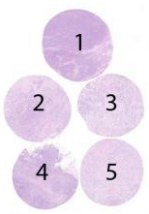
Assessment Run H11 2017

HER-2 ISH

Material

Table 1. **Content of the multi-block used for the NordiQC HER-2 ISH assessment, run H11**

	HER-2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	**HER-2/chr17 ratio¤**	**HER-2/chr17 ratio¤**	**HER-2 copies**
1. Breast carcinoma	0	0.8	0.8 – 1.0	< 4
2. Breast carcinoma	2+	2.3	2.8 – 3.3	> 6
3. Breast carcinoma	3+	8.0	6.5 – 8.5	> 6
4. Breast carcinoma	2+	1.1	1.0 – 1.2	≥ 4 and < 6
5. Breast carcinoma	1+	1.5	1.2 – 1.5	< 4



* PATHWAY® (Ventana), data from two reference labs.
** Inform HER-2 Dual ISH kit (Ventana), range of data from one reference lab.
*** HER-2 FISH pharmDX™ Kit (Dako) and HER-2 FISH (Zytovision), range of data from one reference lab.
¤HER-2/chr17: HER-2 gene/chromosome 17 ratio

All tissues were fixed for 24 - 48 hours in 10% neutral buffered formalin according to the ASCO/CAP 2013 guidelines for tissue preparation of breast tissue for HER-2 ISH analysis.

HER-2 BRISH, Technical assessment

The main criteria for assessing a BRISH HER-2 analysis as technically **optimal** were the ability to interpret the signals and thus evaluate the HER-2/chr17 ratios in all five tissues.

Staining was assessed as **good**, if the HER-2/chr17 ratios could be evaluated in all five tissues, but the interpretation was slightly compromised e.g. due to excessive retrieval, weak or excessive counterstaining or focal negative areas.

Staining was assessed as **borderline** if one of the tissues could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

Staining was assessed as **poor** if two or more of the tissue cores could not be evaluated properly e.g. due to weak signals, large negative areas with no signals (> 25% of the core) or a low signal-to-noise ratio due to excessive background staining.

HER-2 BRISH and FISH interpretation

For both BRISH and FISH, participating laboratories were asked to submit a scoring sheet with their interpretation of the HER-2/chr17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyse scoring consensus.

Consensus scores from the NordiQC FISH reference laboratories

- Breast ductal carcinomas, no. 1 and 5: non-amplified
- Breast ductal carcinomas, no. 4: non-amplified or equivocal
- Breast ductal carcinoma no. 2 and 3: amplified

The ASCO/CAP 2013 guidelines were applied for the interpretation of the HER-2 status

Unamplified: HER-2/chr17 ratio < 2.0 using a dual probe assay or an average < 4 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Equivocal: HER-2/chr17 ratio of < 2.0 using a dual probe assay with an average of ≥ 4 and < 6 HER-2 gene copies per cell/nucleus (both dual and single probe assay)

Amplified: HER-2/chr17 ratio ≥ 2.0 using a dual probe assay or an average ≥ 4 HER-2 copies per cell/nucleus. Using a single probe assay an average of ≥ 6 HER-2 copies per cell/nucleus.

Participation

Number of laboratories registered for HER-2 BRISH	124
Number of laboratories returning slides	121 (98%)
Number of laboratories returning scoring sheet	112 (90%)
Number of laboratories registered for HER-2 FISH	64
Number of laboratories returning scoring sheet	54 (84%)

Results BRISH, technical assessment

In total, 121 laboratories participated in this assessment. 73 laboratories (60%) achieved a sufficient mark (optimal or good). Results are summarized in table 2.

Table 2**. HER-2 BRISH systems and assessment marks for BRISH HER-2 run H11.**

Two colour HER-2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER-2 Dual ISH **800-4422**	91	Ventana	32	24	15	20	62%	65%
INFORM™ HER-2 Dual ISH + IHC **800-4422 + HER-2 IHC**	14	Ventana	3	4	6	1	50%	58%
DuoCISH pharmDx™ **SK109**	3	Dako	1	1	1	0	-	-
Zyto*Dot*® 2C **C-3022 / C-3032**	5	ZytoVision	2	2	1	0	80%	100%
One colour HER-2 systems								
INFORM™ HER-2 SISH **780-4332**	3	Ventana	0	2	0	1	-	-
Zyto*Dot*® **C-3003**	5	ZytoVision	1	1	1	2	40%	75%
Total	121		39	34	24	24	60%	-
Proportion			32%	28%	20%	20%		

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

Comments

In this assessment, optimal demonstration and evaluation of the HER-2 gene amplification status in all cores of the multi-tissue block could be obtained by all the applied dual-colour systems as shown in table 2. Minor focal staining artefacts were accepted if they did not compromise the overall interpretation in each of the 5 individual tissue cores. Artefacts were silver precipitates, excessive background staining or negative areas most likely caused by technical issues as slides drying out during the staining process or inadequate washing etc. In this run and in concordance with the previous NordiQC runs, the ISH rejection criteria defined in the 2013 ASCO/CAP HER-2 guidelines were applied. In brief, repeated test must be performed if more than 25% of the signals/cells cannot be interpreted due to the artefacts listed above. In these cases, the staining results were rated as insufficient (poor or borderline).

For the most commonly used HER-2 BRISH assay, the INFORM™ HER-2 Dual ISH (Ventana), technical adequate result was thus provided in 65% using appropriate and vendor recommended protocol settings being identified as essential to produce a technical optimal staining result. These data, which have been observed consistently in the latest NordiQC HER-2 BRISH assessments, clearly indicates a general challenge for the present assay to provide a reproducible performance. As this test is being used by 87% of all participating laboratories and is applied by appropriate protocol settings, this significantly affects the pass rate and at present no recommendations how to improve the end result has been identified.

Optimal protocol settings: Two-colour HER-2 systems

For the **INFORM™ Dual ISH system 800-4422** (Ventana), optimal demonstration of HER-2 BRISH was typically based on HIER in Cell Conditioning 2 (CC2) for 24-32 min. at 86-90˚C and subsequent proteolysis in P3 for 8-20 min. at 36-37˚C. The HER-2 and chr17 probe cocktail was typically applied for 6 hours at 44˚C following denaturation at 80˚C for 20 min.

Using these protocol settings, sufficient results (optimal or good) were seen in 65% of the submitted protocols (45 of 69). 24 laboratories used a protocol with optimal settings but, for unexplained reasons, completely false negative staining or excessive background staining (e.g. due to silver precipitates) were seen in the entire slide or in large areas comprising >25% of the neoplastic cells in one or more of the tissue cores. No reason for these insufficient results could be related to the applied protocols, reagents, platforms (BenchMark XT, GX or Ultra) or any other protocol parameter. This observation has been seen in the latest runs and might indicate a less robust and reproducible performance of the protocols on the used instruments.

The remaining insufficient results were characterized by impaired morphology hampering interpretation of the signals. This pattern was typically caused by excessive retrieval in e.g. P3 for 24–28 min. and/or prolonged HIER in CC2 or CC1 and as a consequence the nuclei were almost totally digested and virtually no counterstaining could be seen.

14 laboratories used the **INFORM™ Dual ISH systems 800-4422** (Ventana) in combination with an immunohistochemical demonstration for **HER-2 PATHWAY®** (Ventana). An optimal demonstration of HER-2 BRISH using this assay was based on HIER in Cell Conditioning 2 (CC2) for 28-32 min. at 75-90°C and subsequent proteolysis in P2 for 8-20 min. at 36-37°C. The HER-2 and chr17 probe cocktail was typically applied for 6 hours at 44°C following a denaturation at 80°C for 4 or 20 min. HER-2 PATHWAY® was typically performed with iVIEW as detection system. Both BenchMark ULTRA and XT could be used as stainer platform. Using these protocol settings, sufficient results were seen in 58% of the submitted protocols (7 of 12).

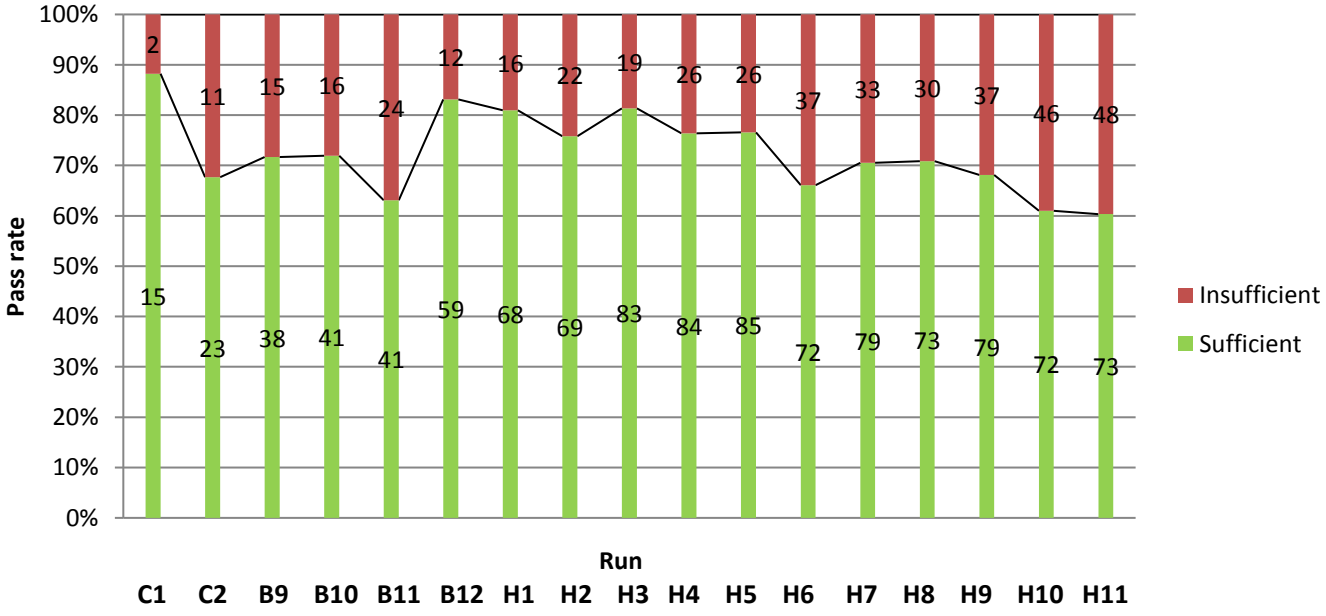
For the **DuoCISH™ system SK109** (Dako), one protocol gave an optimal result.
The protocol was based on HIER in pre-treatment buffer in a waterbath for 15 min. at 95-100°C and subsequent proteolysis in pepsin for 10 min. at RT (both reagents included in the HER-2 DuoCISH pharmDX kit SK109). The HER-2 and chr17 probes were applied for 24 hours at 45°C and visualized by the detection reagents provided in the DuoCISH™ kit SK109.

For the **Zyto*Dot*® 2C system C-3022 / C-3032** (ZytoVision), two protocols gave an optimal result.
The protocols were based on HIER in EDTA pH 8 in a waterbath for 15 min. at 95-98°C, proteolysis in pepsin for 2 min. at room temperature or 5 min. at 37°C, hybridization at 37°C for 18-20 hours and visualization with the ZytoVision detection kit C-3022. Using these protocol settings, sufficient results were seen in 100% of the submitted protocols (4 of 4).

One-colour HER-2 systems
For the **Zyto*Dot*® CISH system C-3003** (ZytoVision), one protocol gave an optimal result.
The protocol was based on HIER in EDTA pH 8 in a waterbath for 15 min. at 100°C, proteolysis in pepsin for 2 min. at room temperature, hybridization at 37°C overnight and visualization with the ZytoVision detection kit C-3003. Using these or similar protocol settings, sufficient results were seen in 75% of the submitted protocols (3 of 4).

Performance history
This was the 17th assessment of HER-2 BRISH in NordiQC and a consistent pass rate at a relatively low level has been observed in the latest runs. Data is shown in Fig. 1.

Fig. 1. **Proportion of sufficient results for HER-2 BRISH in the NordiQC assessments**



HER-2 ISH interpretation and scoring consensus:

Table 3. **NordiQC FISH amplification data**

	NordiQC FISH HER-2/chr17 ratio	**NordiQC FISH HER-2 copies**	**NordiQC HER-2 amplification status**
1. Breast ductal carcinoma	0.8 – 1.0	< 4	Non-amplified
2. Breast ductal carcinoma	2.8 – 3.3	> 6	Amplified
3. Breast ductal carcinoma	6.5 – 8.5	> 6	Amplified
4. Breast ductal carcinoma	1.0 – 1.2	≥ 4 and < 6	Non-amplified / Equivocal
5. Breast ductal carcinoma	1.2 – 1.5	< 4	Non-amplified

* data from 2 different NordiQC reference laboratories.

166 of the 188 (88%) participating laboratories completed scoring sheets. These evaluations were compared to the HER-2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in Figs. 2 and 3. For laboratories performing FISH, the consensus rate was 91% (49 of 54 laboratories) and 84% (94 of 112 laboratories) for laboratories using BRISH. This is an improvement compared to the previous assessment, where the consensus rates were 80% and 78% for FISH and BRISH, respectively.

In general, for both BRISH and FISH, high consensus rates were observed between participants and NordiQC regarding the HER-2 amplification status in the breast carcinomas tissue cores no. 1, 3 and 5. The interpretation of HER-2 amplification status was more challenging for the tissue core no. 2 and 4.
For both BRISH and FISH, disagreement of the interpretation of the HER-2 amplification status between the participants and NordiQC data were both related to "overrating" and "underrating" the HER-2 status and thus an aberrant classification compared to the NordiQC reference data and the majority of other participants.

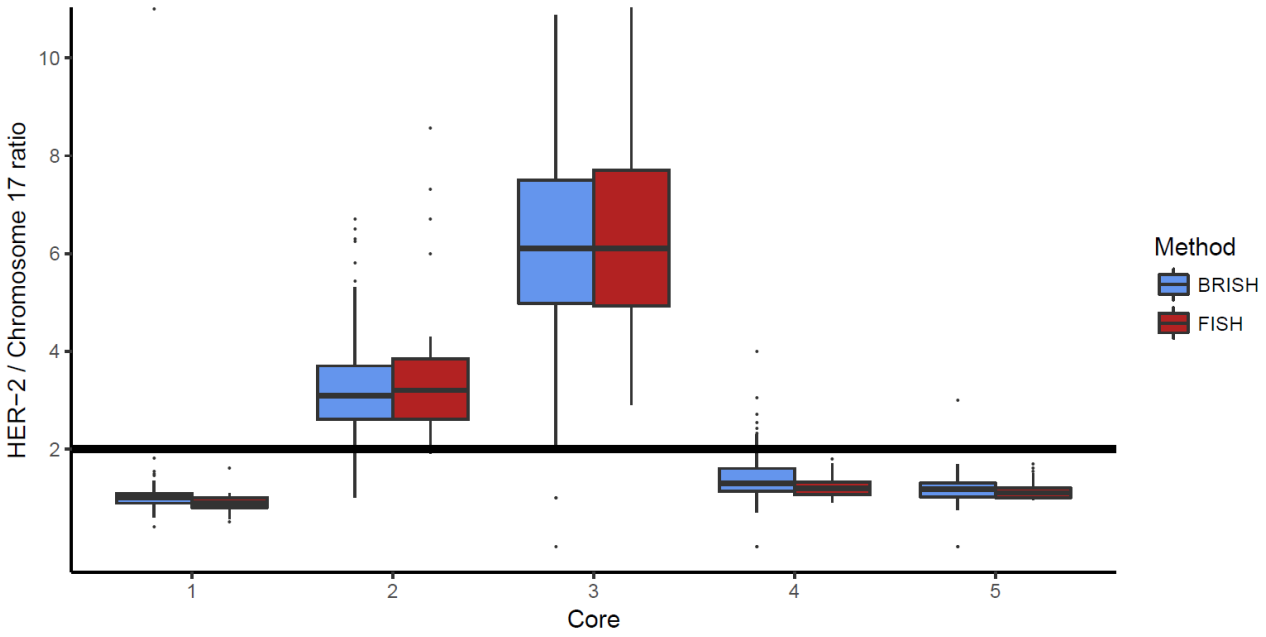
Tumour no. 2 was by the NordiQC reference laboratories classified as amplified with a HER-2 ratio of 2.8 – 3.3 and >6 HER-2 gene copies. This tumour was, by some laboratories using either FISH (3 of 54 laboratories) or BRISH (8 of 112 laboratories) classified as equivocal or non-amplified.

Tumour no. 4 was by the NordiQC laboratories characterized to be non-amplified or equivocal. The tumour showed HER-2 ratio in the range of 1.0 – 1.2 and in areas a level of ≥ 4 but less < 6 HER-2 gene copies was identified. This tumour was, by some laboratories primarily using BRISH classified as amplified. 10 of 112 laboratories performing BRISH and 1 of 54 laboratories performing FISH scored this tumour as amplified.

Participants using FISH tended to have marginally higher level of consensus than participants using BRISH. In general one could speculate that the technical quality of the BRISH staining reaction, as excessive background staining, inadequate counterstaining, chromogen or silver precipitates would compromise the interpretation. However, it was observed that the consensus rates of laboratories producing results assessed as technically sufficient (optimal and good) and insufficient (borderline and poor) were relatively similar (85% and 79%, respectively). This was most likely caused by the ISH rejection criteria applied by NordiQC in the assessment. The criteria (defined in the 2013 ASCO/CAP HER-2 guidelines) require retest, if more than 25% of the signals/cells cannot be interpreted due to artefacts such as silver precipitate, excessive background or negative areas. The material in the assessment consisted of breast tumours with relatively homogenous HER-2 expression, which permitted correct evaluation even in slides with large negative areas. This is not always the case in diagnostic settings with heterogeneous tumours or evaluation in specific "hot-spot areas" identified by HER-2 IHC.

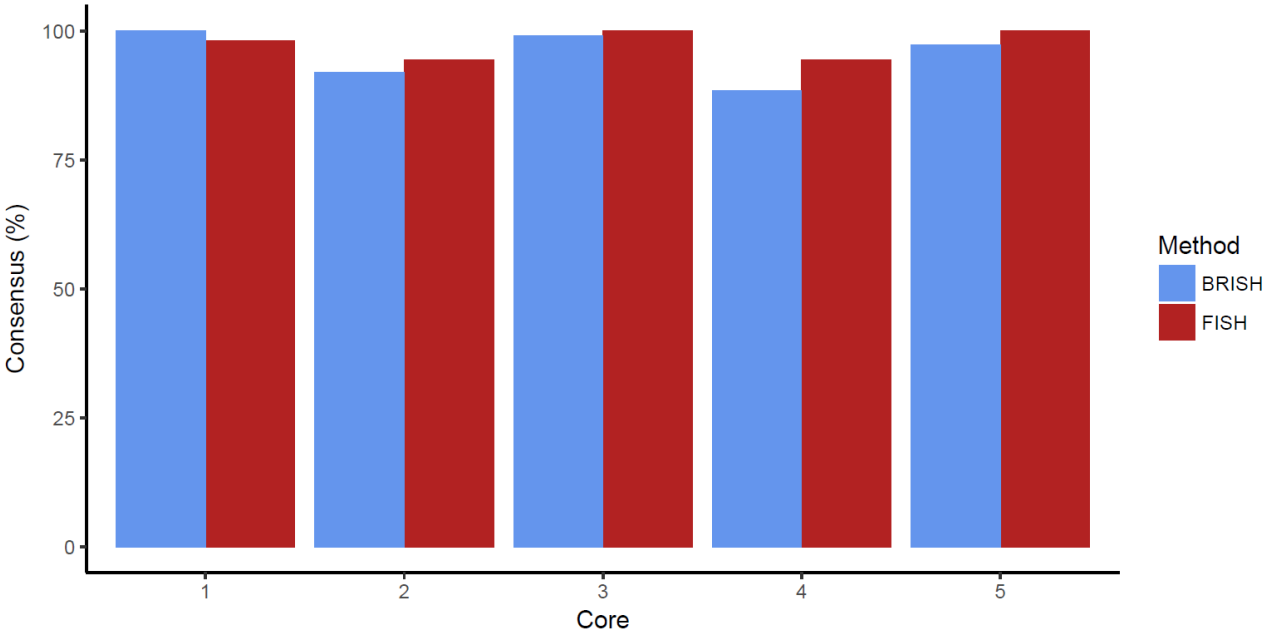
Participants overall interpretation of amplification ratios and consensus rates are shown in Figs. 2 and 3.

Fig. 2



NordiQC HER-2 ISH run H11: participants interpretation of amplification status

Fig. 3



NordiQC HER-2 ISH run H11: consensus between participants and NordiQC

No technical evaluation of FISH protocols was performed. Table 4 shows the FISH assay used by the participants and concordance level to the NordiQC data observed. In this matter it has to be emphasized that it was not possible to identify the cause of an aberrant interpretation of the HER-2 status whether this was related to the technical performance of the FISH assay or the interpretation by the observer(s).

Table 4. **FISH assays used and level of consensus HER-2 status to NordiQC reference data**

Assay	Number	Consensus rate
Pathvysion/Abbot, 6N4630 / 30-161060	16	94% (15/16)
ZytoVision, Z2015 / Z2020/ Z2077	14	86% (12/14)
Dako, K5731	9	89% (8/9)
Leica, TA9217	5	100% (5/5)
Other	10	90% (9/10)

Conclusion

In this assessment and in concordance with previous NordiQC HER-2 ISH runs, technical optimal demonstration of HER-2 BRISH could be obtained by the commercially available two-colour HER-2 systems **INFORM™ HER-2 Dual ISH** (Ventana), **DuoCISH™**(Dako) and **ZytoDot® 2C** (ZytoVision).
The single-colour HER-2 system **ZytoDot®** (ZytoVision) could also be used to produce a technical optimal HER-2 demonstration.
For all systems, retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient demonstration of HER-2 (and chr17 signals) and preserved morphology.
Despite optimal protocol settings being applied, a high proportion of technical insufficient results were seen, indicating that other issues are influencing the quality of the BRISH assays. Especially the capability of present instrumentation and associated HER-2 ISH asssays to provide reproducible performance of the protocols might be a central factor. It was observed that the most commonly used HER-2 BRISH assay, INFORM™ HER-2 Dual ISH (Ventana), only provided a pass rate of 65% and thus a technical inadequate result frequently was seen despite using appropriate and well characterized protocol settings.
Attention must be paid to interpretation in order to obtain correct HER-2 amplification status.
Laboratories performing FISH achieved a slightly higher consensus rate for the interpretation of HER-2 amplification status compared to laboratories performing BRISH.

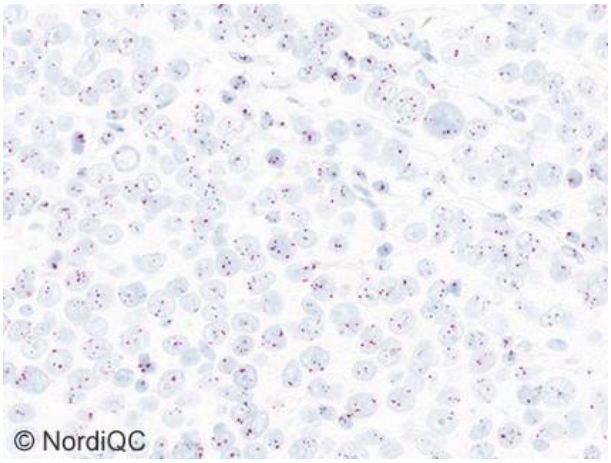


Fig. 1a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 1 without HER-2 gene amplification:
HER-2/chr17 ratio 0.8 – 1.0*.
The HER-2 genes are stained black and chr17 red.
The signals are distinctively demonstrated.
Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies is identified.
NordiQC and the vast majority of participants interpreted this tumour as negative.

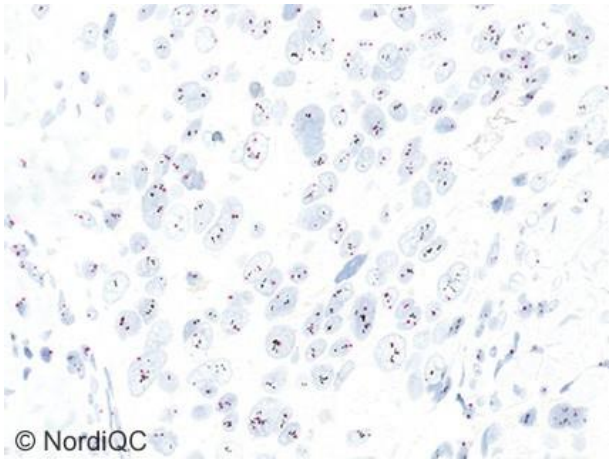


Fig. 1b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 2 with HER-2 gene amplification:
HER-2/chr17 ratio 2.8 - 3.3*.
The HER-2 genes are stained black and chr17 red.
The HER-2 signals are distinctively demonstrated.
NordiQC and virtually all participants interpreted this tumour as positive, low to moderately amplified.

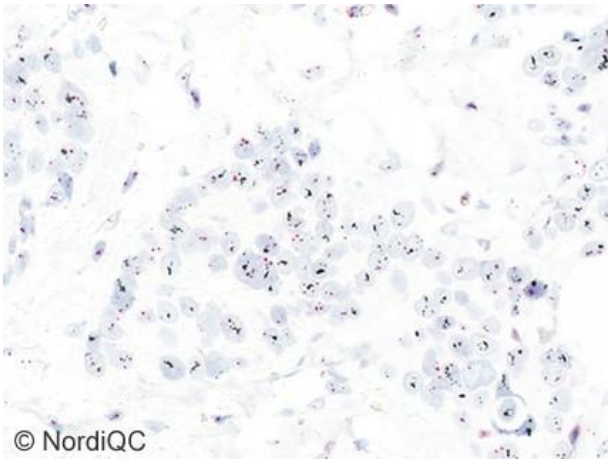


Fig. 2a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 3 with high level HER-2 gene amplification: HER-2/chr17 ratio > 6.5 – 8.5*.
The HER-2 genes are stained black and chr17 red.
The signals are distinctively demonstrated and many HER-2 signals are located in large clusters.
NordiQC and virtually all participants interpreted this tumour as positive, highly amplified.

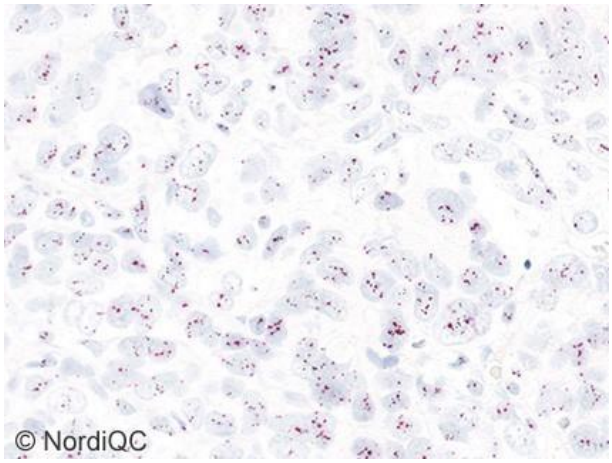


Fig. 2b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 4 without HER-2 gene amplification:
HER-2/chr17 ratio > 1.0 – 1.2*.
The HER-2 genes are stained black and chr17 red.
Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER-2 gene copies is identified.
NordiQC and the vast majority of participants interpreted this tumour as negative or equivocal.

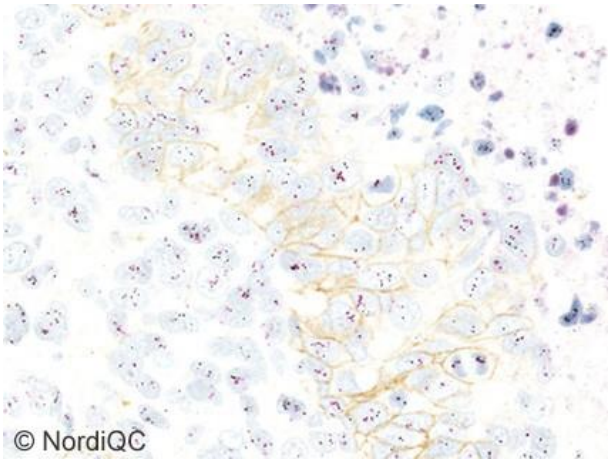


Fig. 3a
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana in combination with HER-2 IHC using PATHWAY, Ventana of the breast carcinoma no. 4 without HER-2 gene amplification:
HER-2/chr17 ratio 1.0 – 1.2*.
The gene protein assay (GPA) label the HER-2 genes black, chr17 red and HER-2 protein brown.
The IHC level is interpreted as 2+ and the GPA assay visualizes IHC hot-spots to evaluate the HER-2 gene status precisely.
The participant interpreted this tumour as negative.

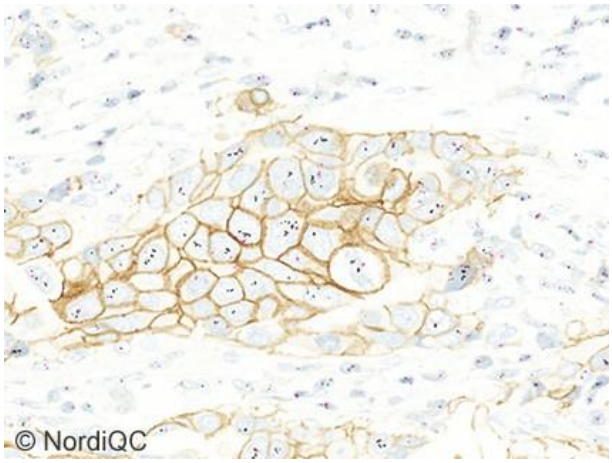


Fig. 3b
Optimal demonstration of the HER-2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana in combination with HER-2 IHC using PATHWAY, Ventana of the breast carcinoma no. 2 with HER-2 gene amplification: HER-2/chr17 ratio > 2.8 – 3.3*.
The gene protein assay (GPA) label the HER-2 genes black, chr17 red and HER-2 protein brown.
The IHC level is interpreted as 2+ and the GPA assay visualizes the HER-2 IHC overexpression and the HER-2 gene status simultaneously.
The participant interpreted this tumour as positive, low amplified.

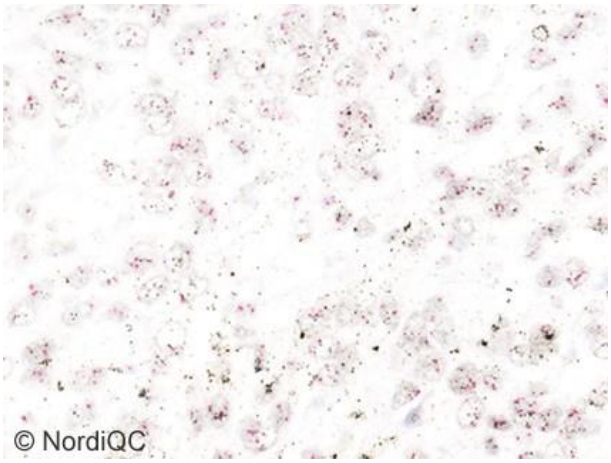


Fig. 4a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 4 without gene amplification: HER-2/chr17 ratio 1.0 – 1.2*.
The vast majority of the neoplastic cells display HER-2 and chr17 signals, but simultaneously silver precipitates are seen outside the cells compromising the interpretation.
This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1-4.

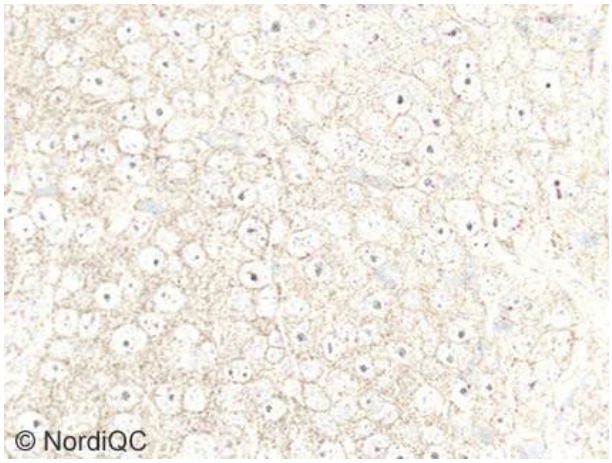


Fig. 4b
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 1 without HER-2 gene amplification:
HER-2/chr17 ratio 0.8 – 1.0*.
Due to extensive silver precipitates the HER-2 gene status cannot reliably be interpreted. This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1-4.

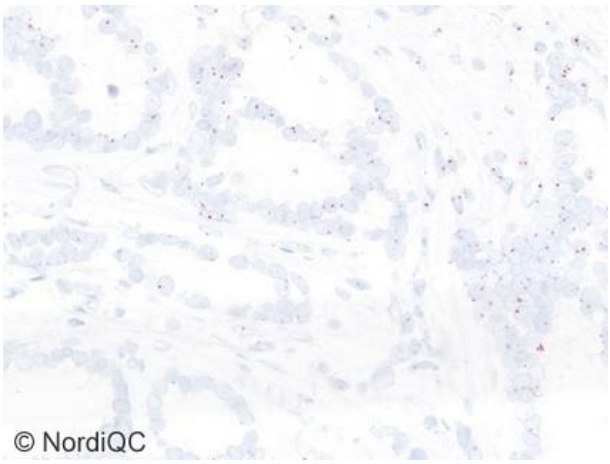


Fig. 5a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 5 without gene amplification: HER-2/chr17 ratio 1.2 – 1.5*.
The majority of the neoplastic cells are negative and only in scattered cells HER-2 and chr17 signals can be identified.
This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1-4.
Negative areas of < 25% in each of the tissue cores were accepted.
The laboratory reported the result as technically insufficient and new test required.

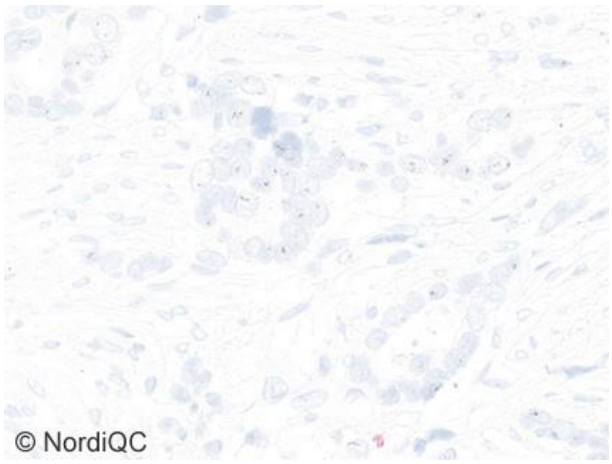


Fig. 5a
Insufficient staining for the HER-2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana of the breast carcinoma no. 3 with gene amplification: HER-2/chr17 ratio > 6.5 – 8.5*.
The majority of the neoplastic cells are negative and only in scattered cells HER-2 signals can be identified.
This aberrant reaction most likely was caused by a technical problem during the staining process in the BenchMark instrument.
Same protocol settings were applied as used in Figs. 1-4.
Negative areas of < 25% in each of the tissue cores were accepted.
The laboratory reported the result as technically insufficient and new test required.

* Reference: HER2 FISH pharmDX™ Kit, Dako & HER2 FISH, Zytovision (range of data from one reference lab.)

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