Assessment Run B1 2006

HER-2

The slide to be stained for HER-2 comprised:

1. Cell line JIMT-1	(Amplified)*
2. Cell line MDA-453	(Amplified)
3. Cell line MCF-7	(Not amplified)
4. Cell line BT474	(Amplified)
5. Breast ductal carcinoma	(Not amplified)
6. Breast ductal carcinoma	(Not amplified)
7. Breast ductal carcinoma	(Amplified)
8. Breast ductal carcinoma	(Amplified)

*Amplification of the HER-2 gene demonstrated by FISH and CISH.

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 cell line no. 4. and the two breast ductal carcinomas no. 7 and 8.
- A clear and unequivocal immunohistochemical staining marked as score 2+ in cell line no. 1 and 2.
- 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 1+ in cell line no. 3.
- Negative staining of normal breast glandular epithelium.
- No or only slight cytoplasmic reaction that did not interfere with the interpretation of the true membranous reaction for HER-2.

67 laboratories submitted stains. At the assessment 15 achieved optimal staining (23 %), 19 good (28 %), 17 borderline (25 %) and 16 (24 %) 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** (Dako, n=36)	15	13	8	0
Pathway **760-2694** (Ventana, n=5)	0	1	1	3
Abs in in-house systems:				
mAb clone **4B5** (Ventana, n=6)	0	2	4	0
mAb clone **CB11** (Novocastra, n=4; NeoMarkers, n=1; Zymed, n=1)	0	1	0	5
mAb clone **SP3** (NeoMarkers, n=2)	0	1	0	1
mAb clone **e2-4001+3B5** (NeoMarkers, n=1)	0	0	0	1
pAb clone **A0485** (Dako, n=9)	0	1	3	6
pAb **28-0004** (Zymed, n=1)	0	0	1	0

Optimal staining for HER-2 in this assessment was only obtained with **Herceptest** used according to the settings recommended by the producer (15 out of 36, 42 %).

The most frequent causes of insufficient stains were (often in combination):
- Less successful primary Abs/systems
- Wrong calibration of the primary Ab concentration giving both false negative and false positive reactions
- Excessive retrieval
- Protocol modifications of the FDA approved systems (Herceptest and Pathway) - i.e. HIER in MWO (instead of water bath) when using the Herceptest, different HIER procedures when using Pathway.

The prevalent feature of an insufficient staining result was either a too weak or false negative reaction in one of the breast carcinomas with gene amplification (i.e., the score decreased from 3+ to 2+ or even 1+), or a too strong, false positive reaction in one of the breast carcinomas without gene amplification (i.e., the score increased from 0/1+ to 2+ or 3+). The insufficient HER-2 staining of the breast carcinoma was in most cases reflected in the staining intensity of the cell lines, especially JIMT-1. In case a false positive staining result was seen in the breast carcinoma with no gene amplification, the JIMT-1 cell line showed a 3+ reaction, and in case of a false negative reaction in the breast carcinoma with gene amplification, the cell line showed a 1+ reaction.

In this assessment the FDA approved system HercepTest (Dako) was the best method to determine the level of the HER-2 protein expression corresponding the gene amplification status. 28 laboratories out of 36 (78 %) laboratories obtained a sufficient staining (optimal or good) for HER-2 using this system. With the FDA approved system Pathway, Ventana, 1 out of 5 (20%) laboratories obtained a sufficient staining. With an in-house immunohistochemical system including a self-established level of sensitivity and specificity, only 5 out of 26 (19 %) obtained a sufficient staining.

The laboratories were asked to send in their own interpretation and scores for the multi tissue sections. 56 out of 67 laboratories sent in their scores. In 37 laboratories (66%) the scores were in concordance with the scores given by the NordiQC assessors. This was an improvement compared to the pilot run in 2005, where the concordance was 58 %. The discrepancies were mainly related to the scoring of the breast ductal carcinoma no. 8 with gene amplification, which was scored as 2+ and not 3+ (8 out of 19), whereas the cell lines were scored unanimously by the laboratories and NordiQC.

In total, only 18 out of 67 laboratories (27%) had a sufficient staining as well as a correct interpretation. This result was exactly the same as in the pilot run, which stresses that the laboratories need to focus both on the immunohistochemical procedures and on the interpretation of the result.

	Optimal / Good		**Borderline / Poor**	
Staining	51% (n=34)		49% (n=33)	
Interpretation in concordance with NordiQC*	**Yes**	**No**	**Yes**	**No**
	62%	38%	70%	30%
	(n=18)	(n=11)	(n=19)	(n=8)

*11 labs did not send in their own score.

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. 78 % was marked as sufficient (optimal or good).

The laboratories which obtained an insufficient stain using an FDA approved system should verify that the staining is performed as indicated by the guidelines given by the system manufactures. The protocol settings - especially HIER (time, temperature, device) - must be validated.

For this run a recommended protocol will not be given, as only the Herceptest gave optimal results. An in-house immunohistochemical system with a self-established level of sensitivity and specificity does not give appropriate and reproducible results.

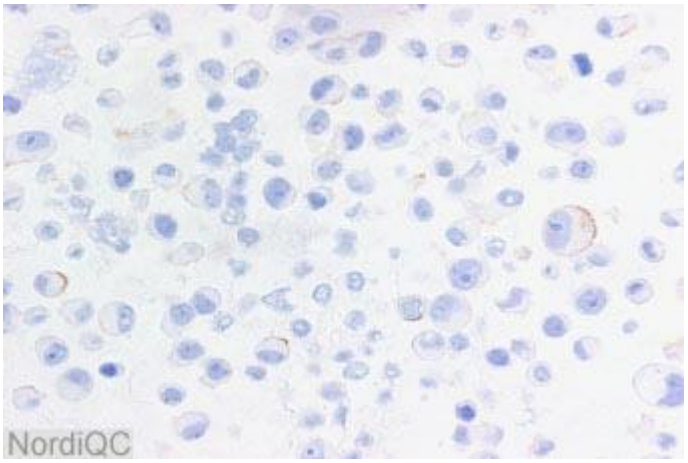

Fig. 1a
Optimal staining for HER-2 of the MCF-7 cell line. The cells show a faint membrane staining corresponding 1+.

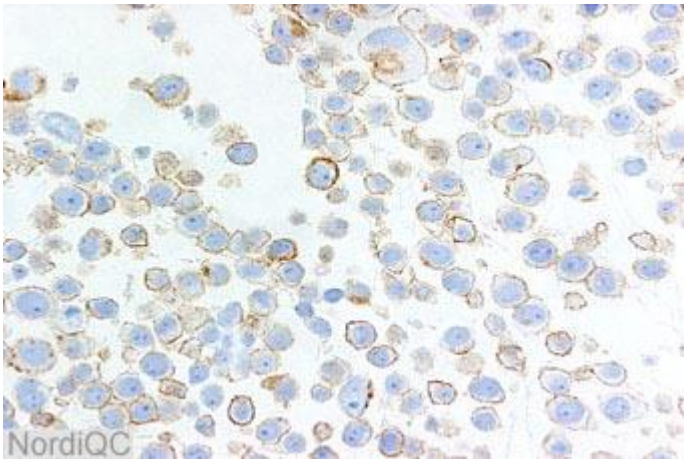

Fig. 1b
Optimal staining for HER-2 of the JIMT-1 cell line. More than 10 % of the cells show a weak to moderate continuous membrane staining corresponding 2+.

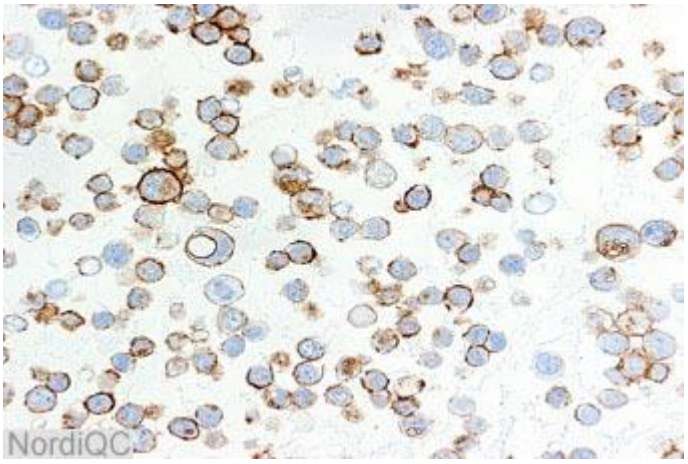

Fig. 2a
Optimal staining for HER-2 of the MDA-453 cell line. More than 10 % of the cells show a continuous membrane staining corresponding to 2+. The cells show a more intense staining than to the other 2+ cell line, JIMT-1, but weaker than the 3+ cell line BT474.

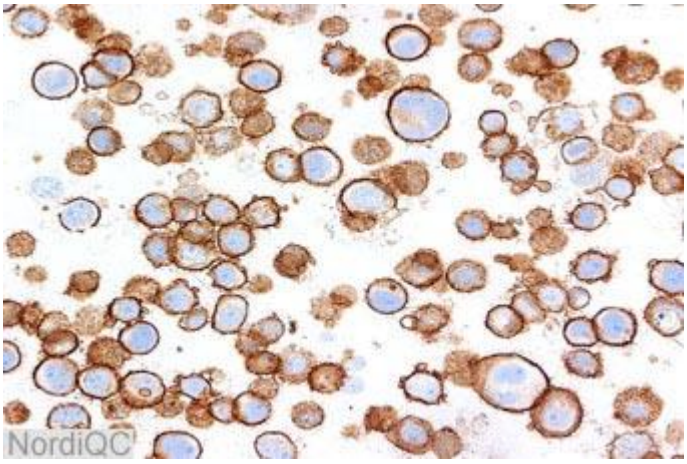

Fig. 2b
Optimal staining for HER-2 of the BT474 cell line. Virtually all cells show a strong complete membrane staining corresponding 3+.

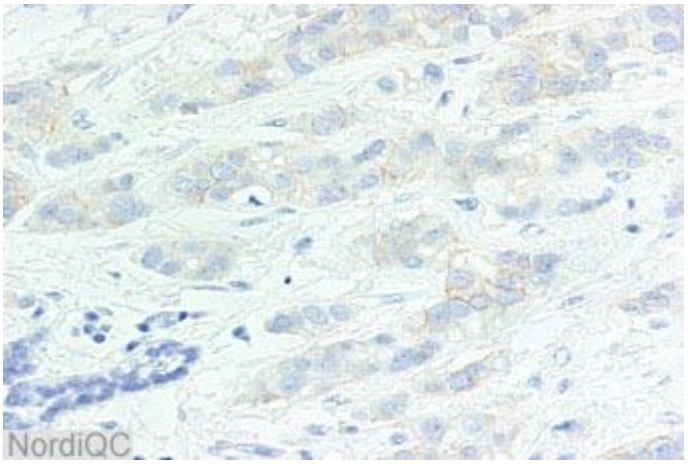

Fig. 3a
Optimal staining for HER-2 of a breast ductal carcinoma without gene amplification. The neoplastic cells show a faint membrane staining corresponding 1+.

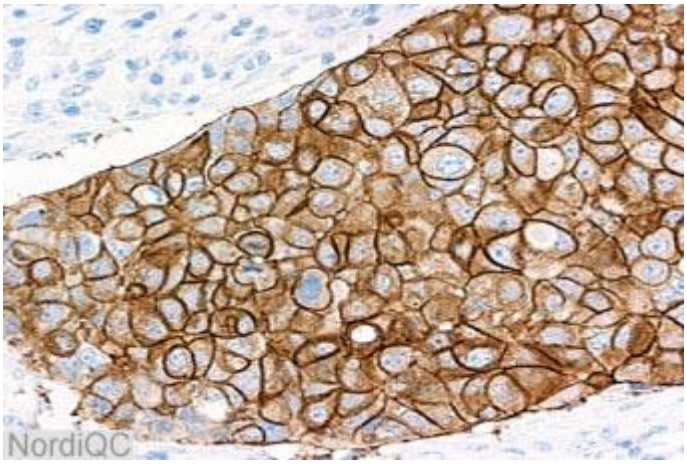

Fig. 3b
Optimal staining for HER-2 of a breast ductal carcinoma with gene amplification. Almost all the neoplastic cells show a strong complete membranous staining corresponding 3+.

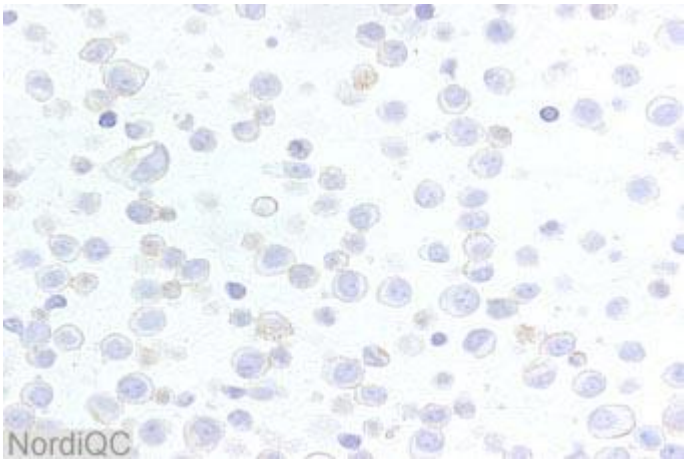

Fig. 4a
Insufficient staining for HER-2 of the 2+ JIMT-1 cell line. The cells only show a weak incomplete membrane staining making this a 1+. Compare with Fig. 1b.
Same protocol results in a false negative staining of the 3+ breast ductal carcinoma with gene amplification, see Fig. 4b.

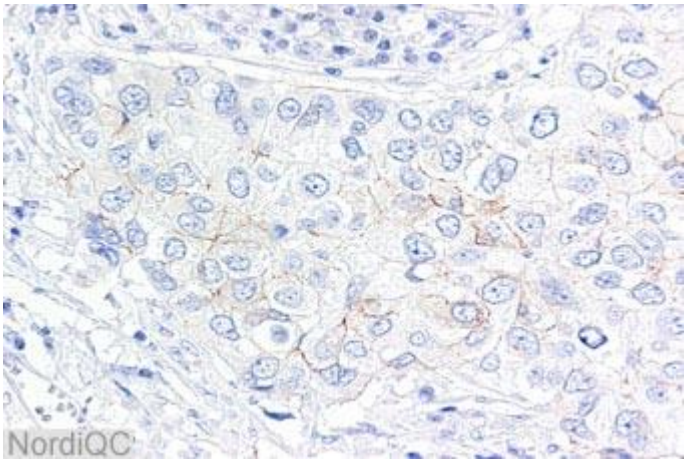

Fig. 4b
Insufficient staining for HER-2 of a breast ductal carcinoma with gene amplification using the same protocol as in Fig. 4a. The neoplastic cells only show a weak, incomplete membrane staining making this a 1+. Same tumor as in Fig. 3b.

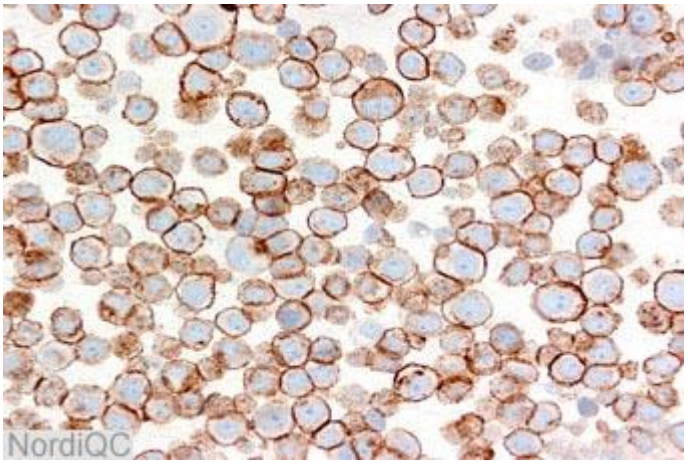

Fig. 5a
Insufficient staining for HER-2 of the 2+ JIMT-1 cell line. The cells show a strong continuous membrane staining making this a 3+. Compare with Fig. 1b.
Same protocol results in a false positive staining of the 1+ breast ductal carcinoma without gene amplification, see Fig. 4b

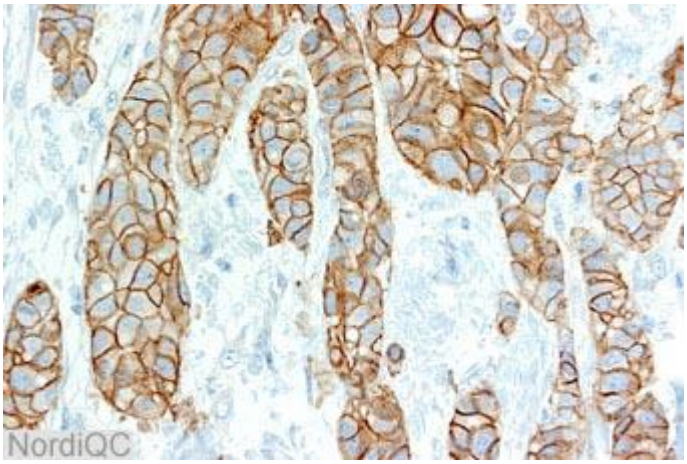

Fig. 5b
Insufficient staining for HER-2 of a breast ductal carcinoma without gene amplification using the same protocol as in Fig. 5a. The neoplastic cells show a strong continuous membrane staining making this a 3+. Same tumor as in Fig. 3a.

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