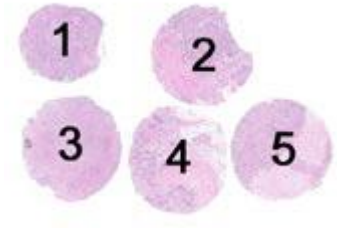
Assessment Run B10 2010

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/chr17 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 (guidelines 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 (HER-2/chr17) ratio as achieved by using HER-2 FISH pharmDX™ Kit, Dako.

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+ or 3+ 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 Scandinavia) 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
201 laboratories participated in this assessment. 87 % 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**	71	Ventana	67	4	0	0	100 %	100 %
HercepTest™ **K5204, K5206, K5207, SK001**	50	Dako	38	3	0	9	82 %	85 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone **CB11, TA9145**	6	Leica	5	0	0	1	83 %	100 %
Abs for in-house HER-2 systems, conc. Ab.								
pAb **A0485**	36	Dako	16	10	1	9	72 %	80 %
mAb clone **CB11**	5 1 1 1	Novocastra/Leica BioGenex Monosan NeoMarkers	3	4	0	1	88 %	88 %
mAb clone **10A7**	1	NovoCastra/Leica	0	1	0	0	-	-
rmAb clone **SP3**	19 3 1 1 1	NeoMarkers Zytomed DCS Spring Vector	19	4	1	1	92 %	100 %
rmAb clone **EP1045Y**	1	Epitomics	0	1	0	0	-	-
Abs for in-house HER-2 systems, RTU Ab.								
mAb clone **CB11, AM134-5ME**	2	BioGenex	0	0	1	1	-	-
rmAb clone **EP1045Y, PM342**	1	BioCare	0	0	0	1	-	-
Total	201		148	27	3	23	-	-
Proportion			74 %	13 %	1 %	12 %	87 %	-

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): 67 out of 71 (94 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on HIER in Cell Conditioning 1 - mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was in the range of 8 – 40 min and as detection kit either iView or UltraView was used. Using these protocol settings all of 71 (100 %) laboratories produced a sufficient staining (optimal or good).

HercepTest™ (Dako): 38 out of 50 (76 %) stains were assessed as optimal. The protocols giving an optimal result were typically based on HIER at 97 - 99°C for 40 min in a water bath or PT Link and an incubation time of 25-30 min in the primary Ab. Using these protocol settings 40 out of 47 (85 %) 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 Bond Epitope Retrieval Solution 1 and using Refine as detection kit.

CE IVD approved systems

Oracle™ (Leica) mAb clone **CB11**: 5 out of 6 (83 %) stains were assessed as optimal. The protocols giving an optimal result were based on HIER in Bond Epitope Retrieval Solution 1 for 25-30 min. and an incubation time of the mAb clone CB11 as Ready-To-Use (RTU) format for 30 min.

Abs for in-house systems

pAb **A0485**: 16 out of 36 (44 %) obtained an optimal mark. All protocols resulting in an optimal staining were based on HIER using either TRS pH 6.1 (Dako) (10/20)*, TRS pH 9 (3-in-1,Dako) (1/3), Cell Conditioning 1 (BenchMark, Ventana) (1/3), Tris-EDTA/EGTA pH 9 (3/6) or Citrate pH 6 (1/2). The pAb A0485 was typically diluted in the range of 1:200-1:700 depending on the total sensitivity of the protocol employed. Using these settings 20 out of 25 (80 %) laboratories produced a sufficient staining.
* (number of optimal results/number of laboratories using this buffer)

mAb **CB11**: 3 out of 8 stains were assessed as optimal. The protocols giving an optimal staining were based on HIER using Cell Conditioning 1 (BenchMark, Ventana),(2/2) or Tris-EDTA/EGTA pH 9 (1/6) The mAb CB11 was typically diluted in the range of 1:70-1:400 depending on the total sensitivity of the protocol employed. Using these settings 7 out of 8 (88 %) laboratories produced a sufficient staining.

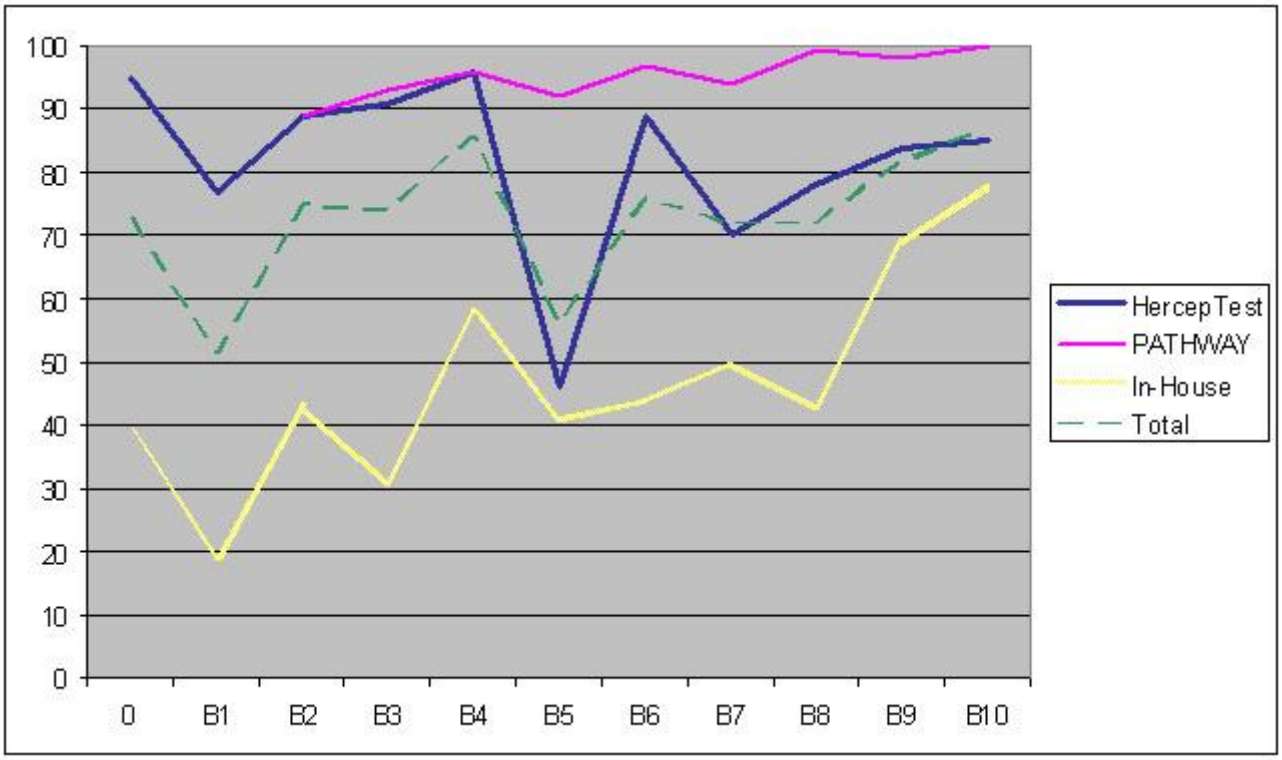
rmAb **SP3**: 19 out of 25 (76 %) stains were assessed as optimal. The optimal protocols were based on HIER using either Tris-EDTA/EGTA pH 9 (8/9), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1) or Citrate pH 6 (8/10) as HIER buffer. The Ab was typically diluted in the range of 1:20-200 depending on the total sensitivity of the protocol employed. Using these settings 22 out of 22 (100 %) laboratories produced a sufficient staining.

Comments
In this assessment the material circulated were identical to that used in run B9, i.e., the same donor blocks were used in both runs. Similar to the observations in run B9, the prevalent feature of an insufficient HER-2 staining in run B10 was a too weak or false negative reaction, which particularly and most critical was observed as a 0/1+ IHC 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 2.5 – 2.9). The weak or false negative reactions were seen in 22/26 of the insufficient results (85%) whereas 4/26 (15%) of the insufficient results were due to a false positive staining and/or a poor signal-to-noise ratio in the 3 HER-2 non-amplified tumours, no. 1, 2 & 3. The weak or false negative results were seen both with in-house protocols and systems as HercepTest™, Dako, while the false positive results were only seen when an in-house protocol was applied. The weak and false negative results were for the in-house systems typically related to a too low sensitivity of the protocol e.g. a too low concentration of the primary Ab, or use of a RTU Ab not applied within a system for which this product was calibrated. Regarding the insufficient results for the 9 laboratories using HercepTest™, Dako, 2 laboratories did not follow the protocol guidelines from Dako, as HIER was shortened or performed in a microwave oven instead of a calibrated waterbath, whereas no cause could be identified for the remaining 7 protocols.

Grouped together, the FDA approved and CE IVD labelled IHC systems gave a pass rate of 92 % (117 out of 127 laboratories), which was identical to the pass rate obtained in run B9. The pass rate for the in-house systems as a group was 78 % (58 out of 74 laboratories), which was an improvement compared to the pass rate of 69 % in run B9 for this group. The pass rate of the in-house systems has now shown an improvement in the two last assessments for HER-2 IHC and has reached a level close to the pass rate of HercepTest™, Dako. As also observed in run B9, the improvement in this run was especially related to the high number of sufficient protocols based on the rmAb clone SP3, of which 92 % (23/25) were assessed as sufficient. Also the mAb clone CB11 showed to be successful, as 89 % (7/8) were assessed as sufficient.

This was the 11th assessment of HER-2 IHC in NordiQC. As illustrated in Fig. 1, the 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 11 runs was 95 % for PATHWAY® (Ventana, rmAb clone 4B5), 82 % for HercepTest™ (Dako) and 47 % for in-house protocols.

Fig. 1. **Pass rates in 11 NordiQC HER-2 IHC assessments.**



In this HER-2 assessment run B10 the over-all pass rate of 87 % was an improvement compared to the pass rate of 82 % in the previous assessment, run B9 2010. In this context it has to be emphasized that the two challenging 2+ tumours have been identical in the last three runs and it is very encouraging that the improvement of the total pass rate has been constant after the implementation of 2+ tumours in run B5 and onwards.

Scoring consensus

The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 123 out of the 171 laboratories (72 %) 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 % (116 out of 149), which was identical to the proportion obtained in run B9.

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 e.g. the rmAb clone SP3 also gave a high proportion of sufficient results. As seen from run B5, The inclusion of the 2+ tumours 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.

Fig.1 shows an example of an optimal result, while Figs. 2 and 3 show examples of insufficient results.

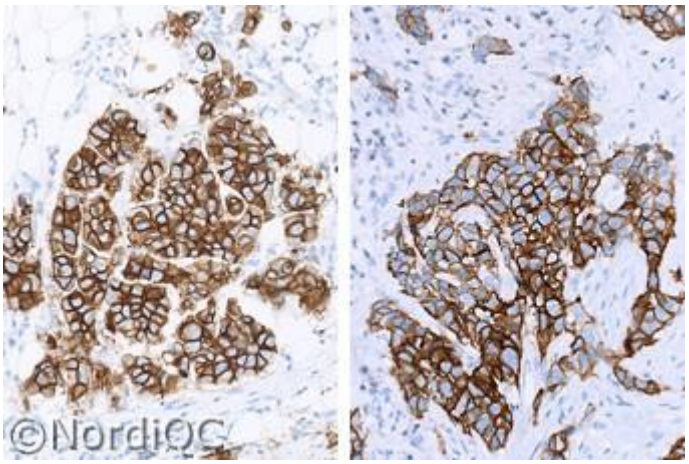


Fig. 1a
Left: Optimal HER-2 staining of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio of > 6.0.
> 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.5 – 2.9.
> 10 % of the neoplastic cells show a moderate complete membranous staining corresponding to 2+.

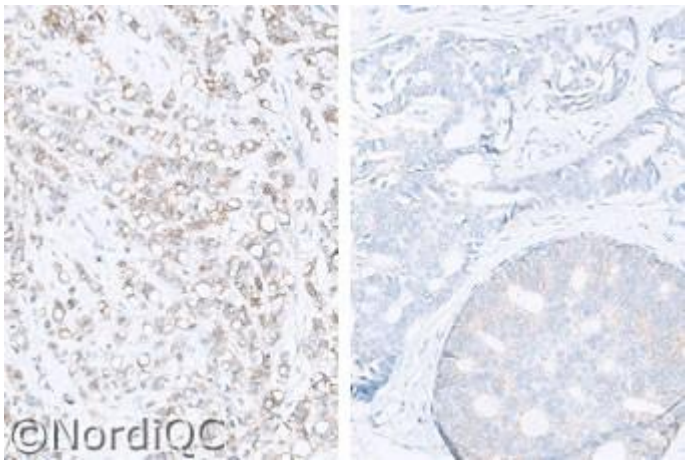


Fig. 1b
Left: Optimal HER-2 staining of the breast lobular carcinoma no. 3 with a HER-2/chr17 ratio of of 1.2 – 1.5. > 10 % of the tumour cells show a weak to moderate complete membranous staining corresponding to 2+.
Right: Optimal HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.1 – 1.3.
The tumour cells show a faint membranous staining corresponding to 1+. The ductal carcinoma in situ (DCIS) component focally show a 2+ staining.

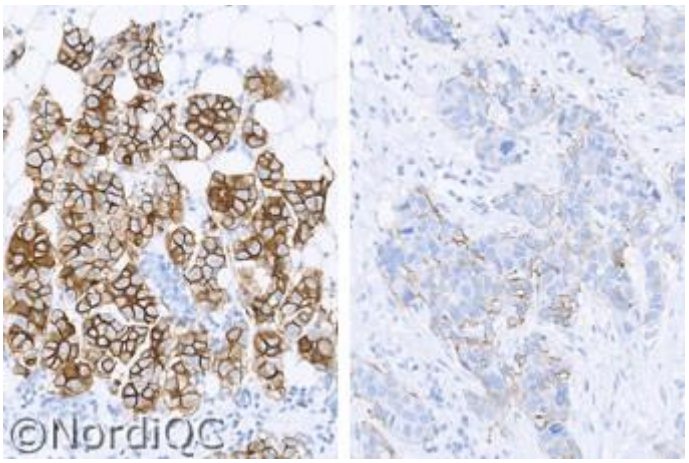


Fig. 2a
Left: HER-2 staining of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: Insufficient HER-2 staining of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.5 – 2.9.
> 10 % of the neoplastic cells show an incomplete membrane staining corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be referred to ISH.

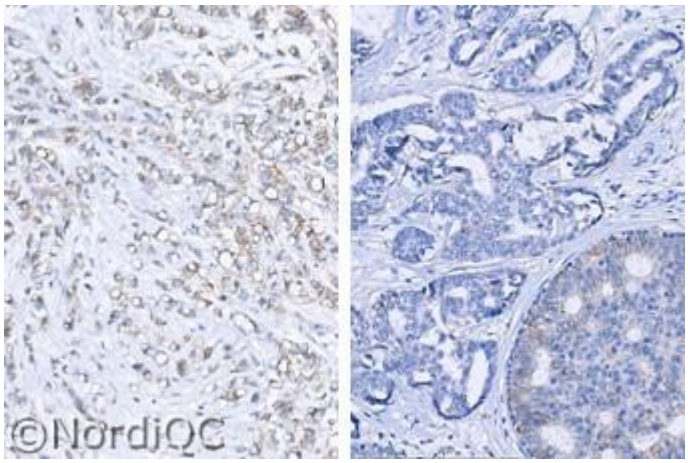


Fig. 2b
Left: HER-2 staining of the breast lobular carcinoma no. 3 with a HER-2/chr17 ratio of 1.2 – 1.5. > 10 % of the neoplastic cells show a moderate but incomplete membrane staining corresponding to 1+.
Right: HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2/chr17 ratio of 1.1 – 1.3. < 10 % of the tumour cells show a membranous staining corresponding to 0, whereas the DCIS component show a 1+ staining.

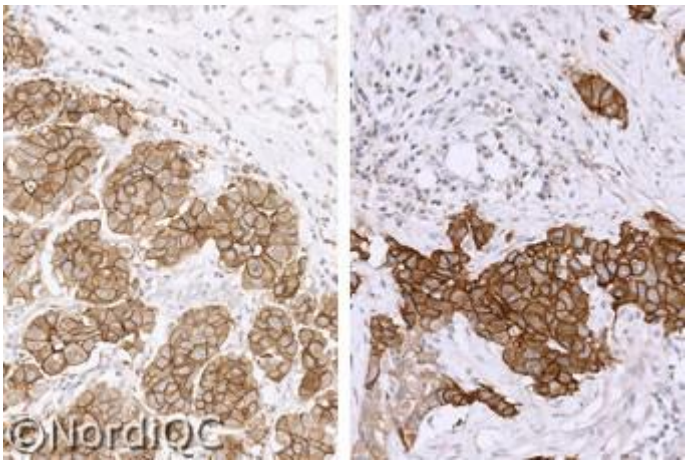


Fig. 3a
Left: HER-2 staining of the breast ductal carcinoma no. 5 with a HER-2/chr17 ratio of > 6.0. > 30 % of the neoplastic cells show a strong and complete membranous staining corresponding to 3+.
Right: HER-2 staining of the breast ductal carcinoma no. 4 with a HER-2/chr17 ratio of 2.5 – 2.9. > 10 % of the tumour cells show a moderate cytoplasmic staining but no distinct membranous staining can be identified. Also compare the results in Figs. 3b left and right.

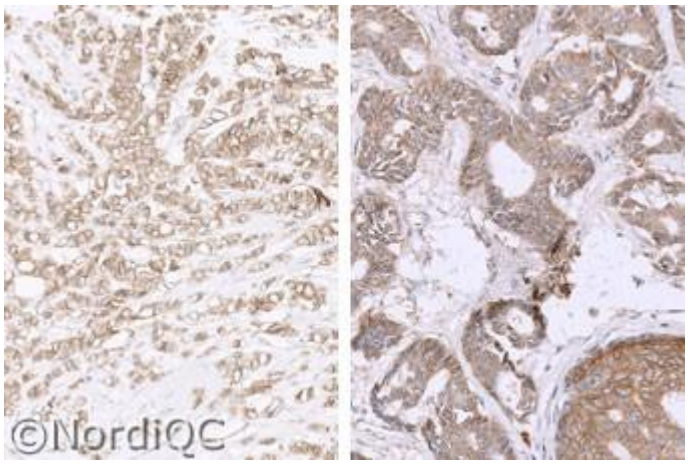


Fig. 3b
Left: Insufficient HER-2 staining of the breast lobular carcinoma no. 3 with a HER-2/chr17 ratio of 1.2 – 1.5. It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.
Right: HER-2 staining of the breast ductal carcinoma no. 2 with a HER-2/chr17ratio of 1.1 – 1.3. It is not possible to interpret the membranous staining due to an excessive and diffuse cytoplasmic staining.

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