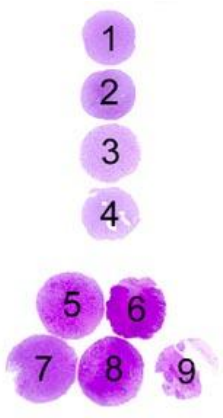
Assessment Run B3 2007
HER-2

The slide to be stained for HER-2 comprised:

	IHC*	FISH*
	HER-2 Score (0, 1+, 2+, 3+)	**Mean HER-2 Copy number**
1. Cell line SK-BR3	3+	Clusters > 3
2. Cell Line MDA-MB-453	2+	2.4
3. Cell Line MDA-MB-175	1+	1.3
4. Cell Line MDA-MB-231	0	1.1
5. Breast ductal carcinoma	3+	Clusters > 3
6. Breast ductal carcinoma	3+	Clusters > 3
7. Breast ductal carcinoma	1+/2+**	1.3
8. Breast ductal carcinoma	1+	1.5
9. Breast ductal carcinoma	0	1.1



***** Verified by 4 NordiQC reference laboratories.
** The score varied through the block.

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 histological specimens corresponding to the gene status. The staining of the additional slide containing 4 cell lines (provided by UK NEQAS) was used to evaluate if they could be included for future HER-2 quality control.

The IHC scoring system was as follows:

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 IHC staining as optimal included:

- A 3+ staining of the breast ductal carcinomas no. 5 and 6.
- A 1+ or 2+ staining of the breast ductal carcinoma no 7.
- A 1+ staining of the breast ductal carcinoma no 8.
- No staining of the breast ductal carcinoma no. 9 and normal breast glandular tissue.
- In cells with a membraneous reaction, no more than a faint cytoplasmic reaction that did not affect the interpretation of the former.

70 laboratories participated in the assessment. 37 laboratories achieved an optimal staining (53 %), 15 good (21 %), 14 borderline (20 %) and 4 (6 %) poor staining.

Table 1. **Scores given for each system/Ab (histological specimens).**

	Score (tissue slide)			
	Optimal	**Good**	**Borderline**	**Poor**
FDA approved systems:				
HercepTest K5204, K5206, K5207, SK001 (Dako, n=34)	23	8	2	1
PATHWAY 790-2991, 800-2996 rmAb clone 4B5 (Ventana, n=15)	12	2	1	0

PATHWAY 760-2694 mAb CB11 (Ventana, n=2)	0	0	2	0
Abs in in-house systems:				
pAb clone A0485 (Dako, n=10)	1	3	5	1
mAb clone CB11 (NeoMarkers, n=1)	0	0	1	0
rmAb clone SP3 (NeoMarkers, n=2; Diagnostic Biosystems, n=1)	0	0	1	2
mAb clone e2-4001+3B5 (NeoMarkers, n=2)	0	0	2	0
mAb clone TAB250 (Zymed, n=1)	0	1	0	0
mAb clone 3B5 (NeoMarkers, n=1)	0	1	0	0
SISH system*				
INFORM HER2 DNA Probe (Ventana, n=1)	1	0	0	0
Total	37	15	14	4

* Silver In Situ Hybridization system, accepted as equivalent to IHC.

As shown in Table 1, with an FDA approved system, 35 out of 51 (69%) obtained an optimal mark, while with an in-house (home made) system only 11% (2 out of 19) obtained this.

HercepTest (Dako): 23 out of 34 (68%) obtained an optimal mark. The procedure was in all cases performed according to the company's instructions, including Heat Induced Epitope Retrieval (HIER) in a water bath. With these settings 31 out of 34 (91%) of the laboratories obtained a sufficient (optimal or good) mark.

PATHWAY rmAb clone **4B5 (RTU)** (Ventana): 12 out of 15 (80%) using this newly FDA-approved system obtained an optimal mark. The optimal protocols were all based on HIER in either Cell Conditioning 1 pH 8,4 (CC1; Benchmark, Ventana)(11 out of 13) or EDTA/EGTA pH 8 (1 out of 2). With CC1 as HIER buffer all of 14 laboratories (100%) obtained a sufficient mark.

Using one of these two systems 92% was marked as sufficient.

pAb **A0485** (Dako): 1 out of 10 (10%) obtained an optimal mark. The Ab was used with a dilution of 1:250 and Cell Conditioning 2 pH 6 (Benchmark, Ventana) was used as HIER buffer. 4 out of 10 (40%) using A0485 obtained a sufficient mark.

Grouped together, using one of the six in-house systems, 6 out of 18 (33%) of the stains obtained a sufficient mark.

INFORM HER2 DNA Probe (SISH)(Ventana): One laboratory used this method and was marked optimal. The FISH results were used as reference and the SISH slide was scored according to the instructions from the vendor.

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 inappropriate staining was typically a too weak or false negative reaction in the breast carcinomas with gene amplification, 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 asked to send in their scores on the multitissue section and the cell line stains.
For 38 out of 67 laboratories returning the slip (57 %) the scores on the multitissue sections were in concordance with those given by the NordiQC assessors.

In total 29 out of 67 laboratories (43 %) both had an appropriate staining of the multitissue slide and an interpretation in concordance with the NordiQC assessor group.

As regards the staining of the cell lines, the overall result was almost identical with that of the staining of the tissue sections: 53 out of 69 slides returned (77%) were marked as sufficient compared to 74% of the tissue sections. However, even though the cell lines are processed similarly to tissues in terms of fixation etc., the concordance (sufficient v. insufficient) was not total, as seen in the table.

	Tissues			
Cell lines	Optimal	Good	Borderline	Poor
Optimal	31	5	4	0
Good	3	7	3	0
Borderline	0	1	5	2
Poor	2	2	2	2

For 57 out of 69 laboratories (83%) the tissue and cell line stains were concordant (i.e., both sufficient or both insufficient), while in 12 (17%), a discrepancy was observed. Even though the mounting of the two materials on separate slides may have contributed to this discrepancy (suggesting lack of reproducibility in the laboratory), other explanations must be looked for, such as the nature of the material, i.e., cell cultures behave differently from tissue in the HER-2 stain. However, we found no particular pattern in the discrepancy that could allow for an explanation.

The laboratories' scoring of the cell lines gave a higher concordance with the NordiQC assessors (86% v. 57%) suggesting that the staining pattern of the cell lines are easier to interpret than that of the tissue slides.

Overall, the proportion of sufficient HER-2 stains on the histological slides is about the same as in Run B2, 2006, i.e about 75%. In contrast to previous assessments virtually all the laboratories adhered strictly to the protocol settings described in the FDA approved HER-2 systems, which made these laboratories improve their performance, increasing the proportion of sufficient stains from 86% to 92%. In contrast, laboratories using in-house (home made) systems performed worse, as the proportion of sufficient stains declined from 58% in Run B2 to 33% in the current run.

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. In-house immunohistochemical systems give unacceptable low performance and should be abolished. Training in scoring is still highly warranted.

It is not settled if cell lines (without concomitant histological slides) are sufficiently reliable for quality assurance of HER-2 staining.

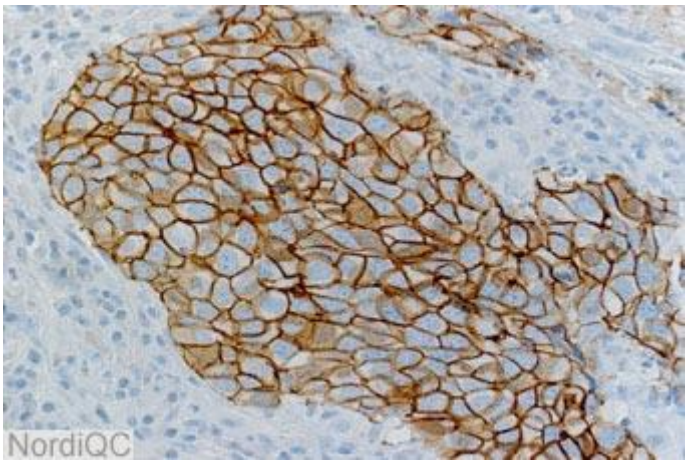


Fig. 1a
Optimal staining for HER-2 of the breast ductal carcinoma no. 6 with gene amplification. Almost all the neoplastic cells show a strong complete membranous staining corresponding to 3+.

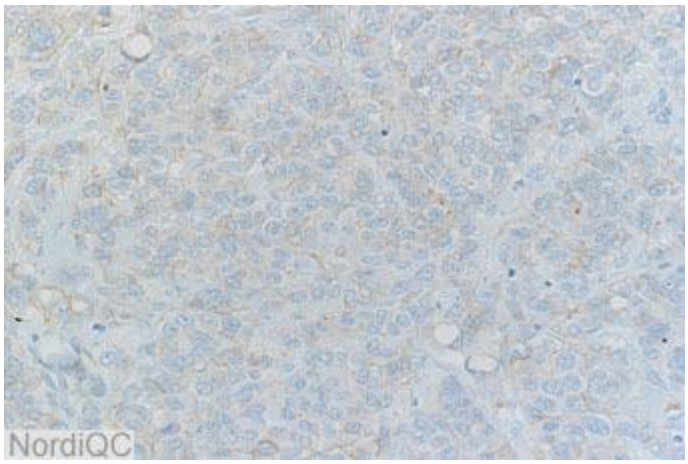


Fig. 1b
Optimal staining for HER-2 of the breast ductal carcinoma no. 8 without gene amplification. The neoplastic cells show a faint incomplete membrane staining corresponding to 1+.

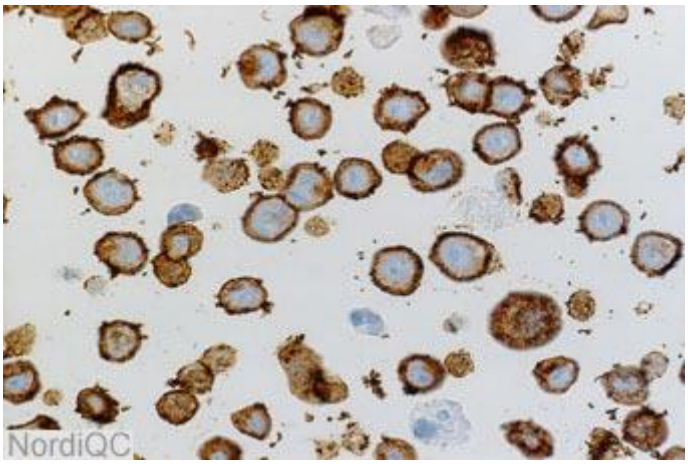


Fig. 2a
Optimal staining for HER-2 of the SK-BR3 cell line. The majority of the cells show a strong complete membrane staining corresponding to 3+. Focally also a cytoplasmic reaction is seen.

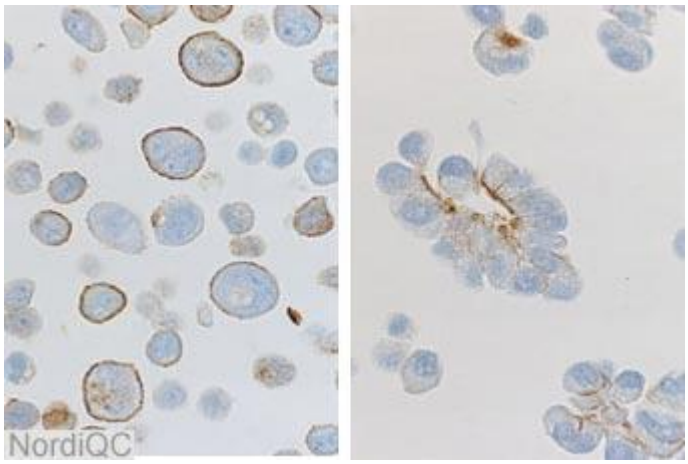


Fig. 2b
Left: Optimal staining for HER-2 of the MDA-MB-453 cell line. More than 10% cells show a weak to moderate complete membrane staining corresponding to 2+.
Right: Optimal staining for HER-2 of the MDA-MB-175 cell line. The majority of the cells predominantly show a partial membrane staining corresponding to 1+.

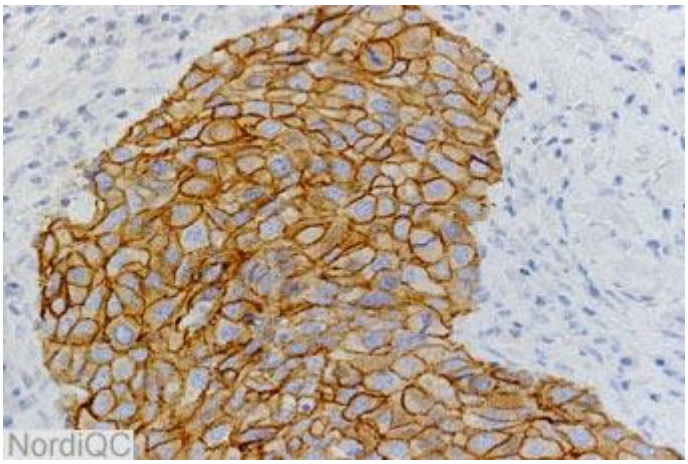


Fig. 3a
Staining for HER-2 of the breast ductal carcinoma no. 6 with gene amplification using an insufficient protocol. Almost all the neoplastic cells show a strong complete membranous staining corresponding to 3+. However compare with Fig. 3b.

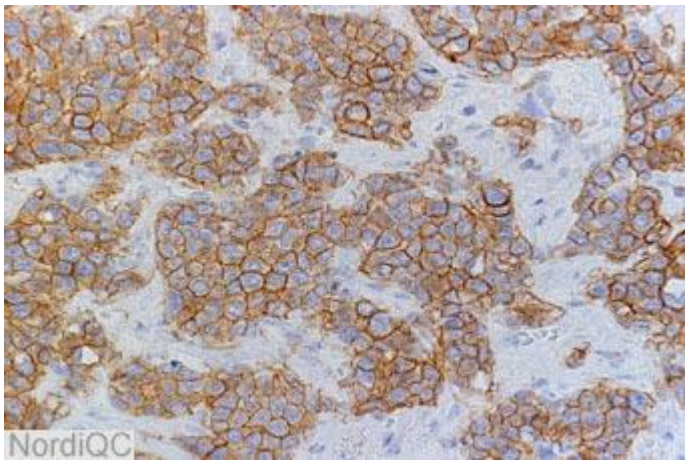


Fig. 3b
Insufficient staining for HER-2 of the breast ductal carcinoma no. 8 without gene amplification using same protocol as in Fig. 3a. The neoplastic cells show a strong continuous membrane staining changing the score form a correct 1+ (Fig. 1b) to a 3+.

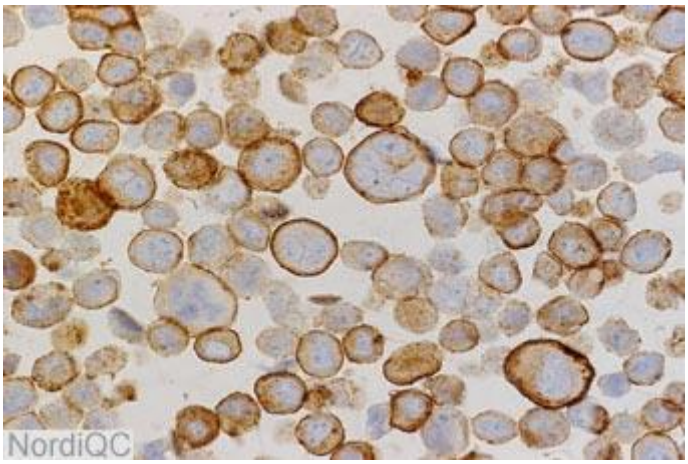


Fig. 4a
Insufficient staining for HER-2 of the 2+ MDA-MB-453 cell line using same protocol as in Figs. 3a-b. The cells show a strong membrane staining making this a 3+. Compare with the optimal staining in Fig. 2b left.

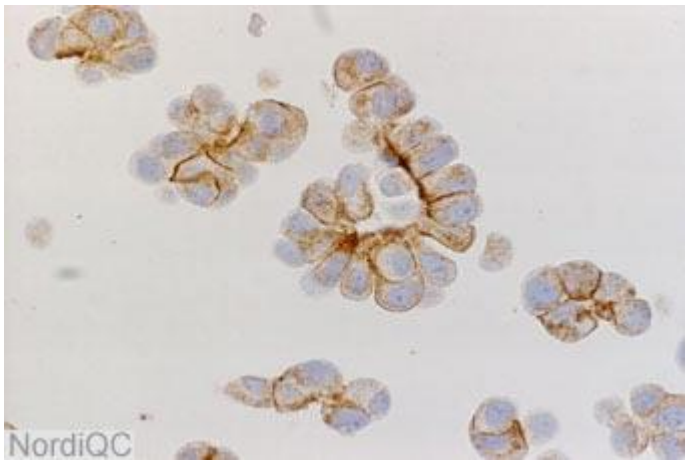


Fig. 4b
Insufficient staining for HER-2 of the 1+ MDA-MB-175 cell line using same protocol as in Fig. 3a & 3b. The cells show a strong membrane and cytoplasmic staining making the result difficult to interpret. Compare with the optimal staining in Fig. 2b right.

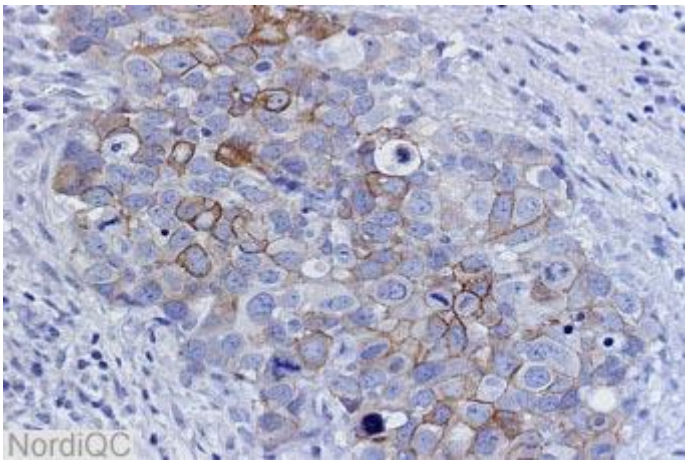


Fig. 5a
Insufficient staining for HER-2 of the breast ductal carcinoma no. 6 with gene amplification. The neoplastic cells only show a moderate membrane staining in scattered cells corresponding to 1+. Compare with the optimal staining in Fig. 1a.

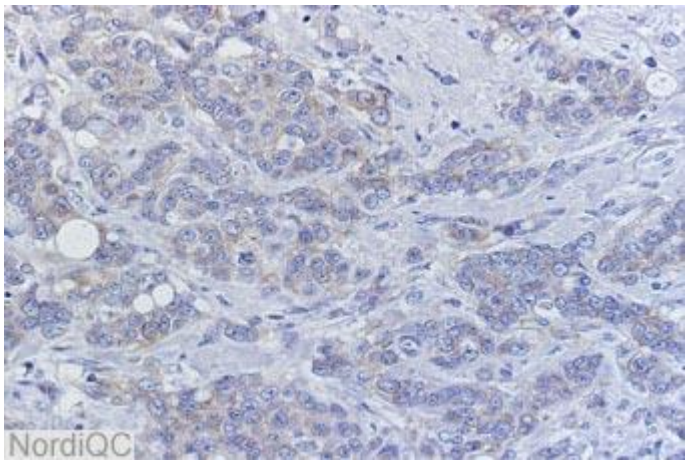


Fig. 5b
Insufficient staining for HER-2 of the breast ductal carcinoma without gene amplification using same protocol as in Fig. 5a. The neoplastic cells show a membrane staining corresponding to 1+. Compare with the result in Fig. 5a. (same score for the the 2 carcinomas).

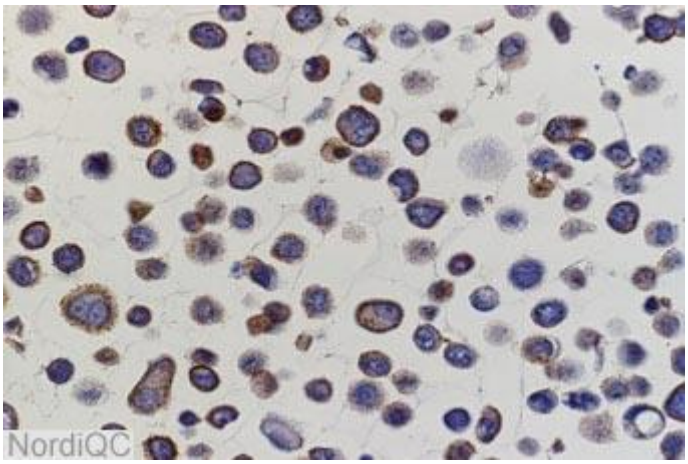


Fig. 6a
Insufficient staining for HER-2 of the 3+ SK-BR3 cell line using same protocol as in Figs. 5a-b. The cells predominantly show a cytoplasmic reaction. A complete membrane reaction is only seen in a few cells. Compare with the optimal staining in Fig. 2a.

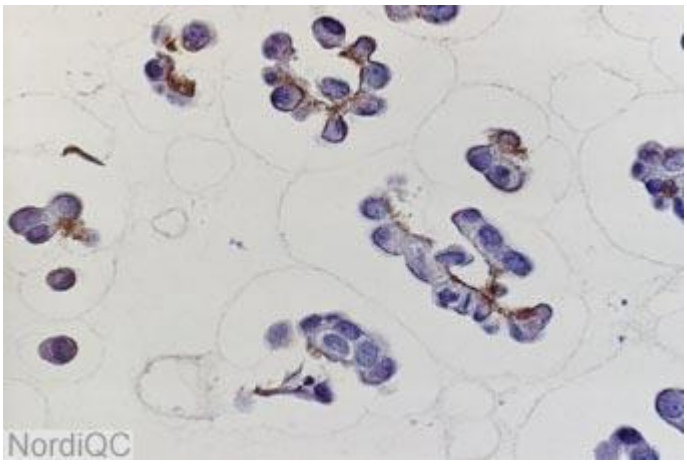


Fig. 6b
Staining for HER-2 of the 1+ MDA-MB-175 cell line using same insufficient protocol as in Figs. 5a-b and 6a.
The staining of the tumours and cell lines are in concordance. Probably insufficient HIER is the reason for the insufficient results.

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