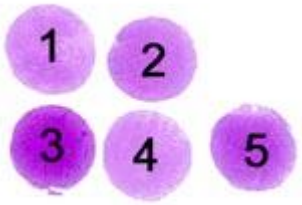
Assessment Run B6 2008
HER-2

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+	5,2 – 5,8
2. Breast ductal carcinoma	2+	3,1 – 3,5
3. Breast ductal carcinoma	2+	1,0 – 1,2
4. Breast ductal carcinoma	1+	1,1 – 1,3
5. Breast ductal carcinoma	0	1,0 – 1,2



* HER-2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits and antibodies (HercepTest™ and PATHWAY®) 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 30% 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 specimens corresponding to the gene status. The cut-off level for a 3+ tumour was in this assessment changed from 10% to 30% of the tumour cells showing a strong complete membrane staining according to the guidelines given by ASCO and CAP.

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

A staining was assessed as good, if the HER-2 gene amplified tumour no. 1 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 (i.e., the 3+ and 2+ tumour with gene amplification showing a 1+/0 reaction) or false positivity (i.e., the 0/1+ tumours and the 2+ tumour without gene amplification showing a 3+ reaction).

Results
105 laboratories participated in the assessment with an IHC HER-2 staining.
75 laboratories achieved an optimal staining (71 %), 5 good (5 %), 2 borderline (2 %) and 23 (22 %) poor staining.
6 laboratories also send in CISH (Zymed) or SISH (Ventana), of which 4 were assessed as optimal and 2 as good. However, these were performed without staining for chromosome (Chr.) 17, wherefore the HER- 2 gene / Chr. 17

ratio was not available.

Table 1. **The IHC systems/Abs used and the scores given**

	Score/tissue			
	Optimal	**Good**	**Borderline**	**Poor**
FDA approved systems:				
PATHWAY® rmAb clone **4B5** (Ventana, n=29)	27	0	1	1
HercepTest™ **K5204, K5206, K5207, SK001** (Dako, n=44)	38	1	0	5
Abs in in-house systems:				
pAb clone **A0485** (Dako, n=16)	6	1	0	9
mAb clone **CB11** (Novocastra/Leica n=4, NeoMarkers/Thermo, n=1)	3	0	0	2
rmAb clone **SP3** (NeoMarkers/Thermo, n=5, Zhongshan Goldenbridge, n=1)	0	1	1	4
mAb clone **3B5** (NeoMarkers/Thermo, n=3)	0	2	0	1
mAb clone **e2-4001+3B5** (NeoMarkers/Thermo, n=2)	0	1	0	1

FDA approved systems:
PATHWAY® rmAb clone 4B5 (RTU) (Ventana): 27 out of 29 (93%) using the FDA approved antibody obtained an optimal mark. The optimal protocols were all based on HIER in Cell Conditioning1 (Benchmark, Ventana, 27 out of 27). Using these settings 97% of the laboratories (27 out of 28) obtained a sufficient staining marked optimal.
HercepTest™ (Dako): 38 out of 44 (86%) using the FDA approved HercepTest™ obtained an optimal mark. In all 44 cases the procedure was performed accordingly to the instructions from the company. Using these settings 89% of the laboratories (39 out of 44) obtained a sufficient staining marked as optimal or good.

In-house systems:
pAb **A0485:** 6 out of 16 (38%) obtained an optimal mark. All protocols resulting in an optimal staining were based on HIER using Citrate pH 6 (2 out of 6), Cell Conditioning1 (Benchmark, Ventana, 3 out of 3) or EDTA/EGTA pH 8.0 (1 out of 1). The pAb A0485 was typically diluted in the range of 1:200-1000. Using these settings 60% (6 out of 10) obtained a sufficient staining marked optimal or good.
mAb **CB11** (Novocastra/Leica): 3 out of 5 (60%) obtained an optimal mark. Using a concentrated Ab, the dilution was in the range of 1:100 – 500 with HIER in either Cell Conditioning1 (Benchmark, Ventana) or Tris-EDTA pH 9.0. Also a Ready-To-Use Ab (Oracle, Leica) could be used to obtain an optimal staining. Using these settings 75 % of the laboratories (3 out of 4) obtained a sufficient staining marked optimal or good.

Comments
In this assessment the prevalent feature of an insufficient staining was a too weak or a false negative reaction, which particularly and most critical was observed as a 1+ reaction in the HER-2 gene amplified breast carcinoma no. 2, but also in the breast carcinoma no. 3. Both were shown to be IHC 2+ in the NordiQC reference laboratories using HercepTest™, Dako and PATHWAY®, Ventana. This reaction pattern was seen in 80% of the insufficient results (20/25) whereas 16 % (4/25) of the insufficient results were caused by a false positive staining giving a 3+ reaction in the breast carcinoma no. 3, which was a HER-2 gene unamplified tumour expected and allowed at maximum to give a 2+ reaction.

Grouped together, the FDA approved IHC systems gave a total pass rate of 92 % as 66 out of 72 laboratories following the vendors protocol recommendations and guidelines obtained a sufficient staining, while the pass rate for an in-house system was 44 %, 14 out of 32 laboratories.

This was the 7th HER-2 assessment in NordiQC and as illustrated in table 2, the two FDA approved systems have given a superior pass rate compared to the in-house HER-2 protocols.
The average pass rate was 39 % for in-house protocols compared to 83 % for HercepTest™ and 94 % for PATHWAY® through all 7 runs.

Table 2. **Pass rates through 7 HER-2 NordiQC runs.**

In contrast to the previous assessment B5, in which two 2+ tumours were included for the first time, and HercepTest™ showed low proportion of sufficient results, the test now yielded a pass rate of 89 % in line with previous assessments. The reason for the aberrant result of HercepTest™ in run B5 is still unexplained but we consider that may be related to certain batches in combination with the laboratory handling.
However, from the participants' protocol data no major changes can be identified between the two runs and it is not possible to give any solid evaluation of any lot-to-lot variation or other differences in the kits, as only 4 laboratories used same lot in the two runs. The recommendation given to verify the protocol settings inclusive the verification of temperature of the water bath and HIER buffer can not be tracked in the protocols.

The pass rate in B6 is still inferior to the result in B4 (86% pass rate), but the design and composition of the multitissue block was changed in B5 as two 2+ IHC tumours were included, which has challenged the laboratories performance compared to the multitissue blocks used in the previous assessments based on 0/1+ and 3+ tumours.
The laboratories were requested to send in their own scores on the stained sections. For 59 out of 96 laboratories (62 %) returning the slip, the scores on the multi-tissue sections were in concordance with those given by the NordiQC assessors, which is a slight improvement from 58 % in the previous assessment.

Conclusion

The two FDA approved HER-2 systems HercepTest™, Dako and the PATHWAY® rmAb clone 4B5, Ventana were the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression, producing a sufficient staining in 89 % and 97 %, respectively, while in-house protocols produced a sufficient staining in 44 % only. The inclusion of 2+ tumours in the assessment material seems to be valuable in efficiently monitoring the IHC HER-2 performance.

Figures

Figs. 1a and 1b – optimal staining results, same protocol
Figs. 2a and 2b – insufficient staining results – false negative, same protocol
Figs. 3a and 3b – insufficient staining results – false positive, same protocol.

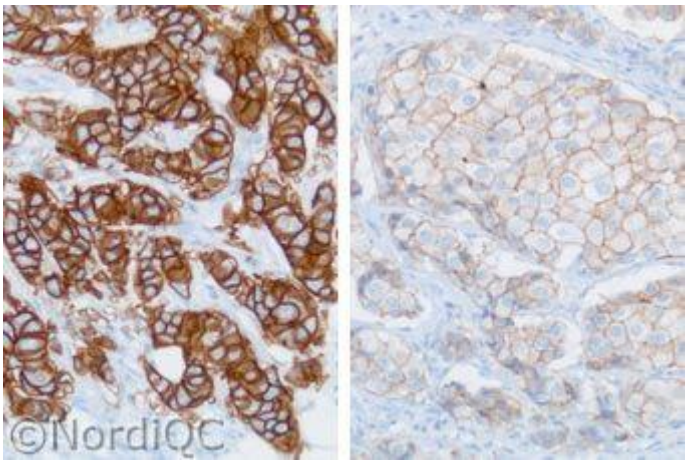


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 1 with HER-2 / Chr. 17 ratio of 5.2 – 5.8. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chr. 17 ratio of 3.1 – 3.5. > 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

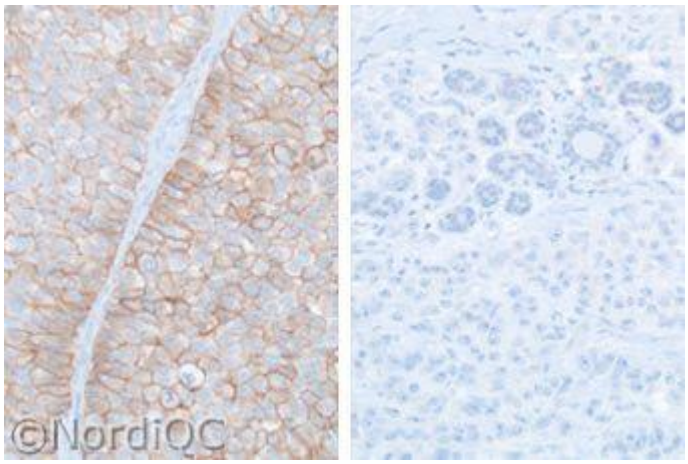


Fig. 1b
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 3 a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
The neoplastic cells are all negative corresponding to 0. Also note the benign glands are unstained.

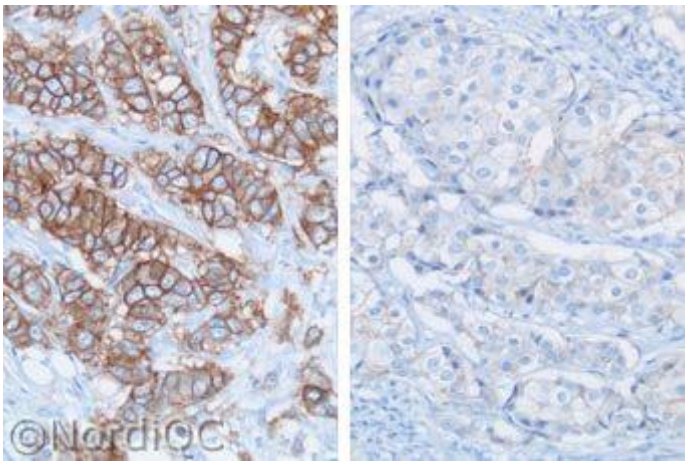


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2 / Chr. 17 ratio of 5.2 – 5,7.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chr. 17 ratio of 3.1 – 3.5.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+.

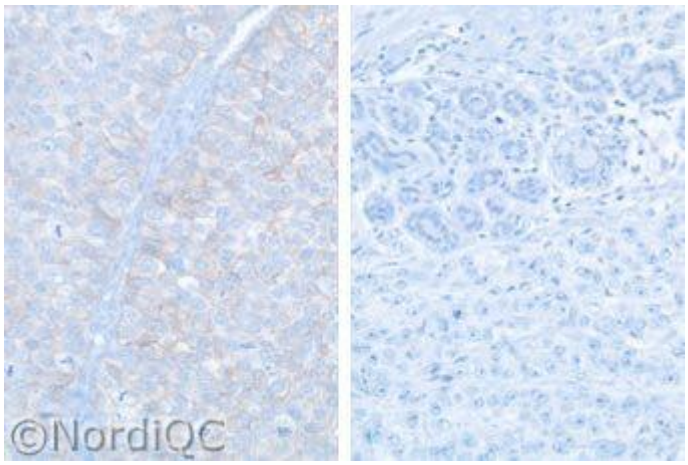


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
The neoplastic cells are all negative corresponding to 0.

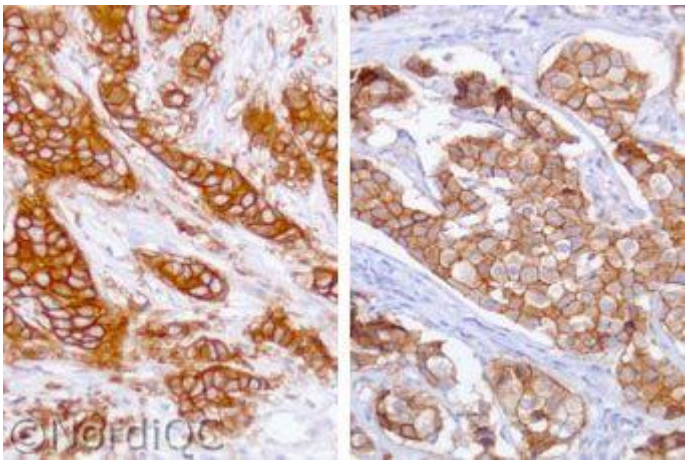


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2 / Chr. 17 ratio of 5.2 – 5.7.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 2 with a HER-2 / Chr. 17 ratio of 3.1 – 3.5.
> 10 % of the neoplastic cells show a strong and complete membranous staining corresponding to 2+.

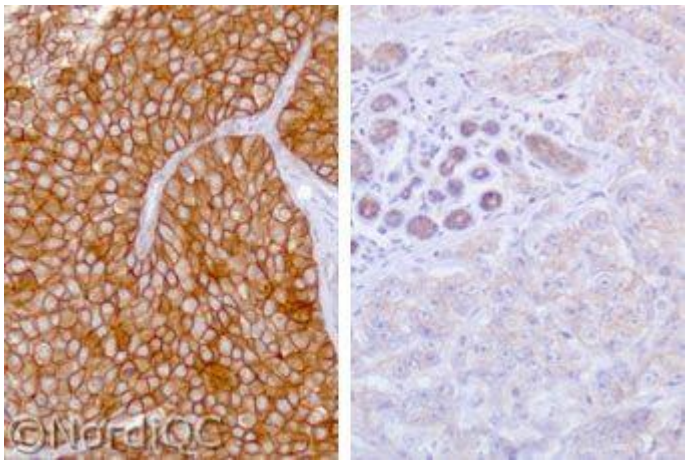


Fig. 3b
Left: Insufficient staining for HER-2 of the breast ductal carcinoma no. 3 a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2 / Chr. 17 ratio of 1.0 – 1.2.
The neoplastic cells show a faint perceptible staining corresponding to 1+, while the benign glands show a moderate staining.

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