Assessment Run G1 2011

HER-2 IHC

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:

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	**HER-2 gene/chr 17 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 the two FDA approved kits/antibodies, HercepTest™ (Dako) & PATHWAY® (Ventana) in 3 NordiQC reference laboratories.
** HER-2 gene/chromosome 17 Ratio as achieved by HER-2 FISH pharmDX™ Kit (Dako) and Dual colour SISH (Ventana) in a NordiQC reference laboratory (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 IHC staining marked as score 0/1+ in the gastric carcinoma no. 3, 4, 5*, 6, 8, 9, 10** and 11
- A clear and unequivocal IHC staining marked as score 2+ in the gastric carcinoma no 2 and 8
- A clear and unequivocal IHC 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

55 laboratories participated in this assessment. 93 % achieved a sufficient mark, but only 53% were optimal. 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**	28	Ventana	20	8	0	0	100 %	100 %
HercepTest™ **K5204, K5207, SK001**	12	Dako	6	6	0	0	100 %	100 %
CE IVD approved HER-2 systems								
Oracle™ mAb clone	3	Leica/Novocastra	0	3	0	0	-	-

CB11, TA9145								
Abs for in-house HER-2 systems, conc. Ab.								
pAb **A0485**	7	Dako	3	3	0	1	86 %	80 %
rmAb clone **SP3**	4	NeoMarkers	0	2	0	2	-	-
rmAb clone **EP1045Y**	1	Epitomics	0	0	0	1	-	-
Total	55		29	22	0	4	-	-
Proportion			53 %	40 %	-	7 %	93 %	-

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): 20 out of 28 (71 %) protocols were assessed as optimal. The protocols giving an optimal result were based on HIER in Cell Conditioning 1 (20/24)* mild or standard in the BenchMark XT or Ultra. The incubation time for the primary Ab was in the range of 16 – 32 min using either iView (760-091, Ventana) or UltraView (760-500, Ventana) as detection kit. Using these protocol settings all of 25 (100 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

HercepTest™ (Dako): 6 out of 12 (50 %) protocols were assessed as optimal. The protocols giving an optimal result were based on HIER using Epitope Retrieval Solution, HercepTest at 97 - 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 all of 11 (100 %) laboratories produced a sufficient staining.

Abs for in-house systems

pAb **A0485**: 3 out of 7 (43 %) protocol were assessed as optimal. All protocols resulting in an optimal staining were based on HIER using either Target Retrieval Solution pH 9 (Dako) (1/2), Target Retrieval Solution pH 6.1 (Dako) (1/2) or Citrate pH 6 (1/1). 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 4 out of 5 (80 %) laboratories produced a sufficient staining.

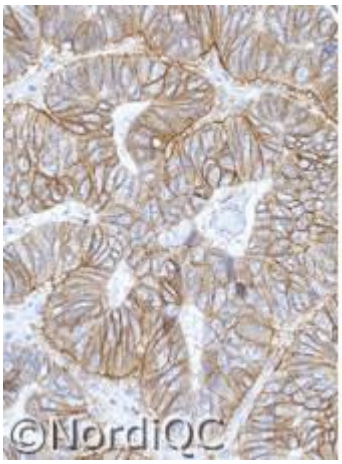
Comments
In this first gastric cancer pilot module for HER-2 IHC a pass rate of 93 % was obtained. Only 4 out of 55 laboratories obtained an insufficient mark: 3 were assessed as false negative and 1 as false positive. The false negative reaction was in particular and most critical observed as a 0/1+ 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 (ratio 2.5). The false positive reaction was observed as a 3+ IHC reaction in the HER-2 gene non-amplified carcinomas no. 2 and 5, shown to be respectively IHC 2+ and 1+ in the reference laboratories.
If the HER-2 IHC protocols were separated in two groups as CE-IVD labelled HER-2 IHC systems versus in-house HER-2 IHC systems, the CE-IVD labelled systems (PATHWAY®, HercepTest™ and Oracle™) showed a pass rate of 100 % (43 out of 43) compared to a pass rate of 67 % (8 out of 12) for the in-house systems.
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™ (Fig. 4b). In contrast, carcinoma no. 10 showed a moderate granular cytoplasmic staining with the HercepTest™ while this carcinoma was negative with PATHWAY® (Fig. 4a).

Scoring consensus
The laboratories were requested to send in their own scores (0, 1+, 2+, 3+) on the stained sections. For 30 out of the 48 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 66 % (29 out of 44). The relatively low scoring consensus most likely is related to the interpretation guidelines modified from the well established guidelines for HER-2 IHC in breast carcinoma. The most frequent discrepancies were related to the carcinoma no. 2 (HER-2 2+, non-amplified) and the carcinoma no. 7 (HER-2 3+, amplified). These two carcinomas were typically given a lower score by the laboratories than the NordiQC assessors. The carcinomas no. 5 and 10 giving an aberrant non-membranous staining pattern were by many laboratories scored as 2+. This was acceptable as

all equivocal results must be re-tested with another assay.

Conclusion
The CE-IVD labelled HER-2 systems PATHWAY® (Ventana), HercepTest™ (Dako) and Oracle™ (Leica), 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 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. Training in scoring is highly warranted and image analysis assisted scoring has to be taken in consideration to improve and facilitate the interpretation.

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



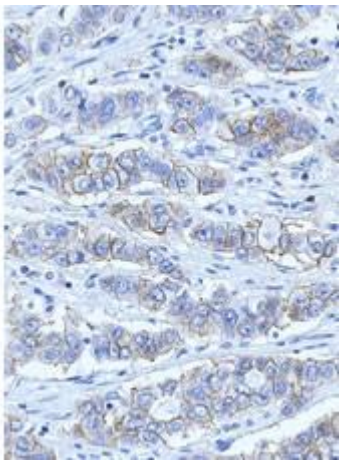
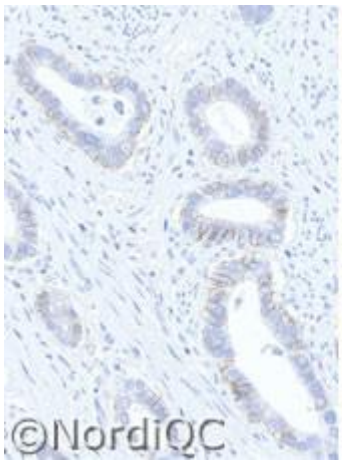


Fig. 1a
Left: Optimal HER-2 staining of the carcinoma no. 7 with a HER-2/Chr17 ratio > 6.0. ≥ 10 % of the neoplastic cells show a strong, lateral and focally a complete membranous staining corresponding to 3+.

Right: Optimal HER-2 staining of the 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+.



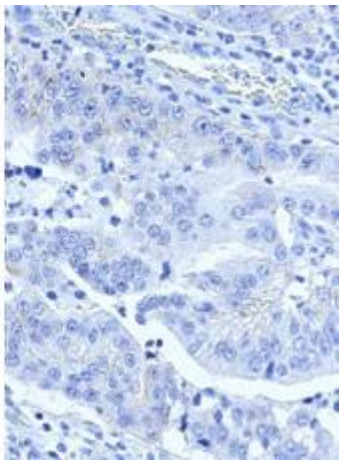


Fig. 1b
Left: Optimal HER-2 staining of the carcinoma no. 2 with HER-2/Chr17 ratio of 1.65. ≥ 10 % of the neoplastic cells show a weak to moderate lateral membranous staining corresponding to 2+.

Right: Optimal HER-2 staining of the carcinoma no. 5 with a HER-2/Chr17 ratio of 0.79. The neoplastic cells show a faint membranous staining corresponding to 1+.

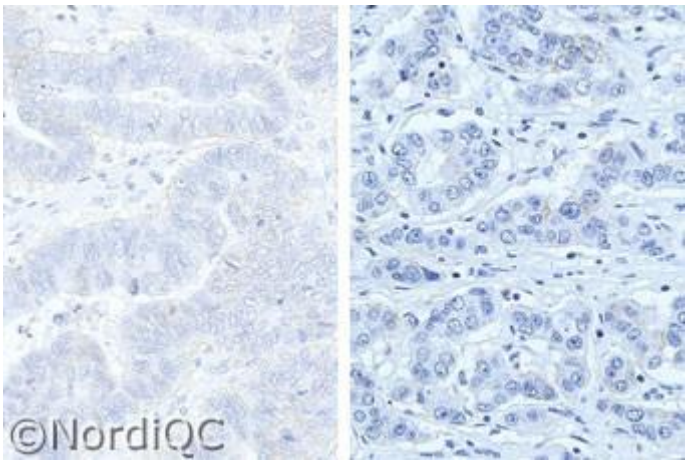


Fig. 2a
Left: Insufficient HER-2 staining of the carcinoma no. 7 with a HER-2/chr17 > 6.0. ≥ 10 % of the neoplastic cells show a weak primary basolateral membranous staining corresponding to 2+.
Right: Insufficient HER-2 staining of the carcinoma no. 8 with a HER-2/Chr17 ratio of 2.5. The neoplastic cells show a faint membranous staining corresponding to 1+, but does not meet the criteria to be classified as 2+. Thus it will not be referred to ISH.

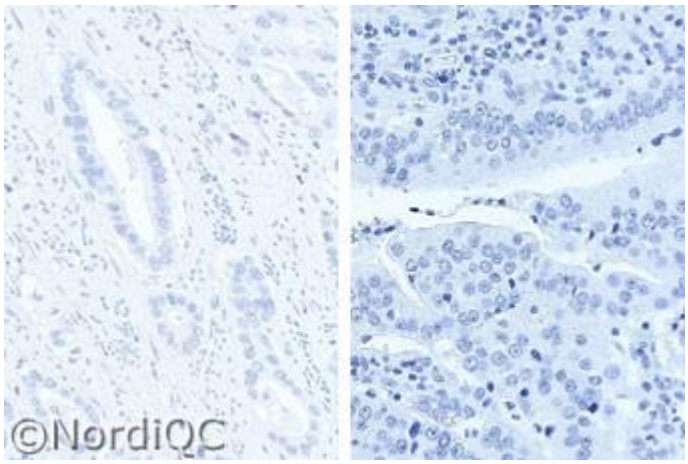


Fig. 2b
Left: HER-2 staining of the carcinoma no. 2 with a HER-2/chr17 ratio of 1.65. No staining is observed, corresponding to 0.
Right: HER-2 Staining of the carcinoma no. 5 with a HER-2/ chr17 ratio of 0.79. No staining is observed, corresponding to 0.

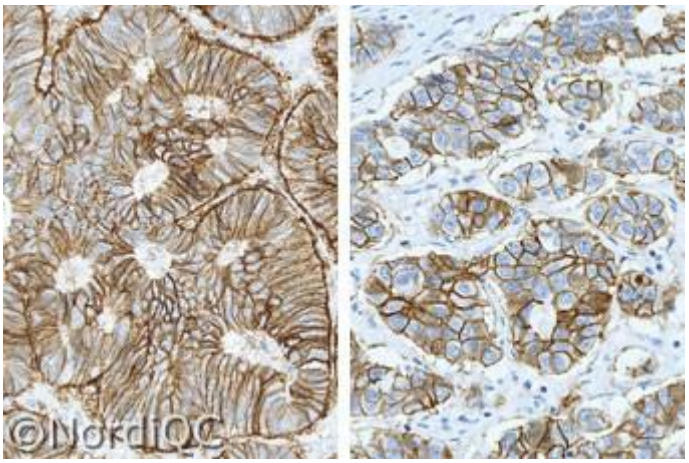


Fig. 3a
Left: HER-2 staining of the carcinoma no. 7 with a HER-2/ chr17 ratio > 6.0. ≥ 10 % of the neoplastic cells show a strong, lateral and a complete membranous staining corresponding to 3+.

Right: HER-2 staining of the carcinoma no. 8 with a HER-2/ chr17 of 2.5. ≥ 10 % of the neoplastic cells show a moderate lateral and focally a complete membranous staining corresponding to 3+.

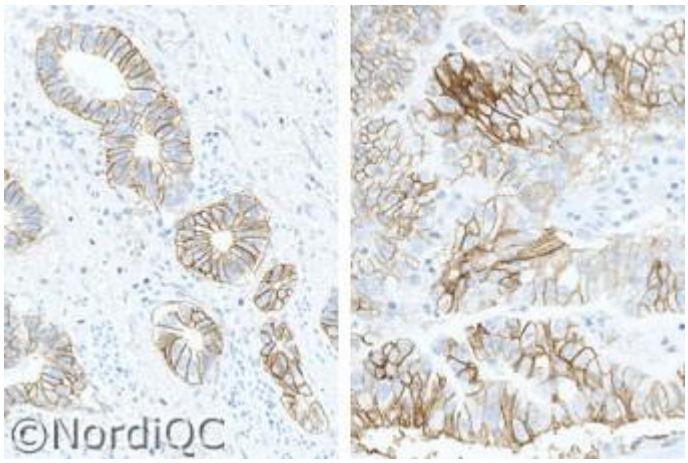


Fig. 3b
Left: Insufficient HER-2 staining of the carcinoma no. 2 with HER-2/chr17 of 1.65. ≥ 10 % of the neoplastic cells show a strong lateral membranous staining corresponding to 3+.

Right: Insufficient HER-2 staining of the carcinoma no. 5 with a HER-2/chr17 ratio of 0.79. ≥ 10 % of the neoplastic cells show a strong, lateral and a complete membranous staining corresponding to 3+.

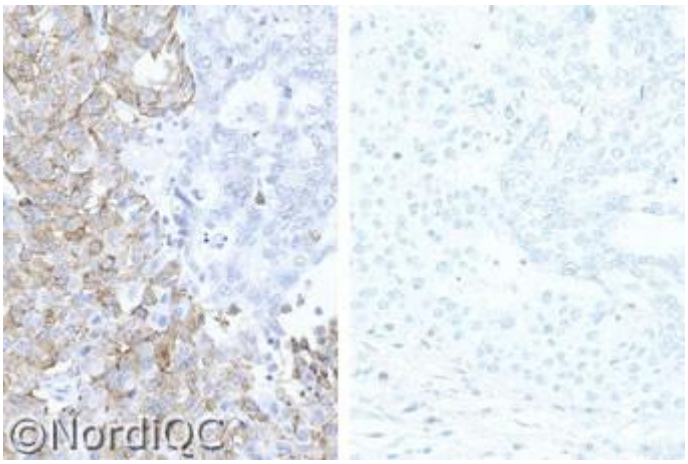


Fig. 4a
Left: HER-2 staining of the gastric carcinoma no. 10 with a HER-2/chr17 of 1.02 using the HercepTest™ (Dako). A moderate to strong granular cytoplasmic staining is seen in a high proportion of the neoplastic cells.

Right: HER-2 staining of the same carcinoma using PATHWAY® (Ventana). No staining is seen corresponding to a score 0.

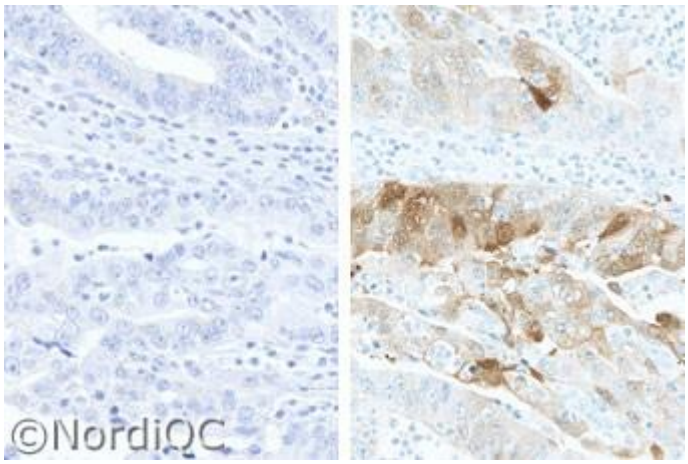


Fig. 4b
Left: HER-2 staining of the carcinoma no. 5 with a HER-2/chr17 of 0.79 using the HercepTest™ (Dako).
The neoplastic cells show a faint membranous staining corresponding to 1+.

Right: HER-2 staining of the same carcinoma using PATHWAY®, Ventana. A moderate to strong cytoplasmic and nuclear staining is seen. The staining is due to cross reaction with an unknown target (not HER-4 as previously believed).

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