Assessment Run G2 2011
(Gastric cancer pilot module)
HER-2 IHC

Material

The slide to be stained for HER-2 comprised the following 11 tissue cores from gastric/GEJ resection specimens, all fixed in neutral buffered formalin for 48-96 h (same material as circulated in run G1):

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2/chr17 ratio****
1. Tonsil	-	-
2. Tubular adenocarcinoma	2+	1.65
3. Tubular adenocarcinoma	0	1.15
4. Tubular adenocarcinoma	1+	1.32
5. Tubular adenocarcinoma	1+	0.79
6. Tubular adenocarcinoma	0	1.21
7. Tubular adenocarcinoma	3+	>6
8. Tubular adenocarcinoma	2+	2.50
9. Signet ring cell adenocarc.	1+	1.07
10. Tubular adenocarcinoma	0	1.02
11. Tubular adenocarcinoma	0	0.95

*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 and Inform Dual ISH, Ventana (average of the two systems).

IHC scoring system applied (cut-off values as recommended for resection material):

Score 0	No staining is observed or cell membrane staining is observed in <10% of the tumour cells.
Score 1+	A faint perceptible membrane staining can be detected in ≥10% of the tumour cells. The cells are only stained in part of their membrane.
Score 2+	A weak to moderate basolateral, lateral or complete membrane staining is observed in ≥10% of the tumour cells.
Score 3+	A strong basolateral, lateral or complete membrane staining is observed in ≥ 10 % 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 gastric carcinoma no. 3, 4, 5*, 6, 8, 9, 10** and 11.
- A clear and unequivocal immunohistochemical staining marked as score 2+ in the gastric carcinoma no 2 and 8.
- A clear and unequivocal immunohistochemical staining marked as score 3+ in the gastric carcinoma no 7.

*a cytoplasmic and nuclear staining was accepted for the HER-2 system PATHWAY®, Ventana
**a cytoplasmic staining was accepted for the HER-2 system HercepTest™, Dako

51 laboratories participated in this assessment. 96 % 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:**

CE-IVD / FDA approved HER-2 systems	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.[1]**	**Suff. OPS[2]**
PATHWAY® rmAb clone **4B5, 790-2991**	21	Ventana	20	1	0	0	100 %	100 %
CONFIRM™, rmAb clone **4B5, 800-2996**	8	Ventana	6	2	0	0	100 %	100 %
HercepTest™ **SK001**	8	Dako	5	3	0	0	100 %	100 %
HercepTest™ **K5204**	1	Dako	0	0	0	1	-	-
HercepTest™ **K5207**	5	Dako	3	1	0	1	80 %	80 %
Oracle™ mAb clone **CB11, TA9145**	1	Leica	0	1	0	0	-	-
Abs for in-house HER-2 systems, conc. Ab.								
rmAb clone **SP3**	3	Thermo/NeoMarkers	2	1	0	0	-	-
pAb clone **A0485**	4	Dako	2	2	-	-	-	-
Total	51		38	11	0	2	-	-
Proportion			75 %	21 %	0 %	4 %	96 %	-

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.

CE-IVD / FDA approved systems:
PATHWAY® rmAb clone **4B5** (Ventana): 20 out of 21 (95 %) protocols were assessed as optimal. The protocols giving an optimal result were typically based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1), mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was in the range of 16 – 32 min. and either iView, UltraView or OptiView were used as the detection kit. Using these protocol settings 20 out of 20 (100%) laboratories produced a sufficient staining (optimal or good).

CONFIRM™ rmAb clone **4B5** (Ventana): 6 out of 8 (75 %) protocols were assessed as optimal. The protocols giving an optimal result were typically based on HIER in CC1 mild in the BenchMark XT or Ultra. The incubation time for the primary Ab was typically in the range of 12 – 32 min. and UltraView was used as the detection kit. Using these protocol settings 6 out of 6 (100%) laboratories produced a sufficient staining.

HercepTest™ SK001 (Dako): 5 out of 8 (63 %) protocols were assessed as optimal. The protocols giving an optimal result were typically based on HIER in HercepTest epitope retrieval solution at 95 - 99°C for 40 min in a water bath or PT Link and an incubation time of 20-30 min in the primary Ab. Using these protocol settings 8 out of 8 (100 %) laboratories produced a sufficient staining.

HercepTest™ K5207 (Dako): 3 out of 5 (60 %) protocols were assessed as optimal. The protocols giving an optimal result were typically based on HIER in HercepTest epitope retrieval solution at 95 - 99°C for 40 min in a water bath or PT Link and an incubation time of 30 min in the primary Ab. Using these protocol settings 4 out of 5 (80 %) laboratories produced a sufficient staining.

Abs for in-house systems:
rmAb **SP3**: 2 out of 3 (67 %) protocols were assessed as optimal. All protocols resulting in an optimal staining were based on HIER using either Bond Epitope Retrieval Solution 2 (BERS2; Bond, Leica) (1/1)* or Citrate pH 6.7 (1/1)*. The rmAb SP3 was diluted in the range of 1:50-60 depending on the total sensitivity of the protocol employed. Using these settings 2 out of 2 laboratories produced an optimal staining.
*number of optimal results/number of laboratories using this buffer).

pAb **A0485**: 2 out of 4 (50 %) protocols were assessed as optimal. All protocols resulting in an optimal staining were based on HIER using Target Retrieval Solution pH 6.1 (Dako) (2/3). The pAb A0485 was diluted in the range of 1:200-1:300 depending on the total sensitivity of the protocol employed. Using these settings 2 out of 2 laboratories produced an optimal staining.

Comments
In this second run for HER-2 IHC in the gastric cancer pilot module a pass rate of 96 % was obtained. Only 2 out of 51 laboratories obtained an insufficient mark due to a false negative result.
The false negative reaction was in particular and most critically observed as a 0/1+ IHC reaction in the HER-2

gene amplified gastric carcinoma no. 8 shown to be IHC 2+ in the NordiQC reference laboratories using both HercepTest™, Dako, and PATHWAY®, Ventana, with a low level of HER-2 gene amplification (HER-2/chr17 ratio 2.5). No reason for the insufficient result could be identified, as the applied protocol settings were similar to the protocols giving the optimal staining results.
The two most widely used assays for HER-2 PATHWAY®, Ventana and HercepTest™, Dako showed an almost identical membrane staining reaction in all the carcinomas. However, in the carcinoma no. 5 a moderate to strong cytoplasmic and nuclear staining reaction was seen with PATHWAY® complicating the interpretation of the specific membrane staining (1+), whereas this tumour showed a staining reaction easily interpreted as 1+ with HercepTest™ (Figs. 3a & 3b).
It was noteworthy that all 7 out of 7 protocols based on an in-house HER-2 system gave a sufficient result. In run G1 only 8 out of 12 protocols (75 %) based on an in-house HER-2 system gave a sufficient result.

Scoring consensus
The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 24 out of the 44 laboratories (55 %) 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 57 % (24 out of 42). The relative low scoring consensus most likely is related to the modified interpretation guidelines compared to the well established guidelines for HER-2 IHC within breast pathology. The most frequent discrepancies were related to the carcinoma no. 2 (HER-2, 2+, non-amplified) and the carcinoma no. 8 (HER-2, 2+, amplified). The carcinomas no. 5 and 10 giving an aberrant non-membranous staining pattern were by many laboratories scored as 2+. This was accepted as all equivocal results must be retested with another assay.

This was the second run for HER-2 IHC in the gastric cancer pilot module and a similar pass rate compared to the first run was obtained as seen in table 2.

Table 2. **Proportion of sufficient results for HER-2 IHC in the two NordiQC runs performed**

	Run G1 2011	Run G2 2011
Participants, n=	55	51
Sufficient results	93 %	96 %

Conclusion
In both the G1 and G2 assessment, a very high pass rate for HER-2 IHC was seen. Both the CE-IVD / FDA approved HER-2 systems and in-house HER-2 systems could be used to obtain an optimal staining result and no significant difference regarding the pass rates could be identified between in-house systems or CE-IVD / FDA approved systems. The inclusion of the 2+ tumours with and without HER-2 gene amplification is essential to evaluate the HER-2 IHC performance and the robustness of the protocols used by the participants. Training in scoring is highly warranted and image analysis assisted scoring has to be taken in consideration to improve and facilitate the interpretation.
The gastric cancer pilot module will not be implemented as a module in 2012.

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

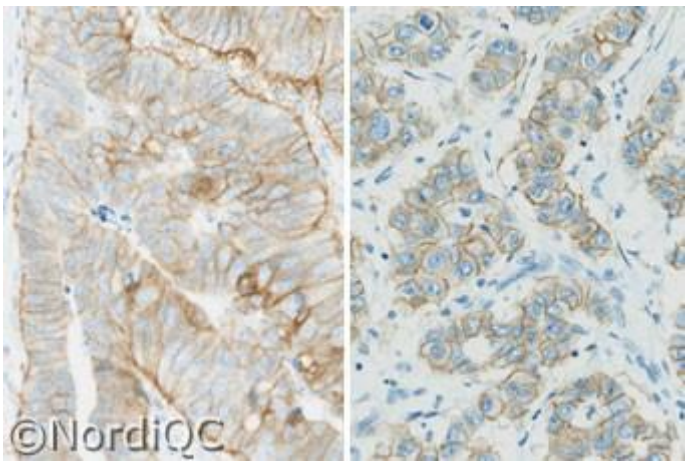

Fig. 1a
Left: Optimal staining for HER-2 of the gastric carcinoma no. 7 with HER-2/chr17 ratio of > 6.0.
≥ 10 % of the neoplastic cells show a strong, lateral and focally a complete membranous staining corresponding to 3+.

Right: Optimal staining for HER-2 of the gastric carcinoma no. 8 with HER-2/chr17 ratio of 2.5. ≥ 10 % of the neoplastic cells show a moderate lateral membranous staining corresponding to 2+.

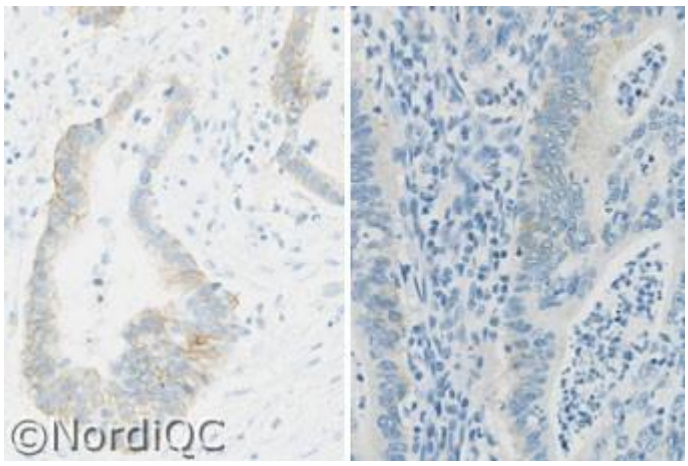

Fig. 1b
Left: Optimal staining for HER-2 of the gastric carcinoma no. 2 with a HER-2/chr17 ratio of 1.65.
≥ 10 % of the neoplastic cells show a weak to moderate lateral membranous staining corresponding to 2+.

Right: Optimal staining for HER-2 of the gastric carcinoma no. 4 with a HER-2/chr17 ratio of 1.32. The neoplastic cells show a faint membranous staining corresponding to 1+.

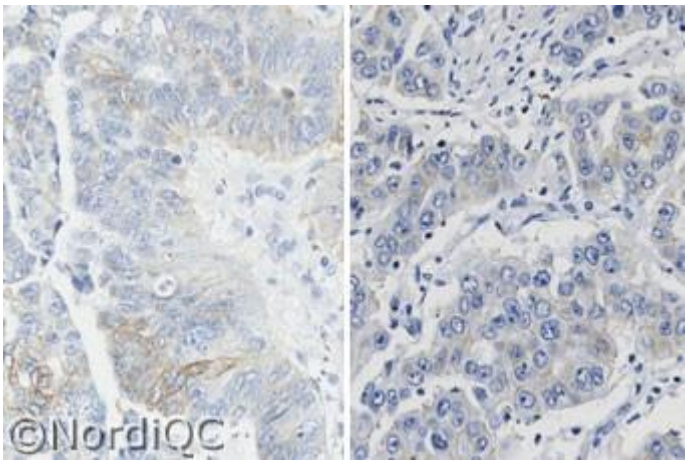

Fig. 2a
Left: Insufficient staining for HER-2 of the gastric carcinoma no. 7 with a HER-2/chr17 ratio of > 6.0. ≥ 10 % of the neoplastic cells show a weak primary basolateral membranous staining corresponding to 2+.

Right: Insufficient staining for HER-2 of the gastric carcinoma no. 8 with a ratio of HER-2 / Chromosome 17 of 2.5. The neoplastic cells show a faint membranous staining corresponding to 1+, but does not meet the criteria to be classified as 2+ and will not be referred to ISH.

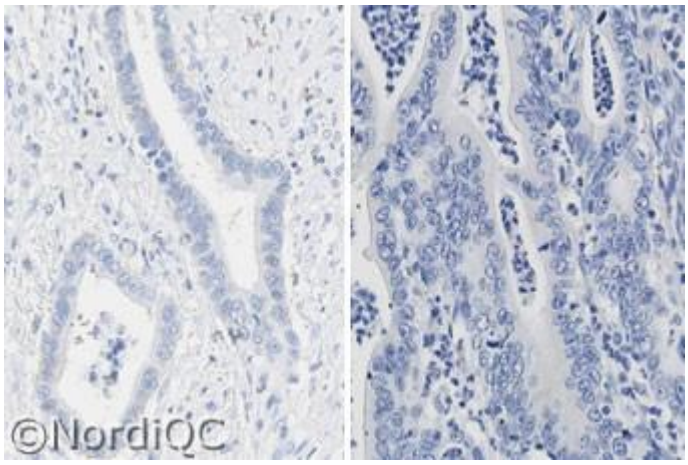

Fig. 2b
Left: Staining for HER-2 of the gastric carcinoma no. 2 with a HER-2/chr17 ratio of 1.65. No staining is observed, corresponding to 0.

Right: Staining for HER-2 of the gastric carcinoma no. 4 with a HER-2/chr17 ratio of 1.32. No staining is observed, corresponding to 0.

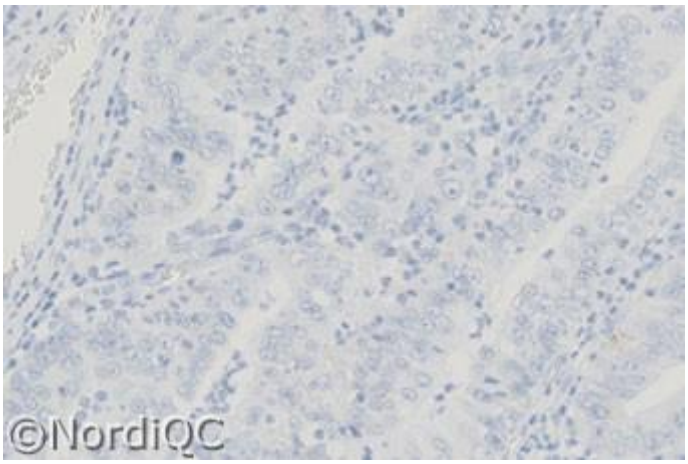

Fig. 3a
Staining for HER-2 of of the gastric carcinoma no. 5 with a HER-2/chr17 ratio of 0.79 using the HercepTest™, Dako. The neoplastic cells show a faint membranous staining corresponding to 1+.

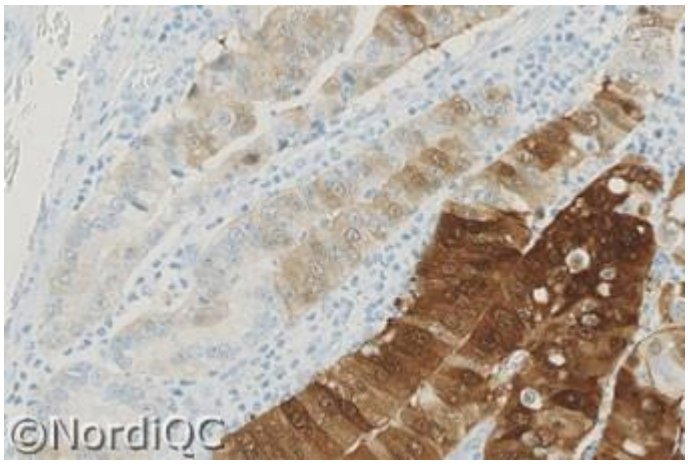

Fig. 3b
Staining for HER-2 of of the gastric carcinoma no. 5 with a HER-2/chr17 ratio of 0.79 using PATHWAY®, Ventana – same field as in Fig. 3a. A moderate to strong cytoplasmic and nuclear staining is seen. The staining reaction is due to cross reaction with an unknown target (not HER-4 as previously believed).

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