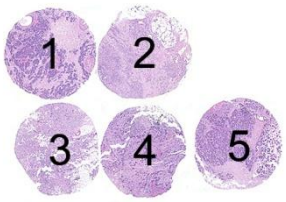
Assessment Run B8 2009

HER-2 IHC

The slide to be stained for HER-2 comprised the following 5 tissues:

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2 gene/chr.17 ratio****
1. Breast ductal carcinoma	0	1.0 – 1.2
2. Breast ductal carcinoma	1+	1.1 – 1.3
3. Breast lobular carcinoma	1+/2+***	1.2 – 1,5
4. Breast ductal carcinoma	2+	2.5 – 2.9
5. Breast ductal carcinoma	3+	> 6.0, clusters



* HER-2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits and antibodies (HercepTest™, Dako, and PATHWAY®, Ventana) in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 Ratio achieved by using HER-2 FISH pharmDX™ Kit, Dako
*** Staining varied through the tissue block.

All carcinomas were fixed for 24 - 48 h in 10 % neutral buffered formalin.

IHC scoring system according to the guidelines given by ASCO/CAP:

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.

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

- A clear and unequivocal immunohistochemical staining marked as score 0 or 1+ in the breast ductal carcinomas no. 1 & 2.
- A clear and unequivocal immunohistochemical staining marked as score 1+/2+ in the breast carcinoma no 3.
- A clear and unequivocal immunohistochemical staining marked as score 2+ in the breast ductal carcinoma no 4.
- A clear and unequivocal immunohistochemical staining marked as score 3+ in the breast ductal carcinoma no 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. 5 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by FISH according to the ASCO/CAP guidelines and the national guidelines in Denmark, Norway and Sweden) and the other breast carcinomas showed a reaction pattern as described above.

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 (e.g. the 3+ tumour and the 2+ tumour with gene amplification showed a 1+ reaction) or false positivity (e.g. the 0, 1+ and 2+ tumours without gene amplification showed a 3+ reaction).

Results
136 laboratories participated in this assessment. 72 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

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

FDA approved HER-2 systems	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
PATHWAY® rmAb clone **4B5, 790-2991,** CONFIRM™, rmAb clone **4B5, 800-2996**	40	Ventana	38	2	0	0	100 %	100 %
HercepTest™ **K5204, K5206, K5207, SK001**	49	Dako	34	4	1	10	78 %	81 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	3	Leica	1	0	0	2	-	-
Abs for in-house HER-2 systems								
pAb clone **A0485**	19	Dako	7	1	2	9	42 %	64 %
mAb clone mAb clone **CB11**	1 1 1	Monosan Novocastra NeoMarkers	0	2	0	1	-	-
mAb clone **3B5**	4	NeoMarkers	0	0	0	4	-	-
mAb clone **e2-4001+3B5**	2	NeoMarkers	0	0	0	2	-	-
rmAb clone **SP3**	13 1 1	NeoMarkers Epitomics Zytomed	7	2	4	2	60 %	62 %
rmAb clone **EP1045Y**	1	Biocare	0	0	0	1	-	-
Total	136		87	11	7	31	-	-
Proportion			64 %	8 %	5 %	23 %	72 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

FDA approved systems

PATHWAY® / CONFIRM™ rmAb clone **4B5** (Ventana): 38 out of 40 (95 %) obtained an optimal mark. The protocols giving an optimal result were all based on HIER using Cell Conditioning 1 mild or standard, 1 lab used MWO and Tris-EDTA pH 9. The incubation time for the primary Ab was in the range of 8 - 32 min and as detection kit either iView or UltraView was used. Using these protocol settings all of 40 (100 %) laboratories produced a sufficient staining.

HercepTest™ (Dako): 34 out of 49 (70%) obtained an optimal mark. The protocols giving an optimal result were based on HIER for 40 min using water bath at 96 - 99°C and an incubation time of 25-30 min in the primary Ab. Using these protocol settings 38 out of 47 (81 %) laboratories produced a sufficient staining.

CE IVD approved systems

Oracle™ (Leica) mAb clone CB11: 1 out of 3 obtained an optimal mark. The optimal protocol used HIER in Bond Epitope Retrieval Solution 2 for 25 min. and the mAb clone CB11 in a Ready-To-Use format and an incubation time for 30 min.

Abs in in-house systems

pAb **A0485**: 7 out of 19 (37 %) obtained an optimal mark. All protocols resulting in an optimal staining were based on HIER using either Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (2/3)*, Cell Conditioning 1 (BenchMark, Ventana) (2/3), Citrate pH 6 (1/9), Tris-EDTA/EGTA pH 9 (1/2) or EDTA/EGTA pH 8 (1/1). The pAb A0485 was typically diluted in the range of 1:400-1:1.000 depending on the total sensitivity of the protocol employed. Using these settings 7 out of 11 (64 %) obtained a sufficient staining marked optimal.
* (number of optimal results/number of laboratories using this buffer)

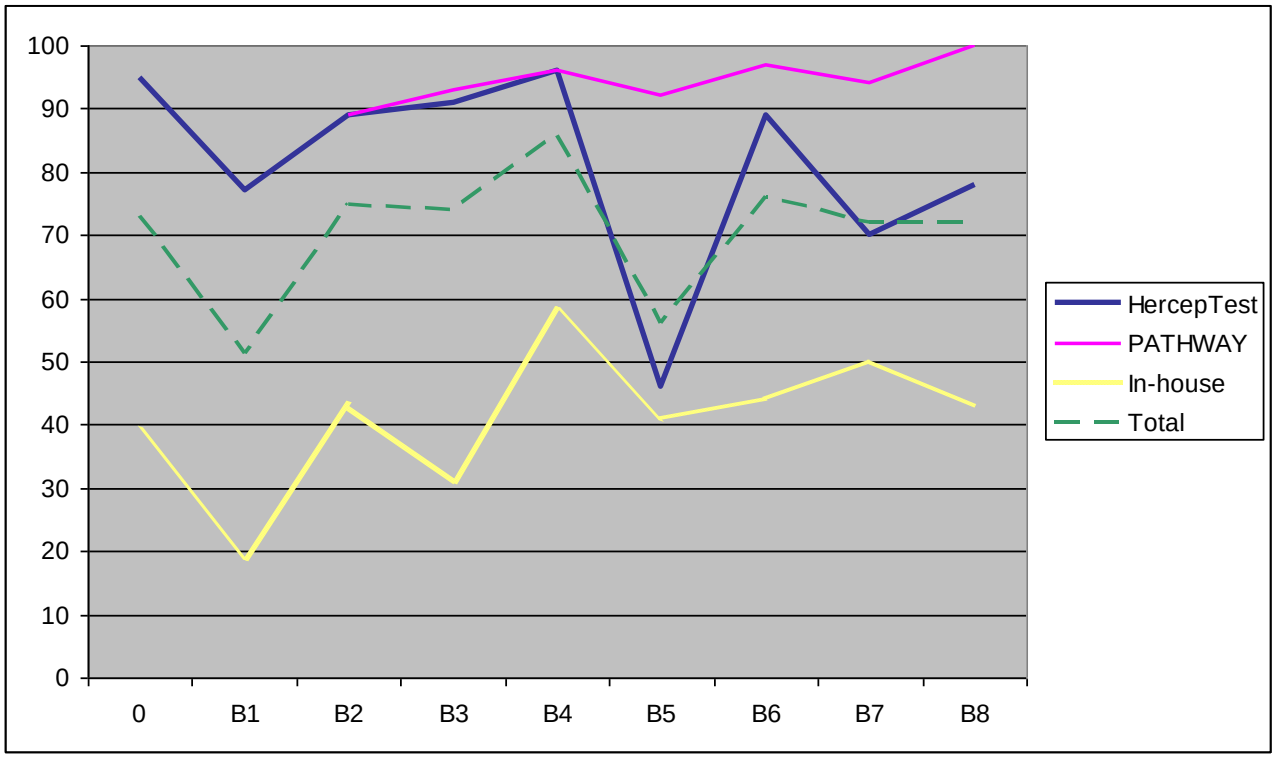
rmAb **SP3**: 7 out of 15 (47 %) obtained an optimal mark. The optimal protocols were based on HIER using either Tris-EDTA/EGTA pH 9 (2/6)*, Cell Conditioning 1 (BenchMark, Ventana) (2/3) or Citrate pH 6 (3/5) as HIER buffer. The Ab was typically diluted in the range of 1:20-200 depending on the total sensitivity of the protocol employed. Using these settings 8 out of 13 (62 %) obtained a sufficient staining (optimal or good).

Comments
In this assessment and in concordance with the previous HER-2 assessments, 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 0 or 1+ reaction in the HER-2 gene amplified breast carcinoma no. 4. This tumour was shown to be IHC 2+ in the NordiQC reference laboratories using both HercepTest™, Dako, and PATHWAY®, Ventana, and showed a low level of HER-2 gene amplification (ratio of 2.5 – 2.9). The weak or false negative reaction was seen in 63 % of the insufficient results (24/38) whereas 27 % (14/38) of the insufficient results were due to a false positive staining and/or a poor signal-to-noise ratio. The weak, insufficient results were seen both when kits like the HercepTest™, Dako, and Oracle™, Leica, were used and when in-house protocols were used. The false positive stains and poor signal-to-noise ratios were virtually only seen when an in-house protocol was applied. The mAb clone 3B5 and the mAb clone cocktail 3B5 + e2-4001 both gave a strong granular cytoplasmic reaction in the majority of the neoplastic cells in all the specimens in the multitissue block hampering the interpretation of the specific membranous reaction. All 6 protocols based on these two Abs gave an insufficient result.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a pass rate of 86 % (79 out of 92 laboratories), while the pass rate for an in-house system was 43 % (19 out of 44 laboratories).

This was the 9th NordiQC HER-2 assessment. As illustrated in Fig. 1, the two FDA approved systems have almost constantly given a superior pass rate compared to the in-house HER-2 protocols.
As shown in Fig. 1. the average pass rate in the 9 runs was 94 % for PATHWAY® (Ventana, rmAb clone 4B5), 81 % for HercepTest™ (Dako) and 41 % for in-house protocols.

Figur 1. **Pass rate through 9 HER-2 assessments**



In this HER-2 assessment the over-all pass rate of 72 % was exactly the same as obtained in the previous assessment, run B7 2009. Many new laboratories participated in the current HER-2 assessment for the first time. For the 35 laboratories participating for the first time, the pass rate was 60 %, whereas the pass rate for the 101 laboratories participating in both run B7 and B8, the pass rate was 76 %.

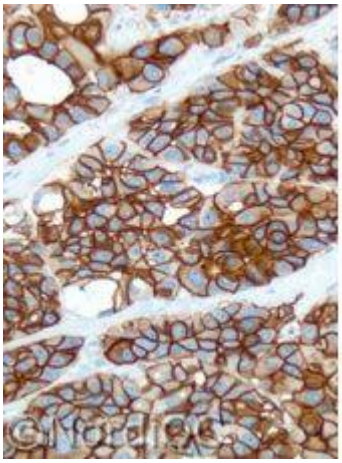
Scoring consensus
The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 81 out of the 126 laboratories (64 %) returning the slip, the scores on all the tissues in the multi-tissue sections were in concordance with the scores given by the NordiQC assessor group. A sufficient staining combined with an interpretation in concordance with the NordiQC assessors was seen in 77 % (75 out of 98), which was a significant improvement from 61 % in run B7.

Conclusion

The two FDA approved HER-2 systems HercepTest™ (Dako) and PATHWAY® rmAb clone 4B5 (Ventana), were in this assessment the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression. The inclusion of the 2+ tumours (from run B5 onwards) with and without HER-2 gene amplification is essential to evaluate the IHC HER-2 performance and the robustness of the protocols used by the participants.

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.



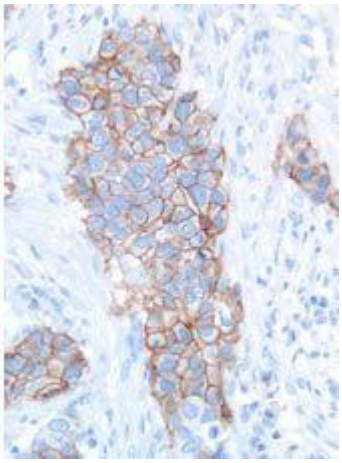
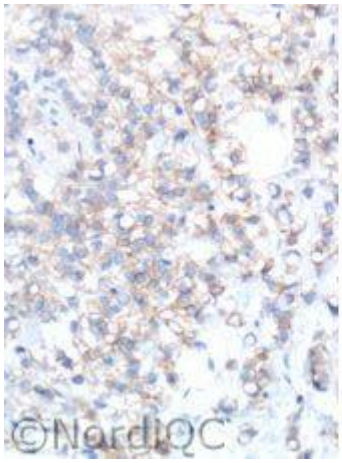


Fig. 1a
Left: Optimal staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/chr. 17 ratio > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio 2.5 – 2.9.
> 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.



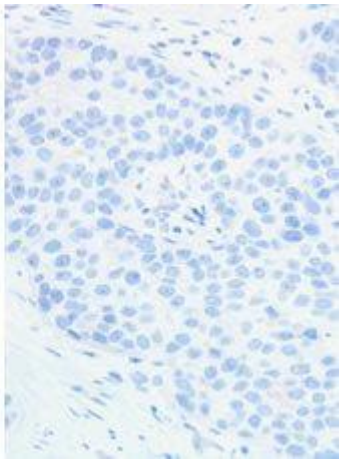
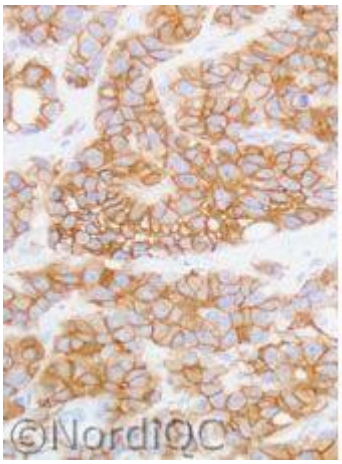


Fig. 1b
Left: Optimal staining for HER-2 of the breast carcinoma no. 3 with a HER-2/Chr. 17 ratio 1.2 – 1.5.
> 10 % of the neoplastic cells show a weak complete membranous staining corresponding to 2+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr. 17 ratio 1.0 – 1.2.
The neoplastic cells are all negative corresponding to 0.



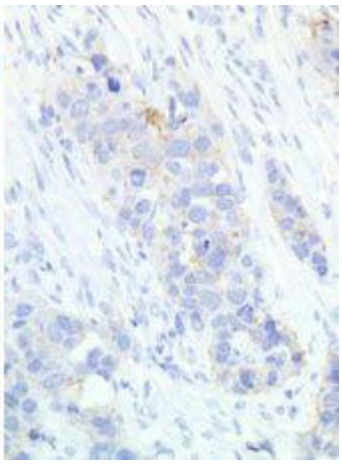
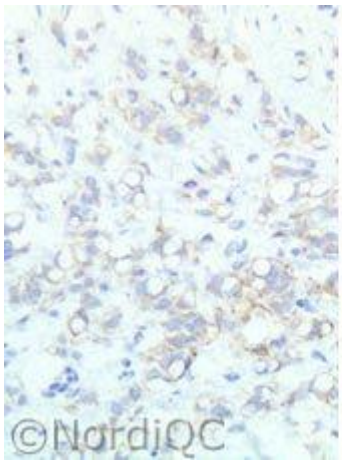


Fig. 2a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a HER-2/chr. 17 ratio > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr. 17 ratio 2.5 – 2.9.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be referred to ISH.



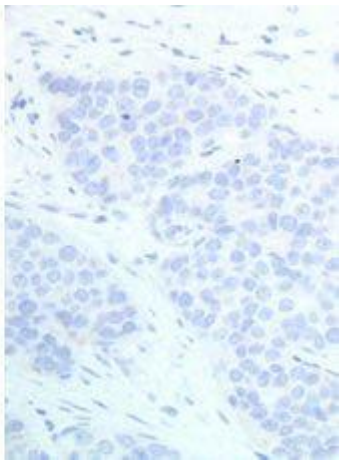


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 3 a HER-2/chr. 17 ratio 1.2 – 1.5.
> 10 % of the neoplastic cells show a faint perceptible membrane staining corresponding to 1+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr. 17 ratio 1.0 – 1.2.
The neoplastic cells are all negative corresponding to 0.

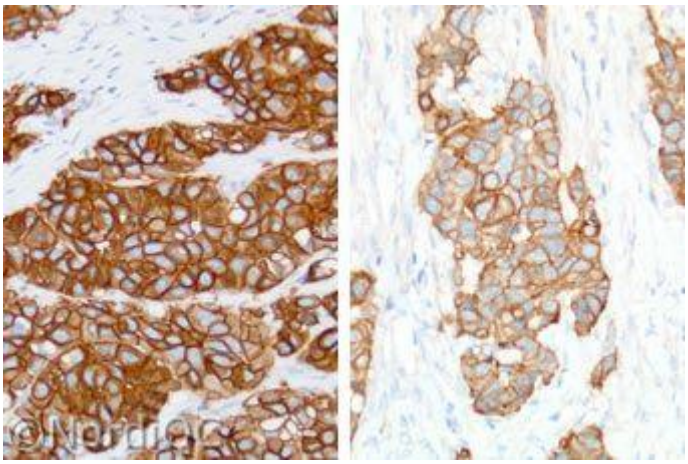


Fig. 3a
Left: Staining for HER-2 of the breast ductal carcinoma no. 5 with a a HER-2/chr. 17 ratio > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Staining for HER-2 of the breast ductal carcinoma no. 4 with a HER-2/Chr.17 ratio 2.5 – 2.9.
> 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+. However also compare the results in Figs. 3b left and right.

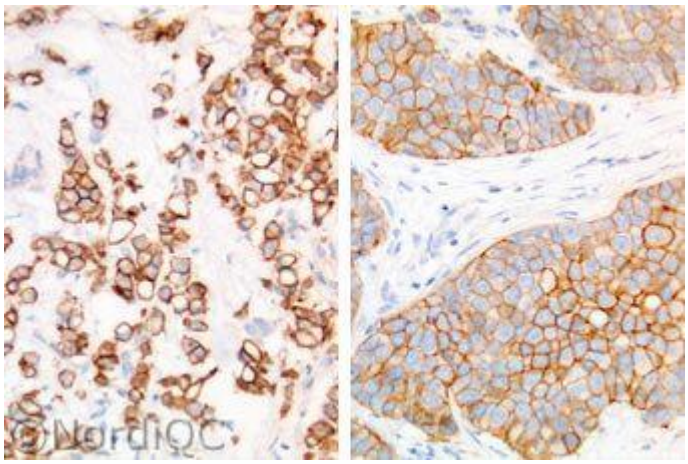


Fig. 3b
Left: Insufficient staining for HER-2 of the breast carcinoma no. 3 with a HER-2/chr. 17 ratio of 1.2 – 1.5.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insuffcient staining for HER-2 of the breast ductal carcinoma no. 1 with a HER-2/Chr.17 ratio 1.0 – 1.2.
> 10 % of the neoplastic cells show a moderate and complete membranous staining corresponding to 2+.

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