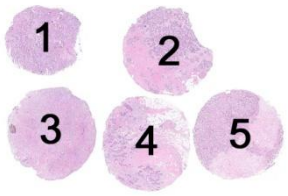
Assessment Run B9 2010

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	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 & PATHWAY®), Ventana in NordiQC reference laboratories.
** HER-2 gene/chromosome 17 Ratio achieved by using HER-2 FISH pharmDX™ Kit, Dako in two NordiQC reference laboratories.

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

Results
189 laboratories participated in this assessment. 82 % 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[3] clone **4B5, 790-2991**, CONFIRM™, rmAb clone **4B5, 800-2996**	57	Ventana	48	8	1	0	98 %	98 %
HercepTest™ **K5204, K5206, K5207, SK001**	50	Dako	39	3	1	7	84 %	88 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	4	Leica	1	3	0	0	-	-
Abs for in-house HER-2 systems								
pAb **A0485**	43	Dako	22	8	2	11	70 %	83 %
mAb clone **CB11**	5 2 1 1	BioGenex Novocastra/Leica Monosan NeoMarkers	1	2	2	5	33 %	-
mAb clone **PN2A**	1	Dako	0	0	0	1	-	-
mAb clone **e2-4001 +3B5**	1	NeoMarkers	0	0	0	1	-	-
rmAb clone **SP3**	19 1 1 1	NeoMarkers Master Diagnostica Spring Zytomed	16	4	0	2	91 %	100 %
rmAb clone **EP1045Y**	1 1	Biocare Epitomics	0	1	0	1	-	-
Total	189		127	29	5	28	-	-
Proportion			67 %	15 %	3 %	15 %	82 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.

FDA approved systems

PATHWAY® / **CONFIRM**™ rmAb clone **4B5** (Ventana): 48 out of 57 (84 %) obtained an optimal mark. The protocols giving an optimal result were all based on HIER in Cell Conditioning 1 - typically mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was in the range of 8 - 32 min. As detection kit either iView or UltraView was used. Using these protocol settings 56 out of 57 (98 %) laboratories produced a sufficient staining.

HercepTest™ (Dako): 39 out of 50 (78%) obtained an optimal mark. The protocols giving an optimal result were based on HIER at 96 - 99°C for 40 min. in water bath or PT link (1 lab used 30 min in a water bath, 1 lab used 20 min in the PT link) and an incubation time of 30 min in the primary Ab (1 lab used 40 min). Using these protocol settings 42 out of 48 (88 %) laboratories produced a sufficient staining. 1 lab obtained an optimal result by applying the primary Ab from the HercepTest on the Bond-max™, performing HIER in Citrate and using Refine as detection kit

CE IVD approved systems

Oracle™ (Leica) mAb clone CB11: 1 out of 4 obtained an optimal mark. The optimal protocol was based on HIER in Bond Epitope Retrieval Solution 1 for 25 min. and incubation of the mAb clone CB11 as Ready-To-Use format for 30 min. Using this protocol setting all of 4 obtained a sufficient staining.

Abs in in-house systems

pAb **A0485**: 22 out of 43 (51 %) obtained an optimal mark. All protocols resulting in an optimal staining were based on HIER using either Target Retrieval Solution pH 6.1 (Dako) (11/17)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (3/7), Cell Conditioning 1 (BenchMark, Ventana) (1/3), Cell Conditioning 2 (BenchMark, Ventana) (1/1), Tris-EDTA/EGTA pH 8 (1/3), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (4/7). The pAb A0485 was typically diluted in the range of 1:200-1:1.000 depending on the total sensitivity of the protocol employed. Using these settings 29 out of 35 (83 %) obtained a sufficient staining.
* (number of optimal results/number of laboratories using this buffe)

rmAb **SP3**: 16 out of 22 (73 %) obtained an optimal mark. The optimal protocols were based on HIER using either Tris-EDTA/EGTA pH 9 (6/6), Cell Conditioning 1 (BenchMark, Ventana) (2/2), Bond Epitope Retrieval Solution 1 (Bond, Leica) (2/2), PTM buffer pH 6 (Thermo)(1/1) or Citrate pH 6 (5/8) 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 19 out of 19 (100 %) obtained a sufficient staining.

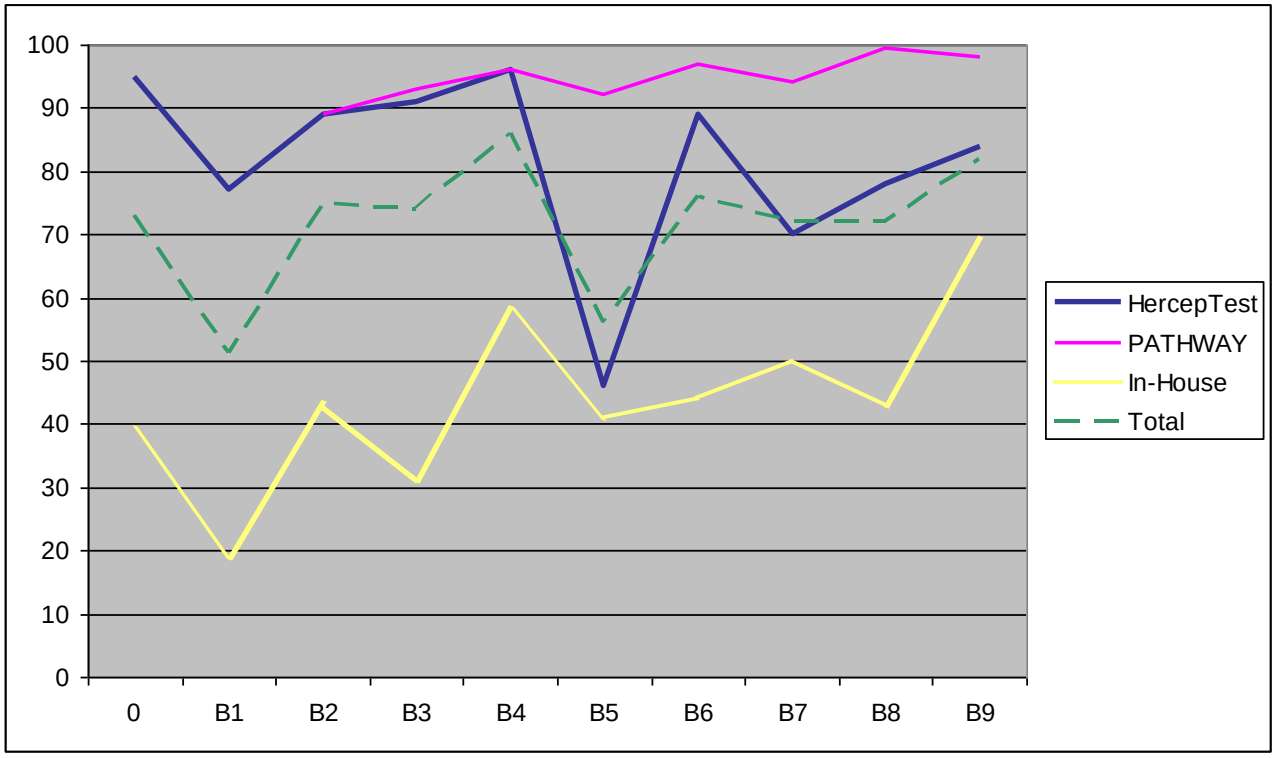
mAb **CB11**: 1 out of 9 obtained an optimal mark. The protocol giving an optimal staining was based on HIER using Cell Conditioning 1 (BenchMark, Ventana), a dilution of 1:40 of the mAb clone CB11 (BioGenex), incubation for 32 min. in the primary Ab and using iView as detection system. With this protocol 1 obtained a sufficient staining.

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 reactions were seen in 28/33 of the insufficient results (85%) whereas 5/33 (15%) 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 with in-house protocols and HercepTest™, Dako. The false positive stains were only seen when an in-house protocol was applied.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a pass rate of 92 % (102 out of 111 laboratories), while the pass rate for the in-house systems was 69 % (54 out of 78 laboratories). The latter was a significant improvement compared to the pass-rate of 44 % in the previous run B8, and seemed mainly related to the higher number of sufficient protocols based on the rmAb SP3, of which 91 % (20/22) were assessed as sufficient.

This was the 10th NordiQC HER-2 assessment. As illustrated in Fig. 1, the two FDA approved systems PATHWAY® (Ventana, rmAb clone 4B5) and HercepTest™ (Dako), have almost constantly given a superior pass rate compared to the in-house HER-2 protocols. The average pass rate in the 10 runs was 94 % for PATHWAY® (Ventana, rmAb clone 4B5), 82 % for HercepTest™ (Dako) and 44 % for in-house protocols.

Fig. 1. Pass rate through 10 HER-2 assessments



The over-all pass rate of 82 % in the current run was an improvement compared to the pass rate of 72 % in the previous run B8 2009. In this context it should be emphasized that the two challenging 2+ tumours are identical in the two runs. In run B9 many new laboratories participated for the first time. The pass rate for these was 68 % (38/56), compared to a pass rate of 89 % (118/133) for laboratories participating previously.

Scoring consensus

The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 130 out of the 180 laboratories (65 %) 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 78 % (115 out of 147), which is in line with the proportion of 77 % in run B8. Thus, the significant improvement from 61 % in run B7 has been maintained.

Conclusion

The FDA approved HER-2 system PATHWAY® rmAb clone 4B5 (Ventana) and the CE IVD labelled kit Oracle™ (Leica), were in this assessment the most reliable methods for the semi-quantitative IHC determination of HER-2 protein expression. In-house systems based on the rmAb clone SP3 gave a high proportion of sufficient results. 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.

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

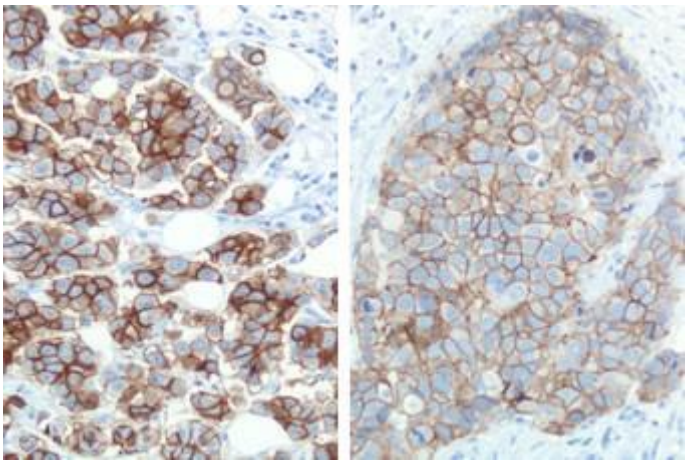


Fig. 1a
Left: Optimal HER-2 staining of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal staining for HER-2 of the breast ductal carcinoma no. 4 with a ratio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

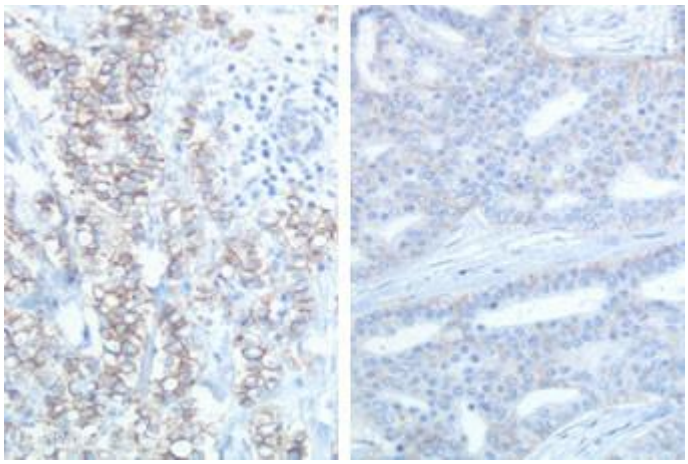


Fig. 1b
Left: Optimal HER-2 staining of the breast lobular carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of of 1.2 – 1.5. > 10 % of the neoplastic cells show a weak to moderate complete membranous staining corresponding to 2+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.1 – 1.3. The neoplastic cells show a faint membranous staining corresponding to 1+.

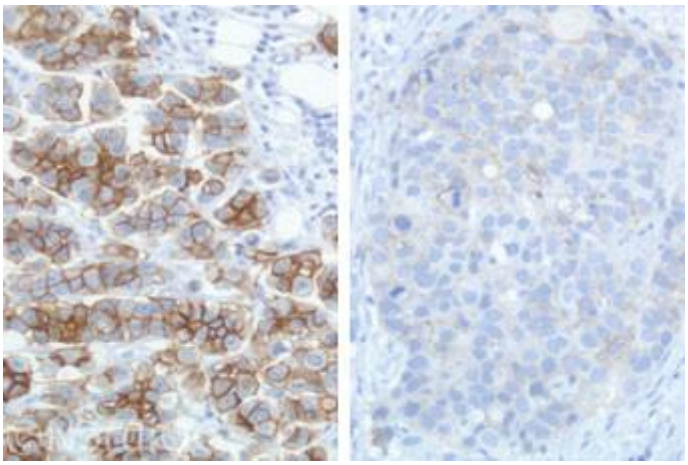


Fig. 2a
Left: HER-2 staining of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome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

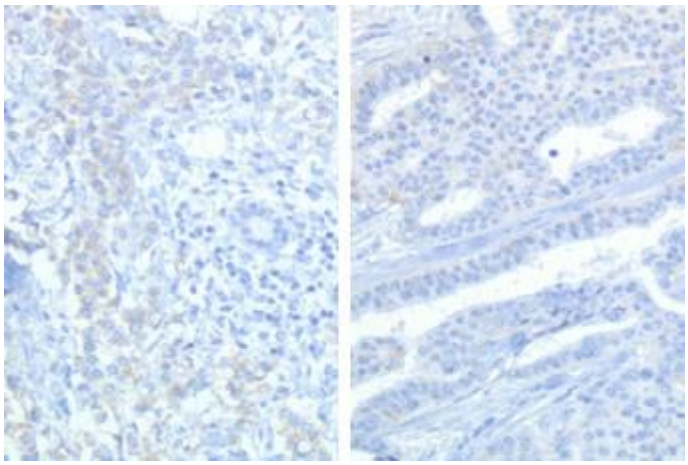


Fig. 2b
Left: Staining for HER-2 of the breast lobular carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.2 – 1.5. > 10 % of the neoplastic cells show a faint membrane staining corresponding to 1+.
Right: HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.0 – 1.2.
< 10 % of the neoplastic cells show a membranous staining corresponding to 0.

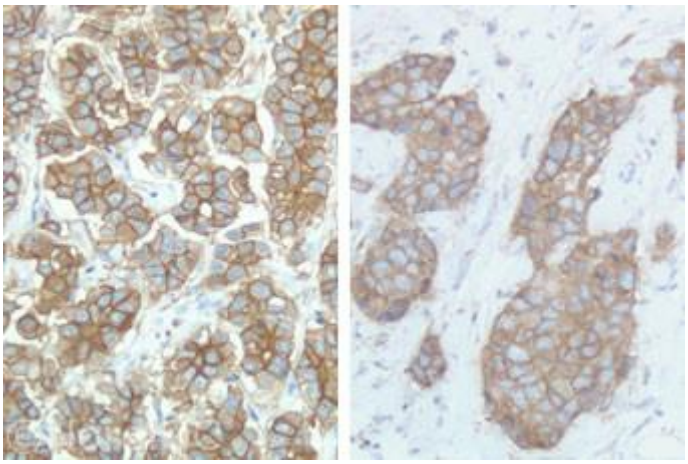


Fig. 3a
Left: HER-2 staining of the breast ductal carcinoma no. 5 with a ratio of HER-2 / Chromosome 17 of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 4 with a ratio of HER-2 / Chromosome 17 of 2.5 – 2.9.
> 10 % of the neoplastic cells show a moderate cytoplasmic staining but no distinct membranous staining can be identified. Also compare the results in Figs. 3b left and right. This pattern was scored both by the participant and NordiQC as 1+. This cytoplasmic reaction pattern was constantly seen for the mAb clone e2-4001+3B5 - both in this and the previous HER-2 assessment.

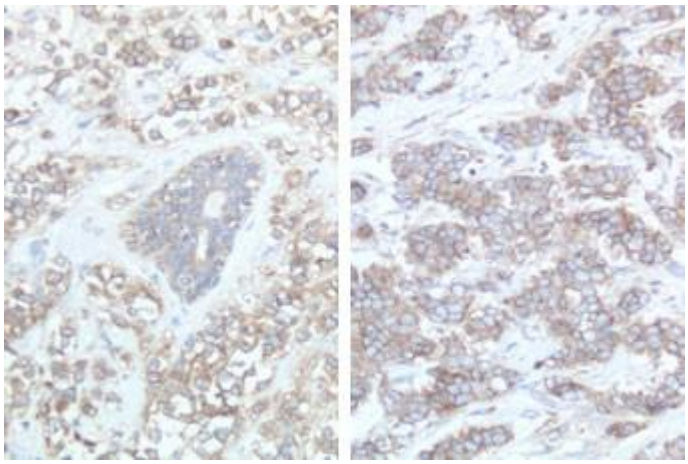


Fig. 3b
Left: HER-2 staining of the breast lobular carcinoma no. 3 with a ratio of HER-2 / Chromosome 17 of 1.2 – 1.5. It is not possible to interpret the membranous staining due to an excessive cytoplasmic staining.
Right: HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2 / Chromosome 17 ratio of 1.0 – 1.2. (HER-2 score 0 in reference lab.). It is not possible to interpret the membranous staining due to an excessive cytoplasmic reaction.

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