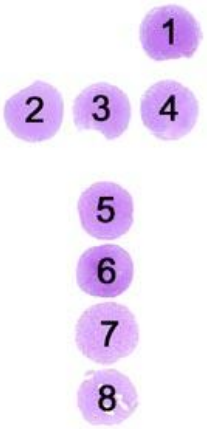
Assessment Run B5 2008
HER-2

The slide to be stained for HER-2 comprised:

	IHC	FISH
	HER-2 Score*	**HER-2 gene / chromosome 17 ratio****
1. Breast ductal carcinoma***	0	1.0
2. Breast ductal carcinoma	3+	cluster > 6
3. Breast ductal carcinoma	2+	3.5
4. Breast ductal carcinoma	2+	1.3
5. Cell line SK-BR3	3+	cluster > 3
6. Cell ine MDA-MB-453	2+	2.4
7. Cell line MDA-MB-175	1+	1.3
8. Cell line MDA-MB-231	0	1.1



* HER-2 immunohistochemical score (see table below) as achieved by using FDA approved kits and antibodies in NordiQC reference laboratories.
** Ratio achieved by using HER2 FISH pharmDX™ Kit, Dako.
*** All carcinomas were fixed 24 - 48 h in 10 % neutral buffered formalin.

IHC scoring system:

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.

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 in accordance with the gene status. The 4 cell lines (provided by UK NEQAS) was included for comparison to evaluate if they could be used for future HER-2 quality control in NordiQC. (Due to more new laboratories attending than expected, 20 of them only received the histological sections).

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

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

A staining was assessed as good, if the tumours no. 2 and 3 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 the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive counterstaining or excessive retrieval hampering the interpretation.

A staining was assessed as poor in case of false negativity (the 3+ and 2+ tumour with gene amplification showing a 1+/0 reaction) or false positivity (the 0/1+ tumour and the 2+ tumour without gene amplification showing a 3+ reaction).

98 laboratories participated in the assessment with an IHC HER-2 staining. Of these 49 laboratories achieved an optimal staining (50 %), 6 good (6 %), 4 borderline (4 %) and 39 (40 %) poor staining.
5 laboratories also send in CISH/SISH of which 2 were assessed as optimal and 3 as good. Table 1 shows the scores in relation to the staining systems used.

Table 1. **Assessment results related to staining systems**

	Mark			
	Optimal	**Good**	**Borderline**	**Poor**
FDA approved systems:				
PATHWAY rmAb clone 4B5 (Ventana, n=25)	23	0	1	1
HercepTest K5204, K5206, K5207, SK001 (Dako, n=44)	19	1	1	23
Abs in in-house systems:				
pAb A0485 (Dako, n=16)	7	2	2	5
mAb clone CB11 (Novocastra, n=3; NeoMarkers, n=1)	0	2	0	2
rmAb clone SP3 (NeoMarkers, n=7)	0	1	0	6
mAb clone 3B5 (NeoMarkers, n=1)	0	0	0	1
mAb clone e2-4001+3B5 (NeoMarkers, n=1)	0	0	0	1

FDA approved systems
PATHWAY rmAb clone **4B5**, (Ventana): 23/25 (92%) using the FDA approved antibody obtained an optimal staining. The optimal protocols were all based on HIER using Cell Conditioning1 on the Benchmark, Ventana (22/23) or HIER in a MWO using Tris-EDTA/EGTA pH 9.0 (1 out of 1). With these settings 23/24 (96 %) obtained a sufficient staining (optimal or good).
HercepTest (Dako): 19/44 (43%) using the FDA approved HercepTest obtained an optimal staining. In 41 out of 44 cases the procedure was performed accordingly to the instructions and protocol settings from the company e.g., HIER in a water bath. With these settings 20/41 (49%) of the laboratories obtained a sufficient staining.

Abs in in-house systems
pAb **A0485** (Dako): 7/16 (44%) obtained an optimal mark. All protocols giving an optimal staining were based on HIER using Citrate pH 6.0 (3/9), Tris-EDTA/EGTA pH 9.0 (2 out of 3), Cell Conditioning1 (Benchmark, Ventana, 1/2) or EDTA/EGTA pH 8.0 (1/1). The pAb A0485 was typically diluted in the range of 1:300-1,500. Using these settings 9/13 (69 %) obtained a sufficient staining.

The laboratories were requested to send in their own scores on the stained sections. For 52/90 laboratories (58 %) returning the slip, the scores on the multi-tissue sections were in concordance with those given by the NordiQC assessors, which is an improvement from 48 % in the previous assessment.

Correlation between cell lines and histology
The correlation between the assessment of the cell lines and the tissues was relatively low (Table 2).

Table 2. **Correlation of overall results of assessment of cell lines and tissues.**

	Tissues			
Cell lines	Optimal	Good	Borderline	Poor
Optimal	37	1	0	12
Good	4	1	0	1
Borderline	3	0	1	8
Poor	0	1	0	11

In only 63/80 cases (79 %) there was a concordance between the results in the cell lines and tissues. This is very much in line with the data from previous run B3, where a concordance of 83% was noticed. Many reasons may contribute to the discrepancy including training and routine to interpret cell lines versus histological sections. However, two patterns were observed:

1. An insufficient (false negative) reaction in the breast ductal carcinoma no. 3 in combination with an optimal staining of the cell lines. This was seen in 13/17 cases.
2. A sufficient staining in the histological specimens in combination with an insufficient staining of the cell lines due to impaired morphology of the cell lines, probably as a results of excessive retrieval.

These data indicate that histological specimens should be the preferred for EQA of HER-2. However, due to potential heterogeneity of tissue material, cell cultures may be valuable as a supplement.

Comments
In this assessment the prevalent feature of an insufficient staining was a too weak reaction, particularly a 1+ reaction in the breast carcinoma no. 3 (amplified tumour expected to give a 2+ reaction). This was seen in 36/42 of the insufficient results (86 %). 10 % of the insufficient results were over-stained as illustrated by a 3+ reaction in the breast carcinoma no. 4 (unamplified tumour expected to give a 2+ reaction).

Protocols based on the FDA approved rmAb clone 4B5 Pathway, Ventana, were the most robust, as 96 % of the laboratories using this obtained a sufficient result on the histological material.
The pass rate of HercepTest (Dako) was found to be low compared to that found in previous assessments of HER-2. In almost all HercepTest cases (23/24) with an insufficient result, the reaction was too weak as indicated above. In 3 cases the laboratory did not follow the recommended protocol guidelines, whereas no other aberrant protocol settings were noticed in the remaining 20 protocols giving insufficient results.
The results of the Herceptest has been presented for Dako, which was supported with various stained slides from the test (with optimal and insufficient staining results) as well as unstained slides and information of the Ab lot numbers used by the participants.
Dako informed NordiQC that no lot-to-lot variation could be detected when NordiQC test slides and control cell lines were stained. Dako notes that the NordiQC 2+ tissue was fixed longer than recommended in the Herceptest protocol. However, the fixation time is within the limits of 48 hours recommended by ASCO.
Dako recommends assessment runs to be performed on cell lines. However, the correlation between cell lines and tissues is not very good in the NordiQC tests, wherefore NordiQC consider the the tissues necessary as the basis for calibration of the system.
At present we have no firm explanation for the low pass rate. However, this was the first NordiQC test including two 2+ cases, of which one was amplified the other not, challenging the systems and the laboratories. It has to be considered if a too low temperature of the water bath may be a cause of the insufficient HerceptTest results, which has not been revealed in previous - maybe less challenging - tests.

It has previously been shown that excessive drying of slides (more than 1 hour at 60°C) prior to IHC for HER-2 can deteriorate the HER-2 antigen for various antibodies. However, we have no reason to believe that this has been a significant cause of suboptimal staining results.

Conclusion
Both FDA approved HER-2 systems PATHWAY and HercepTest as well as an in-house HER-2 method could give an optimal result for the semi-quantitative IHC determination of HER-2 expression. In this assessment PATHWAY was far the most robust method. For all FDA approved HER-systems the protocol has to be followed strictly and the protocol settings should be accurately validated. The unsatisfactory performance of HercepTest in this run should be interpreted with care, as it is based on only one tissue sample. New tests on more 2+ carcinomas must be carried out.

Figures
Figs. 1a and 1b – optimal staining results (a and b same protocol)
Figs. 2a and 2b – insufficient staining results: false negative (a and b same protocol)
Figs. 3a and 3b – insufficient staining results: false positive (a and b same protocol)

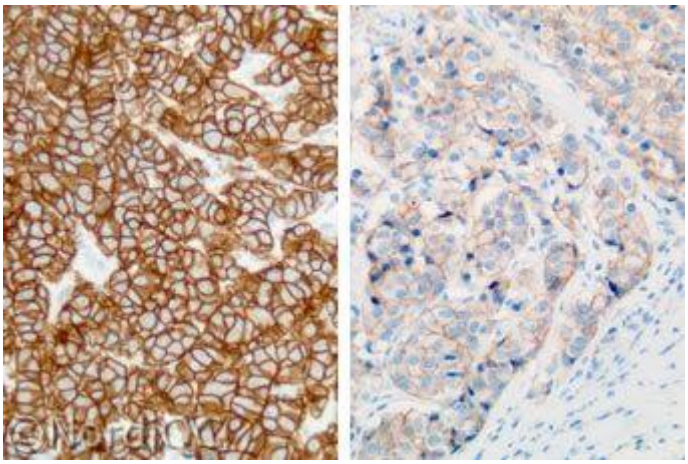


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio > 6 (clusters). > 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/Chr. 17 ratio 3.5.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

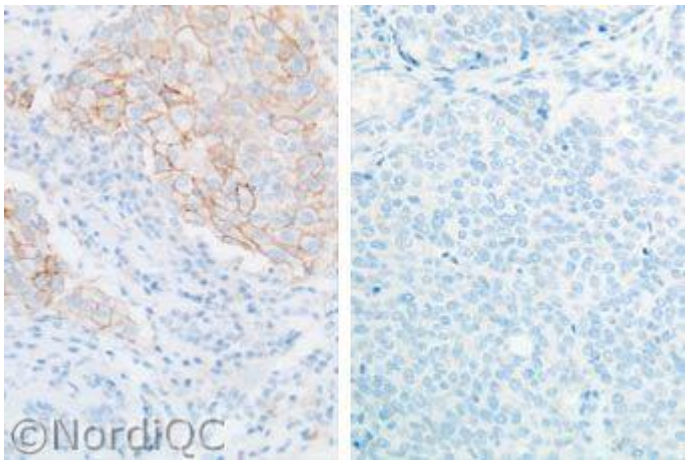


Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio 1.3.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr. 17 ratio 1.0.
The neoplastic cells are all negative corresponding to 0.

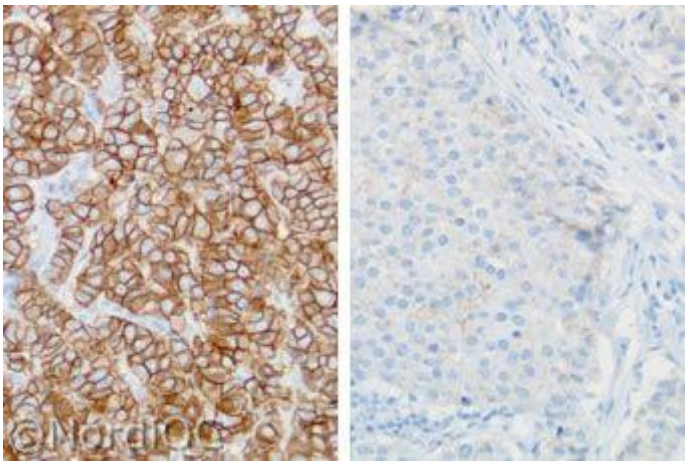


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio > 6 (clusters).
> 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/Chr. 17 ratio 3.5. > 10 % of the neoplastic cells show faint perceptible membrane staining corresponding to 1+.

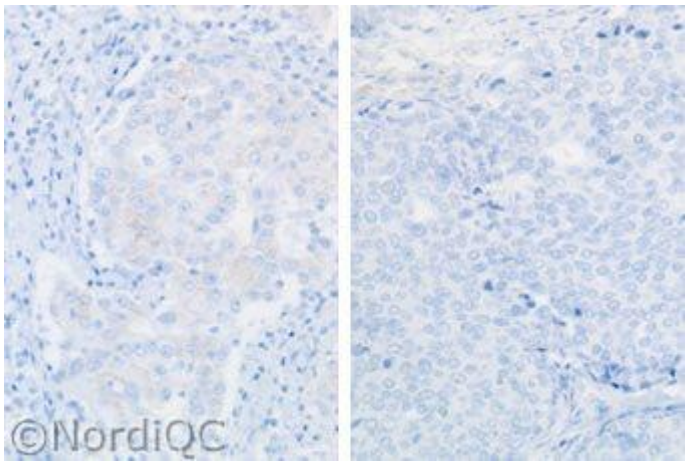


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio 1.3.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr. 17 ratio 1.0.
The neoplastic cells are all negative corresponding to 0.

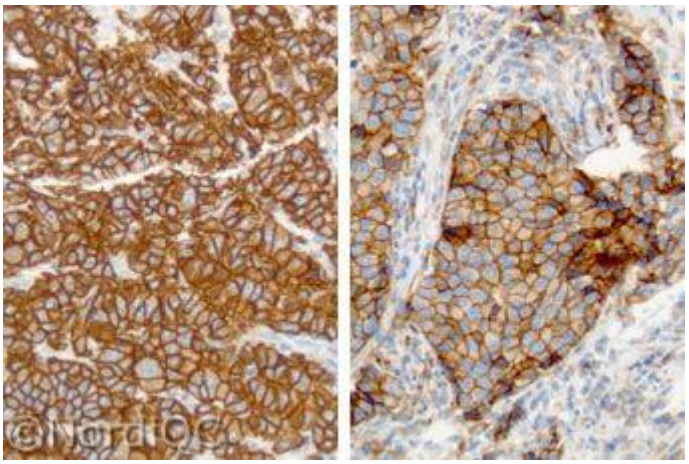


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2/Chr. 17 ratio > 6 (clusters).
> 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 3 with a HER-2/Chr. 17 ratio 3.5. > 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.

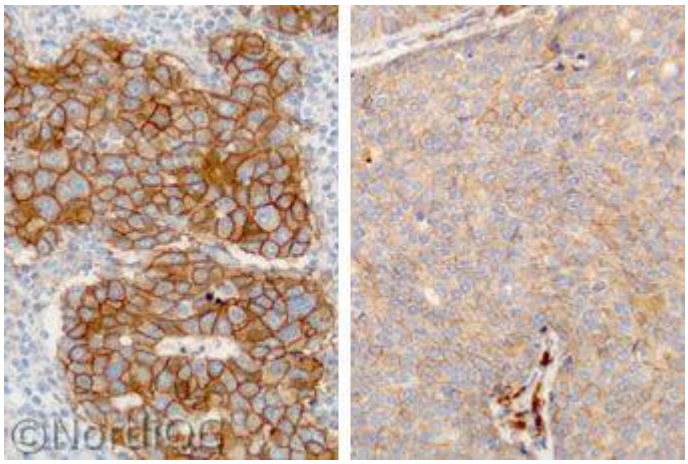


Fig. 3b
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio 1.3.
> 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr. 17 ratio 1.0.
An excessive cytoplasmic staining impedes the interpretation.

SN/HN/MV/LE 4-7-2008