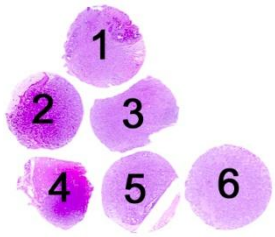
Assessment Run B7 2009
HER-2

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

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2 gene/chr17 ratio****
1. Breast ductal carcinoma	0/1+	1.0 – 1.2
2. Breast ductal carcinoma	0/1+	1.2 – 1.5
3. Breast ductal carcinoma	2+	2.4 – 2,8
4. Breast ductal carcinoma	1+	1.3 – 1.6
5. Breast ductal carcinoma	3+	5.1 – 5.8
6. Breast ductal carcinoma	3+	> 6.0



* 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 HER2 FISH pharmDX™ Kit, Dako.

All carcinomas were fixed 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 2+ in the breast ductal carcinoma no 3.
- A clear and unequivocal immunohistochemical staining marked as score 1+ in the breast ductal carcinoma no 4.
- A clear and unequivocal immunohistochemical staining marked as score 3+ in the breast ductal carcinoma no 5 & 6.
- 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 & 6 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).

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

Results
114 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 scores given**

FDA approved HER-2 systems	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
PATHWAY® rmAb clone **4B5, 790-2991, 800-2996**	33	Ventana	30	1	0	2	94 %	100 %
HercepTest™ **K5204, K5206, K5207, SK001**	47	Dako	33	0	0	14	70 %	75 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	2	Leica	2	0	0	0	-	-
Abs for in-house HER-2 systems								
pAb clone **A0485**	15	Dako	9	1	2	3	66 %	77 %
mAb clone **CB11**	4 1 1 1	Novocastra Biocare NeoMarkers Zytomed	2	1	0	4	43 %	75 %
rmAb clone **SP3**	6 1	NeoMarkers Master Diagnostica	0	2	1	4	29 %	-
rmAb clone **EP1045Y**	1	Biocare	0	0	0	1	-	-
mAb clone **e2-4001+3B5**	2	NeoMarkers	0	1	1	0	-	-
Total	114		76	6	4	28	-	-
Proportion			67 %	5 %	3 %	25 %	72 %	83 %

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

FDA approved systems

PATHWAY® rmAb clone **4B5** (Ventana): 30 out of 33 (91%) obtained an optimal mark. The protocols giving an optimal result were all based on HIER using Cell Conditioning 1 mild or standard and an incubation time of 12-32 min in the primary Ab and iView or ultra View as the detection system. Using these protocol settings all of 31 (100 %) laboratories produced a sufficient staining.

HercepTest™ (Dako): 33 out of 47 (70%) obtained an optimal mark. The protocols giving an optimal result were typically based on HIER for 40 min using water bath at 97 - 99°C and an incubation time of 30 min in the primary Ab. Using these protocol settings 33 out of 43 (75 %) laboratories produced a sufficient staining.

CE IVD approved systems

Oracle™ (Leica) mAb clone CB11: Both of two obtained an optimal mark. These protocols used HIER in Bond Epitope Retrieval Solution 1 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**: 9 out of 15 (60 %) obtained an optimal mark. All protocols resulting in an optimal staining were based on HIER using Citrate pH 6 (2/4)*, EDTA/EGTA pH 8 (2/2)*, Tris-EDTA/EGTA pH 9 (1/4), Target Retrieval Solution (TRS) pH 9 (EnVision FLEX TRS high pH, Dako, (2/2) or TRS pH 6.1 (FLEX TRS low pH, Dako, (2/2). The pAb A0485 was typically diluted in the range of 1:200-1:1.000. Using these settings 10 out of 13 (77 %) obtained a sufficient staining marked optimal or good.
* (number of optimal results/number of laboratories using this buffer)

mAb **CB11**: 2 out of 7 (29 %) obtained an optimal mark. The optimal protocols were based on HIER using Tris-EDTA/EGTA pH 9 (1/3) and Citrate pH 6 (1/1) as HIER buffer. The Ab was typically diluted was 1:200-500. Using these settings 3 out of 4 (75 %) obtained a sufficient staining optimal or good.

Comments

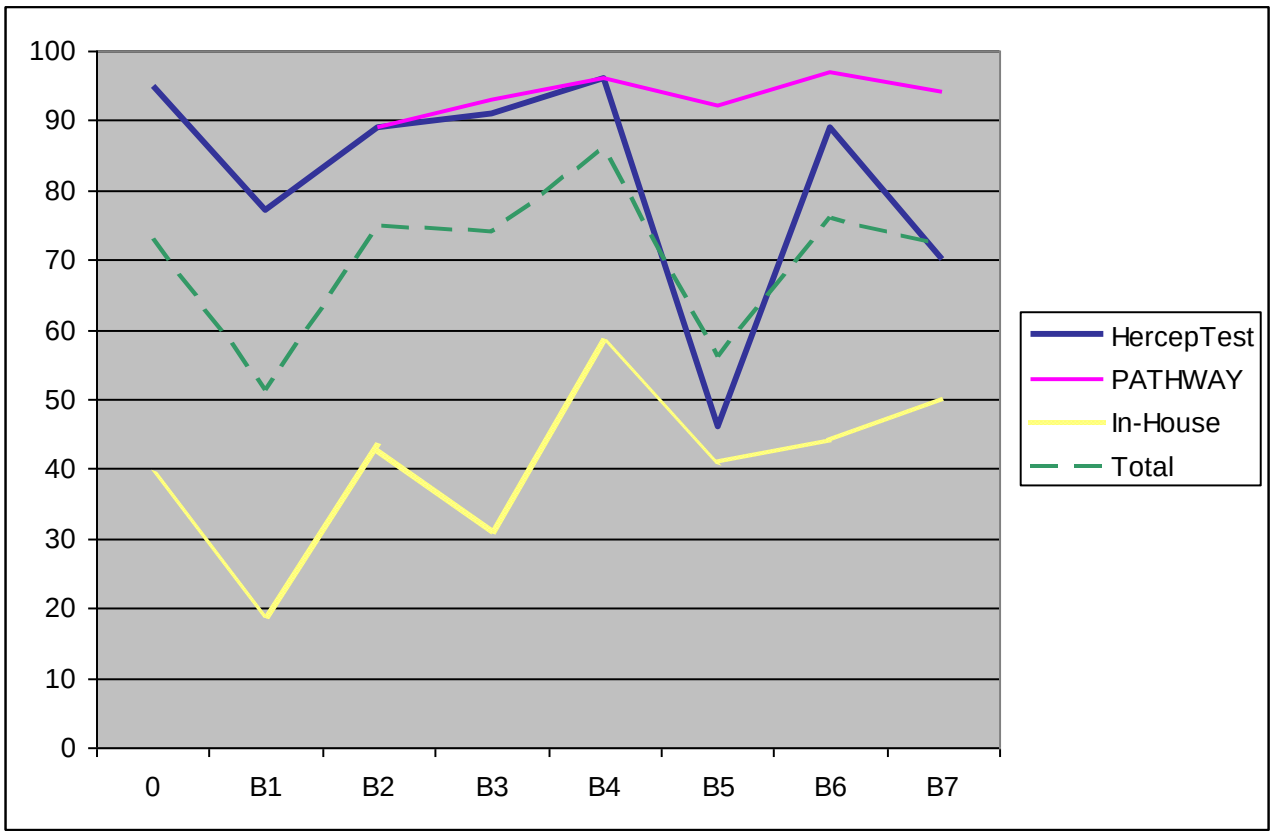
In this assessment the prevalent feature of an insufficient staining was a too weak or a false negative reaction, which particularly and most critical was observed as a 0 or 1+ reaction in the HER-2 gene amplified breast carcinoma no. 3. This tumour was shown to be IHC 2+ in the NordiQC reference laboratories using HercepTest™,

Dako and PATHWAY®, Ventana and showed a low level of HER-2 gene amplification with a ratio of 2.4 – 2.8. The weak or negative reaction was seen in 88% of the insufficient results (28/32) whereas 12 % (4/32) of the insufficient results were caused by a false positive staining and/or a poor signal-to-noise ratio.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a total pass rate of 85 % (66 out of 78 laboratories following the vendors protocol recommendations and guidelines obtained a sufficient staining), while the pass rate for an in-house system was 50 % (16 out of 32 laboratories).

This was the 8th NordiQC HER-2 assessment. As illustrated in table 2, the two FDA approved systems have given a superior pass rate compared to the in-house HER-2 protocols.
As shown in Fig. 1. the average pass rate in 8 runs was 94 % for PATHWAY® (Ventana, rmAb clone 4B5), 82 % for HercepTest™ (Dako) and 41 % for in-house protocols.

Figur 1. **Pass rate through 8 HER-2 assessments**



The slight decline in the overall pass rate for HER-2 in the current run was mainly caused by a lower pass rate of HercepTest™, which in this run was 70 % compared to 89 % in the previous run B6. The decline was in part caused by four laboratories not following the protocol guidelines from Dako (HIER was shortened or performed in a microwave oven instead of a calibrated water bath). For the remaining 10 laboratories obtaining an insufficient result with HercepTest ™ no plausible cause could be identified.

Scoring consensus
The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 82 out of 106 laboratories (77 %) 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. This is an improvement from 58 % and 62 % in run B6 and B7 respectively.
A sufficient staining combined with an interpretation in concordance with the NordiQC assessors was seen in 61% (65 out of 106).

Conclusion
The FDA approved HER-2 systems HercepTest™ (Dako) and PATHWAY® rmAb clone 4B5 (Ventana), and the CE IVD labelled assay Oracle™ (Leica) were in this assessment the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression. The inclusion of 2+ tumours (from run B5 onwards) in the assessment material 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.

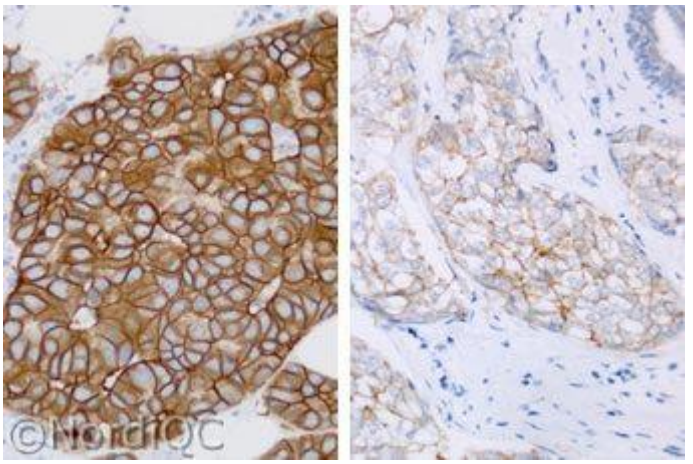


Fig. 1a
Left: Optimal HER-2 staining of the breast ductal carcinoma no. 6 (with HER-2/chr17 ratio > 6.0). More than 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 3 with a HER-2/chr17 ratio 2.4 – 2.8. More than 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.

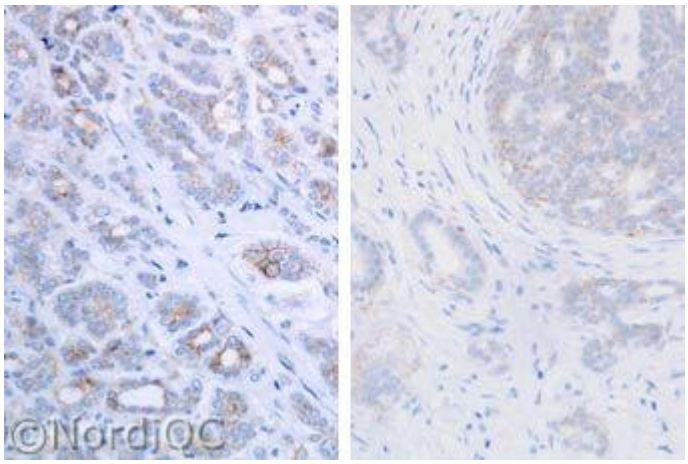


Fig. 1b
Left: Optimal HER-2 staining of the breast ductal carcinoma no. 4 (with a HER-2/chr17 ratio 1.3 – 1.6). More than 10 % of the neoplastic cells show a weak to moderate perceptible membranous staining corresponding to 1+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 1 (with a HER-2/chr17 ratio of 1.0 – 1.2). The neoplastic cells show a faint membranous staining corresponding to 1+. Note the stronger staining in the CIS component.

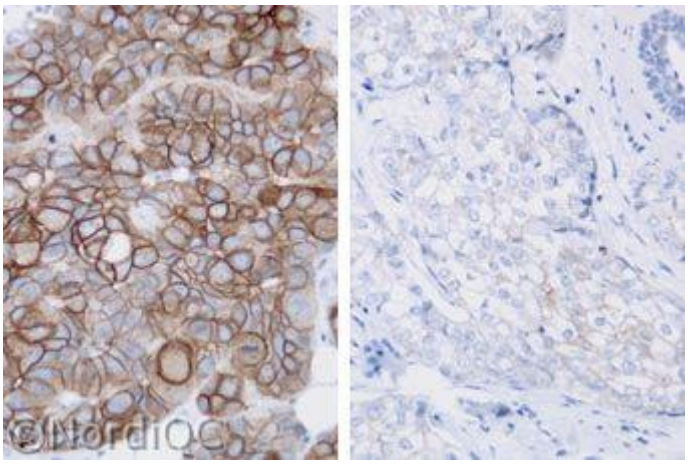


Fig. 2a
Left: HER-2 staining of the breast ductal carcinoma no. 6 (with a HER-2/chr17 ratio > 6.0).
More than 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient HER-2 staining of the breast ductal carcinoma no. 3 (with a HER-2/chr17 ratio 2.4 – 2.8). More than 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 would not be referred to ISH.

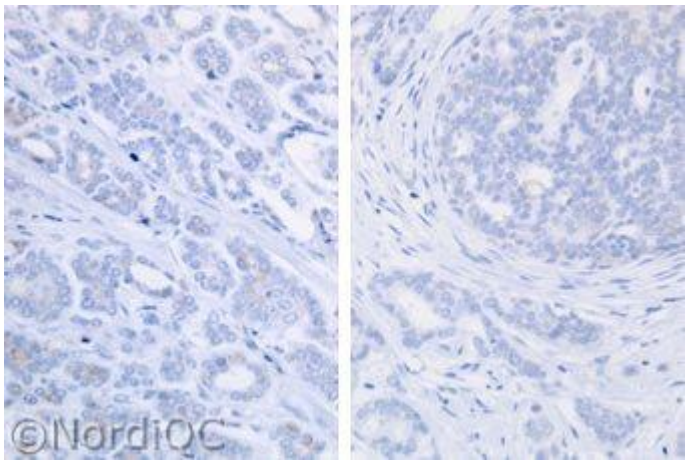
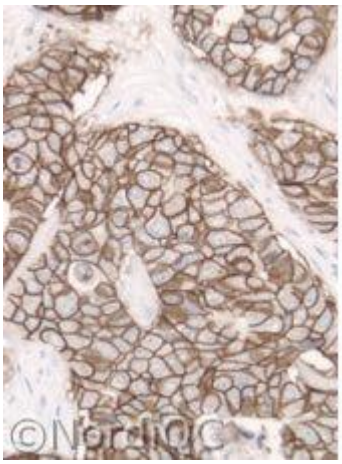


Fig. 2b
Left: Staining for HER-2 of the breast ductal carcinoma no. 4 (with a HER-2/chr17 ratio 1.3 – 1.6). More than 10 % of the neoplastic cells show a faint membrane staining corresponding to 1+.
Right: HER-2 staining for HER-2 of the breast ductal carcinoma no. 1 (with a HER-2/chr17 ratio of 1.0 – 1.2). The neoplastic cells are all negative corresponding to 0. Also note the reduced staining in the CIS component compared to the result obtaining in Fig. 1 b right.



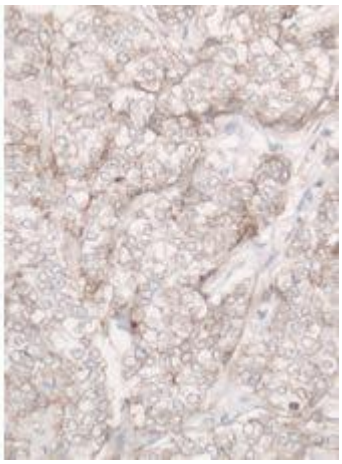
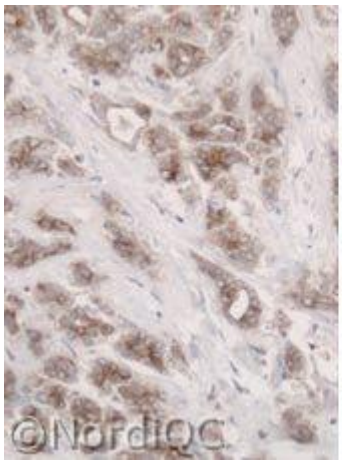


Fig. 3a
Left: HER-2 staining of the breast ductal carcinoma no. 6 (with a HER-2/chr17 ratio > 6.0). More than 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 3 (with a HER-2/chr17 ratio 2.4 – 2.8). More than 10 % of the neoplastic cells show a weak and complete membranous staining corresponding to 2+. However, compare the results in Figs. 3b left and right (same protocol).



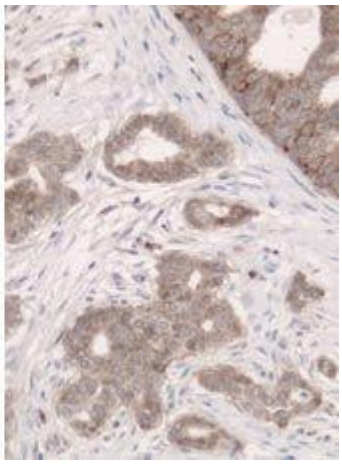


Fig. 3b
Left: Insufficient HER-2 staining of the breast ductal carcinoma no. 4 (with a HER-2/chr17 ratio 1.3 – 1.6). A strong cytoplasmic staining obscures the interpretation of the membranous HER-2 expression.
Right: Insufficient HER-2 staining of the breast ductal carcinoma no. 1 (with a HER-2/chr17 ratio of 1.0 – 1.2). A strong cytoplasmic staining obscures the interpretation of the membranous HER-2 expression.

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