
PATHWAY 760-2694 (Ventana, n=5)	0	3	0	2
In-house systems:				

pAb clone **A0485** (Dako, n=9)	1	4	3	1
pAb **28-0004** (Zymed, n=1)	0	0	1	0
mAb clone **4B5** (Ventana, n=9)	5	3	1	0
mAb clone **CB11** (Novocastra, n=3; NeoMarkers, n=1)	0	2	2	0
rmAb clone **SP3** (NeoMarkers, n=2)	1	0	1	0
mAb clone **10A7** (Novocastra, n=1)	0	1	0	0
mAb clone **e2-4001+3B5** (NeoMarkers, n=1)	0	0	0	1
mAb clone **TAB250** (Zymed, n=1)	0	0	1	0
mAb clone **3B5** (NeoMarkers, n=1)	0	0	0	1
mAb clone **PNA2** (Dako, n=1)	0	0	0	1

Optimal staining for HER-2 in this assessment was obtained with the FDA approved **HercepTest** (Dako) performed accordingly to the instructions from the company in 33 out of 44 cases (75%). Sufficient results (i.e., including those marked good) were obtained in 39/44 (89 %).

The FDA approved **Pathway** (Ventana) gave sufficient results in 3/5 (60%).

Among the in-house systems, optimal staining could be obtained with the mAb clone **4B5** (5 out of 9), the rmAb clone **SP3** (1 out of 2) and the pAb **A0485** (1 out of 9) as follows:

4B5: the optimal protocols were based on HIER in either Cell Conditioning 1 (CC1, Ventana, 4 out of 8) or EDTA/EGTA pH8 (1 out of 1). The mAb was used as a ready-to-use antibody. Using these settings 8 out of 9 obtained a sufficient result.

SP3: the optimal protocol was based on HIER in CC1 (1 out of 1). The rmAb was used in the dilution of 1:200. Using this Ab 1 out of 2 obtained a sufficient result.

A0485: the optimal protocol was based on HIER in Citrate pH 6 (1 out of 5) and the dilution 1:500. Using these settings 5 out of 9 obtained a sufficient result.

Grouped together, in-house immunohistochemical systems with a self-established level of sensitivity and specificity resulted in an optimal staining in 7/31 (23%) and a sufficient staining (optimal or good) in 18/31 (58 %).

The most frequent causes of insufficient staining results were (often in combination):
- Less successful primary Ab
- Wrong calibration of the primary Ab
- Excessive retrieval
- Protocol modifications of the FDA approved systems (Herceptest & Pathway) - i.e. HIER in MWO instead of water bath when using the Herceptest and different HIER procedures and detection systems using Pathway.

The prevalent feature of an inappropriate staining was typically either a too weak or false negative reaction in one of the breast carcinomas with gene amplification (going from the score 3+ to 0, 1+ or 2+), or a too strong and false positive reaction in one of the breast carcinomas without gene amplification (going from the score 0-1+ in the ductal breast carcinoma no. 5 to 2+ and from the score 1+ to 3+ in the ductal breast carcinoma no. 6). Also positivity in the normal breast glandular epithelium cells was seen.

The incorrect level of the HER-2 staining of the breast carcinomas was in most cases simultaneously shown in the cell lines of which especially the cell line JIMT-1 reflected the inappropriate staining level. If a false positive result was seen in the breast carcinoma, the JIMT-1 cell line showed a 3+ reaction and in case of a false negative reaction in the breast carcinoma, the cell line showed a 0 – 1+ reaction. However as the cell lines seemed to be much more fragile to the immunohistochemical procedures and frequently showed an impaired morphology and heterogeneous reaction both within the sections and throughout the multi blocks, the assessor group and NordiQC decided not to use the cell lines for the evaluation of the HER-2 protein expression. The breast carcinoma specimens did not show the same tendency to deterioration of the morphology.

Scoring

The laboratories were also asked to score their own HER-2 staining (to be assessed by NordiQC for concordance with the reference laboratory scores). 77 out of 79 laboratories submitted their scores, and in 37 laboratories (65%) the interpretation and scores were in full concordance with the scores given by the NordiQC assessors. The discrepancy between participant and NordiQC scoring was mainly related to the breast ductal carcinoma no. 8 with gene amplification, which was scored as 2+ and not 3+ (18 out of 28).

In total 37 out of 79 laboratories (47 %) both had a sufficient staining and an interpretation in concordance with

the NordiQC assessor group.

	Optimal / Good		Borderline / Poor	
Staining	75% (n=57)		25% (n=20)	
Interpretation in concordance with NordiQC	**Yes**	**No**	**Yes**	**No**
	65%	35%	60%	40%
	(n=37)	(n=20)	(n=12)	(n=8)

Comparing the staining results with those of run B1 (June, 2006) improvements are seen. The proportion of sufficient results increased from 51 % to 75 % in this run.

In run **B1**, the 33 laboratories that obtained an insufficient mark were given a specific recommendation to improve their protocol. 28 laboratories submitted a new HER2 stain in run B2 and 22 of them changed their protocol according to the recommendations. 15 improved from insufficient to sufficient (68 %). 6 laboratories did not follow the recommendations and none of these obtained a sufficient staining in run B2.

Conclusion

The FDA approved HER-2 system HercepTest (Dako) was in this assessment the most reliable method for the semi-quantitative immunohistochemical determination of HER-2 protein expression with sufficient results in 89%. The laboratories which obtained an inappropriate staining using a FDA approved system should verify that the protocol is performed as indicated by the guidelines given by the system manufactures and validate the protocol settings - especially that HIER is controlled (time, temperature, device), and which immunohistochemical system should be used. Grouped together, in-house systems gave sufficient results in 58%. Apparently the best results were obtained with clone 4B5. It must be emphasized, however, that in-house systems should be very carefully calibrated to obtain reliable results.

The concordance in interpretation between participants and assessors was 63% (almost unchanged from run B1), stressing the need for training in HER-2 scoring, automated quantization of HER-2 stains, and comparison with FISH/CISH studies.

The technical problems with the cell lines and the variation in the expression in one of the carcinomas may hamper the conclusions, and it cannot be excluded that the average marks are slightly too high. However, we consider that laboratories should no be marked down unless we can render it probable that the laboratories should improve their performance.

When comparing the NordiQC and UK NEQAS ICC, in spite of differences in materials and methods, the HER-2 results are very much in line, see Immunocytochemistry, vol 5, page 66-70.

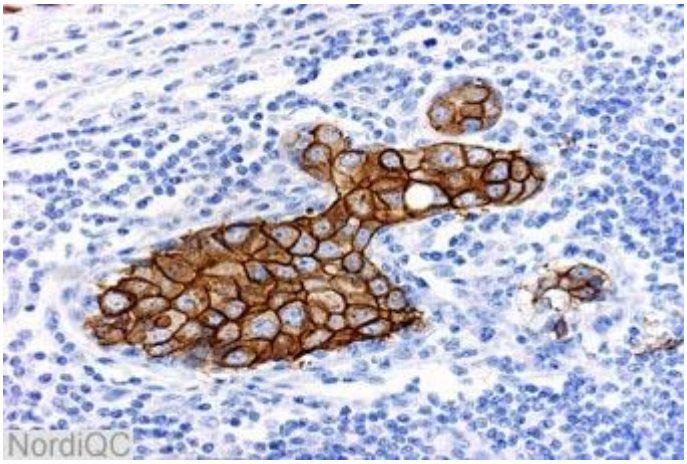

Fig. 1a
Optimal staining for HER-2 of the breast ductal carcinoma no. 8 with gene amplification. Almost all the neoplastic cells show a strong complete membranous staining corresponding to 3+.

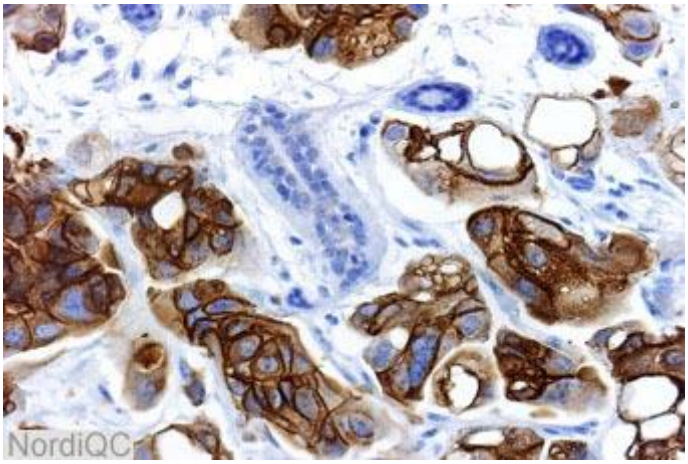

Fig. 1b
Optimal staining for HER-2 of the breast ductal carcinoma no. 7 with gene amplification. The neoplastic cells show a strong complete membranous staining corresponding to 3+, while the entrapped normal ducts and glands are negative.

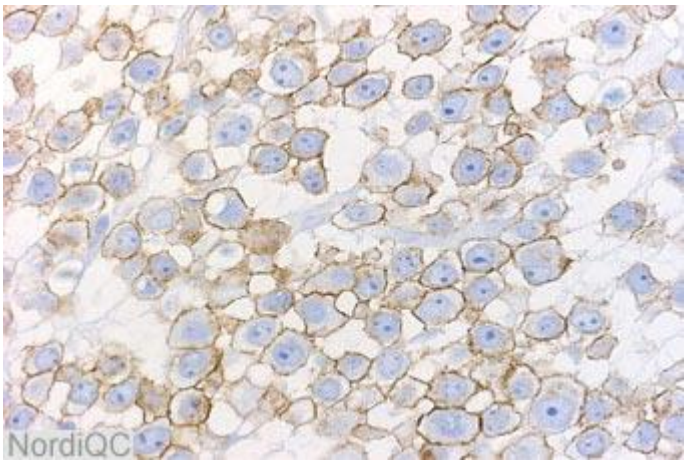

Fig. 2a
Optimal staining for HER-2 of the JIMT-1 cell line. More than 10 % of the cells show a continuous membrane staining corresponding to 2+. The cells do not stain strongly, but display a weak to moderate intensity. The cells have intact membranes and show distinct nuclear details.

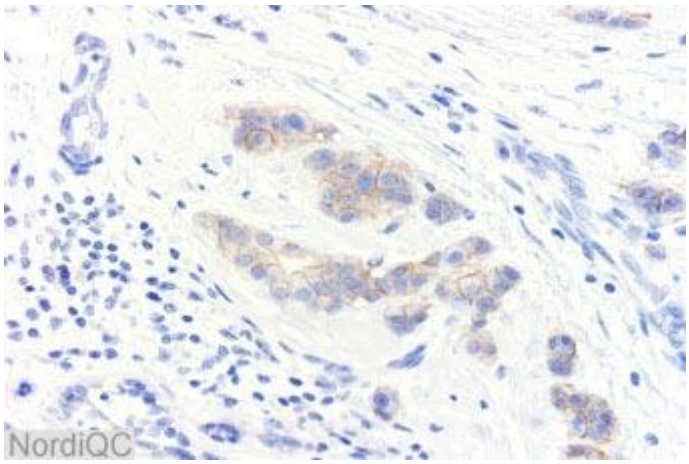

Fig. 2b
Optimal staining for HER-2 of the breast ductal carcinoma no. 6 without gene amplification. The neoplastic cells show a faint membrane staining corresponding to 1+.

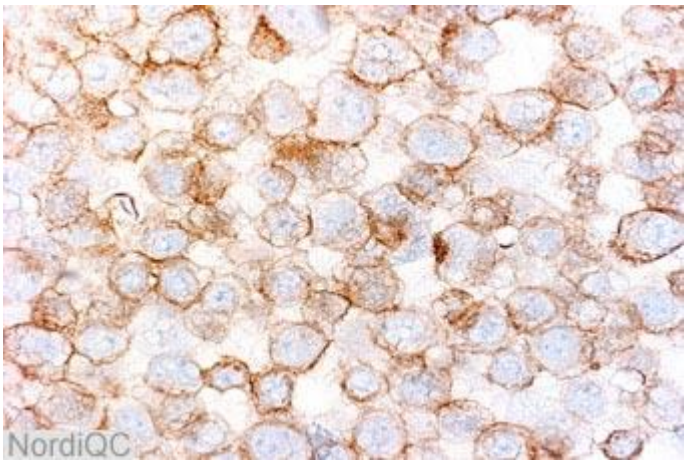

Fig. 3a
Insufficient staining for HER-2 of the 2+ JIMT-1 cell line. The cells show an impaired morphology (in contrast to fig. 2a), as both the membranes and the nuclear details are blurred. This is probably due to a relatively harsh HIER that is calibrated for tissue, not for cell cultures. Compare with Fig. 3b.

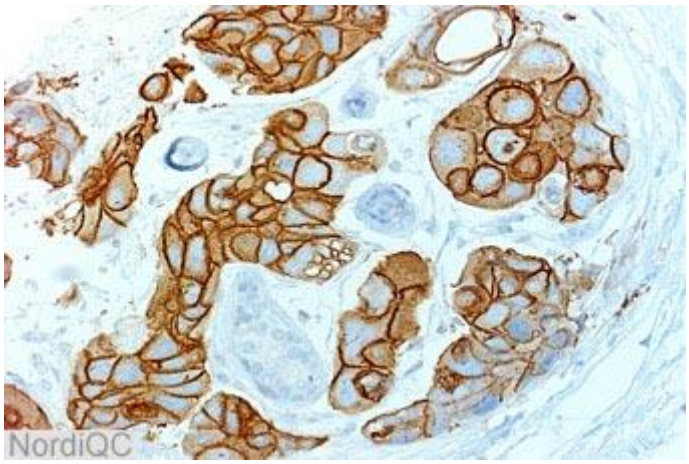

Fig. 3b
Optimal staining for HER-2 of the breast ductal carcinoma no. 7 with gene amplification. The neoplastic cells show a strong complete membranous staining corresponding to 3+. Compare with Fig. 3a. As the main weight was put on the tissue, the total score was sufficient.

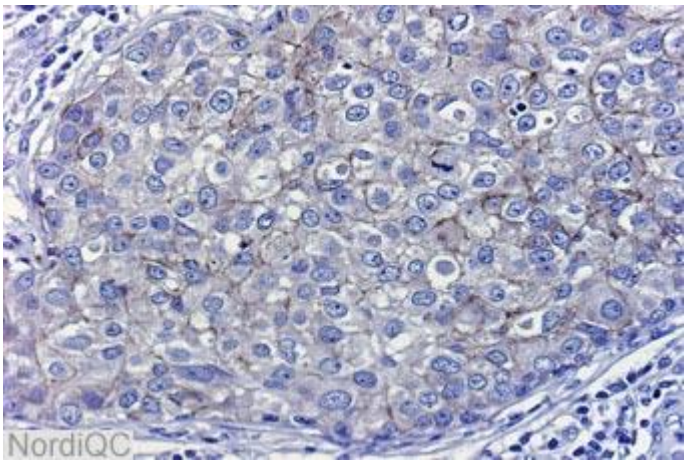

Fig. 4a
Insufficient staining for HER-2 of the breast ductal carcinoma no. 8 with gene amplification. The neoplastic cells only show a weak incomplete membrane staining making this a 1+. Same tumour as in fig. 1a. Same protocol as in fig. 4b

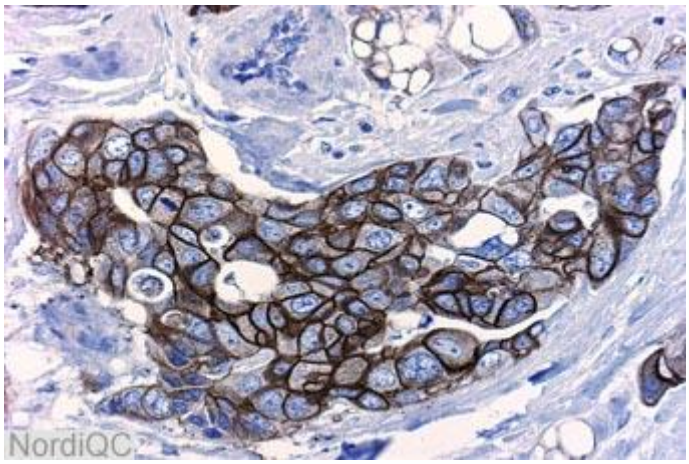

Fig. 4b
Staining for HER-2 of the breast ductal carcinoma no. 7 with gene amplification, using same protocol as in fig. 4a. Almost all the neoplastic cells show a strong complete membranous staining corresponding to 3+. However this tumour showed a higher gene amplification and HER2 protein expression than the tumour in fig. 4a. The final result was marked as insufficient.

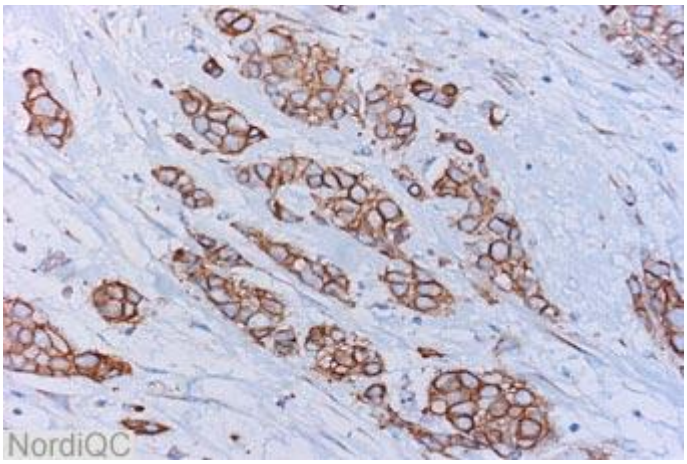

Fig. 5a
Insufficient staining for HER-2 of the breast ductal carcinoma no. 6 without gene amplification. The neoplastic cells show a strong continuous membrane staining making this a 3+. Same tumour as in fig. 2b.

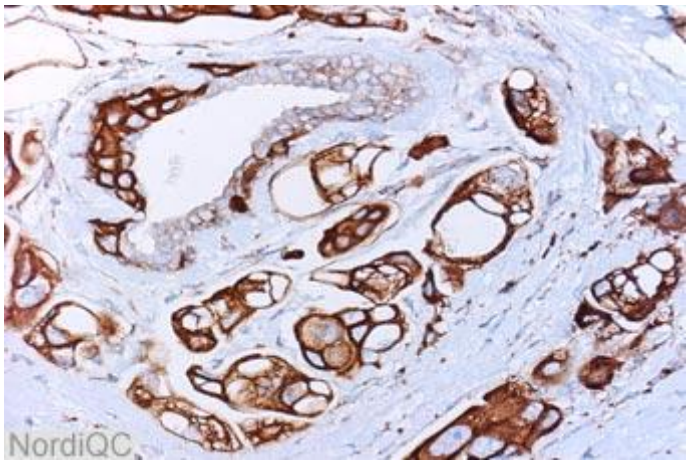

Fig. 5b
Insufficient staining for HER-2 of the breast ductal carcinoma no. 7 with gene amplification using same protocol as in fig. 5a. The neoplastic cells show a strong continuous membrane staining. However, the normal ducts and glands also show a moderate staining, indicating an over-staining of the slide. Compare with fig. 1b, where the normal ducts and glads are unstained.

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