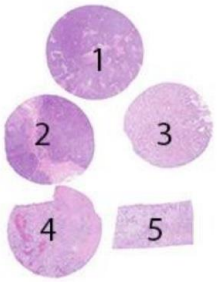
Assessment Run H14 2018

HER2 ISH (BRISH or FISH)

Material

Table 1. **Content of the multi-block used for the NordiQC HER2 ISH assessment, run H14**

	HER2 IHC*	Dual - SISH**	FISH***	FISH***
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	0	0.8 – 0.9	0.8 – 1.0	< 4
2. Breast carcinoma	2+	1.1 – 1.3	1.0 – 1.2	≥ 4 and < 6
3. Breast carcinoma	1+	1.2 – 1.3	1.2	< 4
4. Breast carcinoma	2+	2.3 – 3.0	2.8 – 3.3	> 6
5. Breast carcinoma	3+	6.2 - 8.0	6.5 – 8.5	> 6



* PATHWAY® (Ventana/Roche), data from two reference labs.
** Inform HER2 Dual ISH kit (Ventana/Roche), range of data from one reference lab.
*** HER2 FISH (Zytovision), range of data from one reference lab.
¤HER2/chr17: HER2 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 HER2 ISH analysis.

HER2 BRISH, Technical assessment

The main criteria for assessing a BRISH HER2 analysis as technically **optimal** were the ability to interpret the signals and thus evaluate the HER2/chr17 ratios in all five tissues.

Staining was assessed as **good**, if the HER2/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 (≤ 25% of the core).

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.

HER2 BRISH and FISH interpretation

For both BRISH and FISH, participating laboratories were asked to submit a scoring sheet with their interpretation of the HER2/chr17 ratio. Results were compared to NordiQC FISH data from reference laboratories to analyze scoring consensus.

Consensus scores from the NordiQC BRISH/FISH reference laboratories

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

The ASCO/CAP 2013 guidelines were applied for the interpretation of the HER2 status

Unamplified: HER2/chr17 ratio < 2.0 using a dual probe assay or an average < 4 HER2 gene copies per cell/nucleus (both dual and single probe assay)

Equivocal: HER2/chr17 ratio of < 2.0 using a dual probe assay with an average of ≥ 4 and < 6 HER2 gene copies per cell/nucleus (both dual and single probe assay)

Amplified: HER2/chr17 ratio ≥ 2.0 using a dual probe assay or an average ≥ 4 HER2 copies per cell/nucleus. Using a single probe assay an average of ≥ 6 HER2 copies per cell/nucleus.

Participation

Number of laboratories registered for HER2 BRISH	138
Number of laboratories returning slides	129 (93%)
Number of laboratories returning scoring sheet	118 (91%)
Number of laboratories registered for HER2 FISH	64
Number of laboratories returning scoring sheet	64 (100%)

Results BRISH, technical assessment

In total, 129 laboratories participated in this assessment. 92 laboratories (71%) achieved a sufficient mark (optimal or good). Results are summarized in Table 2.

Table 2**. HER2 BRISH systems and assessment marks for BRISH HER2 run H14.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
INFORM™ HER2 Dual ISH **800-4422/780-4422**	90	Ventana/Roche	40	24	16	10	71%	74%
INFORM™ HER2 Dual ISH + IHC **800-4422 + HER2 IHC**	20	Ventana/Roche	8	7	5	0	75%	73%
Zyto*Dot*® 2C **C-3022 / C-3032**	9	ZytoVision	2	1	5	1	-	-
One colour HER2 systems								
INFORM™ HER2 SISH **780-4332**	6	Ventana/Roche	3	3	0	0	-	-
Zyto*Dot*® **C-3003**	4	ZytoVision	0	4	0	0	-	-
Total	129		53	39	26	11		-
Proportion			41%	30%	20%	9%	71%	

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 HER2 gene amplification status in all five cores of the multi-tissue block could be obtained by all the applied dual-colour systems and the Ventana/Roche HER2 SISH one colour system as shown in Table 2. Minor focal staining artefacts were accepted if they did not compromise the overall interpretation in each of the five individual tissue cores. Artefacts as silver precipitates, excessive background staining or negative areas (see Figs. 5a-5b) were 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 HER2 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 HER2 BRISH assay, the INFORM™ HER2 Dual ISH (Ventana/Roche), a technical adequate result was provided in 71% of the submitted slides using appropriate and vendor recommended protocol settings identified as essential to produce a technical optimal staining result. These data, which have been observed consistently in the latest NordiQC HER2 BRISH assessments, clearly indicates a general challenge for the present assay to provide a reproducible performance. As this test is used by 85% of all participating laboratories applied with appropriate protocol settings, this significantly affects the pass rate. At present, no recommendations on how to improve the end result have been identified.

Optimal protocol settings: Two-colour HER2 systems

For the **INFORM™ Dual ISH system 800-4422** (Ventana/Roche), optimal demonstration of HER2 BRISH was typically based on heat induced epitope retrieval (HIER) in Cell Conditioning 2 (CC2) for 28-40 min. at 86-90˚C and subsequent proteolysis in Protease 3 for 8-20 min. at 36-37˚C. The HER2 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) (see Figs. 1-2) were seen in 74% of the submitted protocols (48 of 65). 17 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) in the entire slide or large areas comprising >25% of the neoplastic cells in one or more of the tissue cores (see Figs. 5a-5b). 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 performance of the protocols on the used instruments. The "negative spot artefact" (large negative areas comprising >25% of the neoplastic cells in one or more of the tissue cores) was seen in 71% (12 of 17) of the laboratories. The "silver precipitate artefact" (large areas with silver precipitates comprising >25% of the neoplastic cells in one or more of the tissue cores) was seen in 24% (4 of 17) of the laboratories.

20 laboratories used the **INFORM™ Dual ISH systems 800-4422** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY®** (Ventana/Roche). Optimal demonstration of HER2 BRISH using this assay was typically based on HIER in CC2 or Cell Conditioning 1 (CC1) for 24-32 min. at 75-100°C and subsequent proteolysis in Protease 2 for 8-20 min. at 36-37°C. The HER2 and chr17 probe cocktail was typically applied for 6 hours at 44°C following a denaturation at 80°C for 4 or 32 min. HER2 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 73% of the submitted protocols (11 of 15) (see Figs. 3a-3b). In the current assessment, the pass rate of this combined **HER2 Dual ISH / HER2 IHC assay** (also known as HER2 gene protein assay / GPA) dropped from 100% in Run H13 (spring 2018) to 75%. The reason for insufficient staining results was in all cases (5 of 20) due to large negative areas comprising >25% of the neoplastic cells in one or more of the tissue cores ("negative spots"). Since the introduction of this combined **HER2 Dual ISH / HER2 IHC assay** in 2014, a total of 102 protocols have been submitted for assessment. 80% (82 of 102) have obtained sufficient staining results. In the same period, 810 protocols based on the **INFORM™ Dual ISH systems 800-4422** have been submitted and 66% (533 of 810) obtained sufficient staining results. Despite the recorded drop in pass rate in the current run, these data suggest that the combined **HER2 Dual ISH / HER2 IHC assay** is somewhat more robust compared to the "classic" **INFORM™ Dual ISH system 800-4422.** At present, the reason for this difference is unknown.

For the **Zyto*Dot*® 2C system C-3022 / C-3032** (ZytoVision), two protocols gave optimal results (see Fig. 4a). Protocols were based on HIER in EDTA pH 8 in a waterbath for 15 min. at 96-97°C, proteolysis in pepsin for 3-5 min. at room temperature or at 37°C, hybridization at 37°C for 18-20 hours following a denaturation at 65-78°C for 5 min. and visualization with the ZytoVision detection kit C-3022. Using these protocol settings, sufficient results were seen in 50% of the submitted protocols (3 of 6).

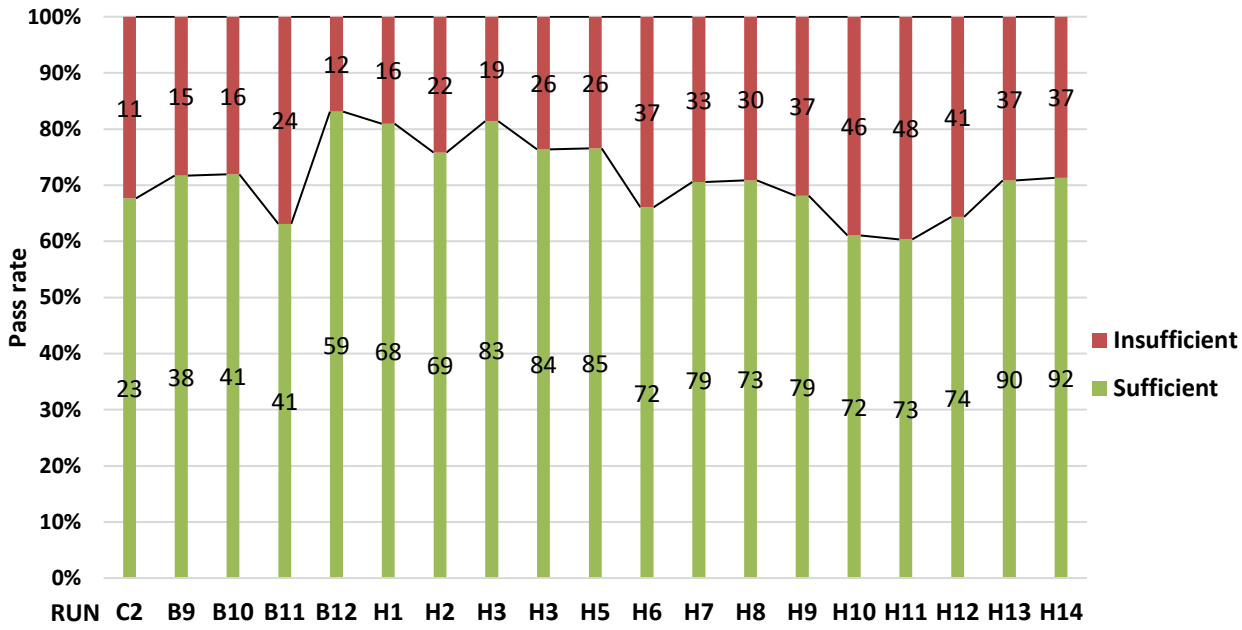
One-colour HER2 systems

For the **INFORM™ SISH system 780-4332** (Ventana/Roche), three protocols gave optimal results (see Fig. 4b). Protocols were typically based on HIER in CC2 for 28-32 min. at 85-86°C and subsequent proteolysis in Protease 3 for 8-12 min. at 36°C. The HER2 SISH probe was applied for 6 hours at 52°C following a denaturation at 93°C for 4-8 min. Using these protocol settings, sufficient results were seen in 100% of the submitted protocols (6 of 6).

Performance history

This was the twentieth assessment of HER2 BRISH in NordiQC and a consistent pass rate at a relatively low level has been observed in the latest runs. Data is shown in Graph 1.

Graph 1. **Proportion of sufficient results for HER2 BRISH in the NordiQC assessment**



HER2 ISH interpretation and scoring consensus

Table 3. **NordiQC FISH amplification data***

	NordiQC FISH HER2/chr17 ratio	NordiQC FISH HER2 copies	NordiQC HER2 amplification status
1. Breast ductal carcinoma	0.8 – 1.0	< 4	Non-amplified
2. Breast ductal carcinoma	1.0 – 1.2	≥ 4 and < 6	Non-amplified / Equivocal
3. Breast ductal carcinoma	1.2	< 4	Non-amplified
4. Breast ductal carcinoma	2.8 – 3.3	> 6	Amplified
5. Breast ductal carcinoma	6.5 – 8.5	> 6	Amplified

* data from one NordiQC reference laboratory.

182 of the 193 (94%) participating laboratories completed scoring sheets on the NordiQC homepage. These evaluations were compared to the HER2 FISH amplification status obtained by the NordiQC reference laboratories, summarized in Graph 2 and 3. For the laboratories performing FISH, the consensus rate was 91% (58 of 64) and 64% (75 of 118) for laboratories using BRISH. This was a small increase for laboratories that used FISH compared to last runs where the consensus rate was 85%. Contrary, this was a significant decrease for labs using BRISH as the consensus rate in run H13 was 93%.

In general, for both BRISH and FISH, high consensus rates were observed between participants and NordiQC regarding the HER2 amplification status in most cores. The most challenges in interpretation of HER2 amplification status were seen in tissue core no. 2, especially for laboratories performing BRISH, and to a lesser extent core 3 and 4.
For BRISH and FISH, disagreement of the interpretation of the HER2 amplification status between the participants and NordiQC data were both related to "overrating" and "underrating" the HER2 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 non-amplified with a HER2 ratio of 1.0– 1.2 and ≥ 4 and < 6 HER2 gene copies. This tumour was, by some laboratories using either FISH (1 of 64 laboratories) or BRISH (33 of 118 laboratories) classified as amplified or indeterminable.

Tumour no. 3 was by the NordiQC reference laboratories characterized as non-amplified. The tumour showed HER2 ratio of 1.2 and < 4 HER2 gene copies were identified. This tumour was, by some

laboratories using either FISH (2 of 64) or BRISH (9 of 118) classified as amplified (n=6) or equivocal (n=5).

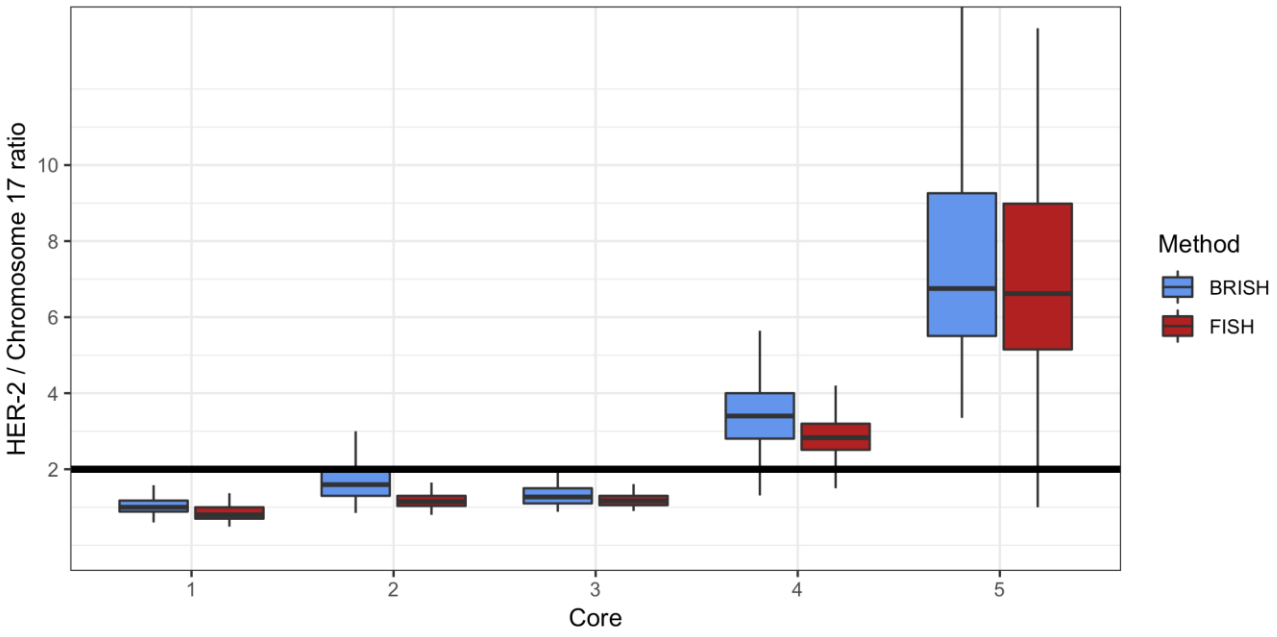
Tumour no. 4 was by the NordiQC reference laboratories characterized to be amplified. The tumour showed HER2 ratio in the range of 2.8-3.3 and > 6 HER2 gene copies were identified. This tumour was, by some laboratories using either FISH (4 of 66) or BRISH (7 of 113) classified as non-amplified (n=5) or equivocal (n=6).

In this assessment participants using FISH had a higher level of consensus on the individual core than participants using BRISH. This is a change compared to the previous assessment, where participants using FISH and BRISH tended to have the same level of consensus on the individual cores.

It was observed that the consensus rates of the individual cores among laboratories that produced staining reaction assessed as technically sufficient (BRISH only) were marginally higher than laboratories with an insufficient mark (92% and 89%, respectively). Despite insufficient staining, laboratories were still able to correctly evaluate the slide. The ISH rejection criteria are applied in NordiQC assessments. The criteria (defined in the 2013 ASCO/CAP HER2 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 HER2 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 HER2 IHC.

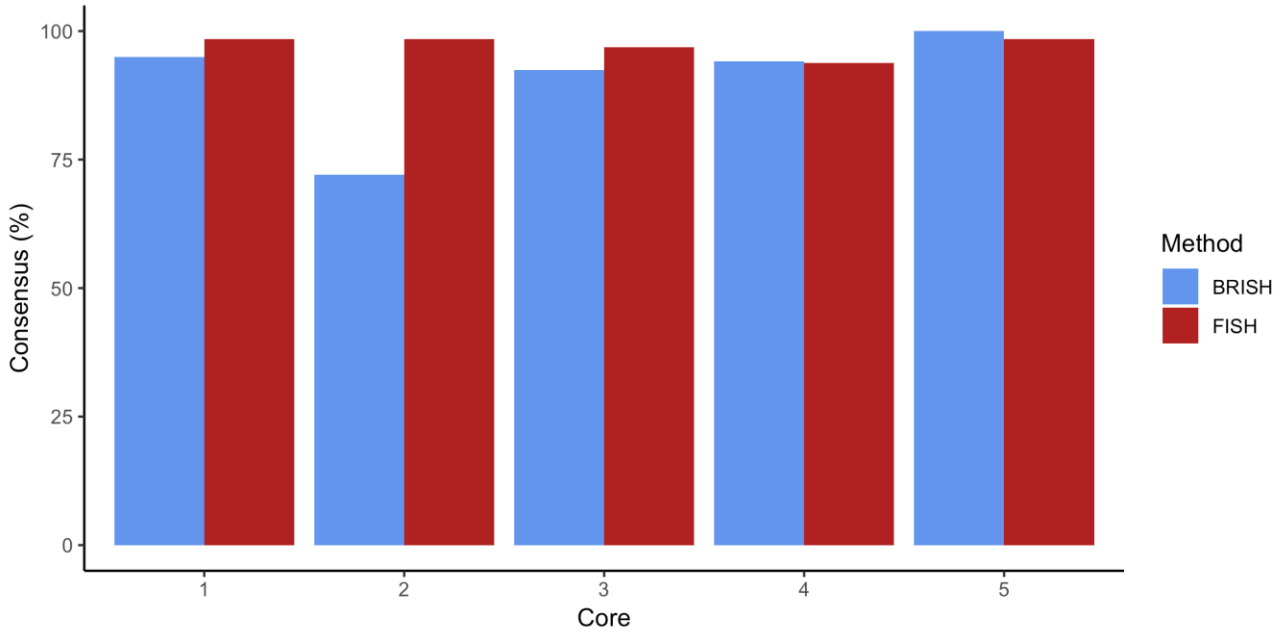
Participants overall interpretation of amplification ratios and consensus rates are shown in Graph 2 and 3.

Graph 2



NordiQC HER2 ISH run H14: Participant interpretation of amplification status

Graph 3



NordiQC HER2 ISH run H14: 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 HER2 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 HER2 status to NordiQC reference data, H14**

Assay	**Number**	**Consensus rate**
Pathvysion/Abbot, 6N4630 / 30-161060	16	94% (15/16)
ZytoVision, Z2015 / Z2020/ Z2077	15	93% (14/15)
Dako, K5731	12	83% (10/12)
Leica, TA9217	6	100% (6/6)
Other	21	90% (19/21)

Conclusion

In this assessment and in concordance with previous NordiQC HER2 ISH runs, technical optimal demonstration of HER2 BRISH could be obtained by the commercially available two-colour HER2 systems **INFORM™ HER2 Dual ISH** (Ventana/Roche) and **ZytoDot® 2C** (ZytoVision).

The single-colour HER2 system **INFORM™ SISH system** (Ventana/Roche) could also be used to produce a technical optimal HER2 demonstration.

For all systems, retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient demonstration of HER2 (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 HER2 ISH assays to provide reproducible performance of the protocols might be a central factor. It was observed that the most commonly used HER2 BRISH assay, INFORM™ HER2 Dual ISH (Ventana/Roche), only provided a pass rate of 71% despite using appropriate and well characterized protocol settings. The combined **HER2 Dual ISH / HER2 IHC assay** (Ventana/Roche) achieved a pass rate of 75% - slightly higher than the "classic" INFORM™ HER2 Dual ISH (Ventana/Roche) – but significantly lower than in the previous HER2 ISH runs.

Laboratories performing FISH achieved a significantly higher consensus rate for the interpretation of HER2 amplification status compared to laboratories performing BRISH.

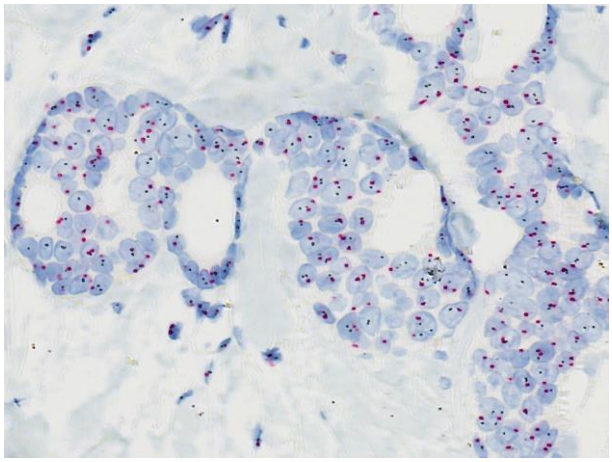


Fig. 1a (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.2*. The HER2 genes are stained black and chr17 red. The signals are distinctively demonstrated. NordiQC and the vast majority of participants interpreted this tumour as non-amplified.

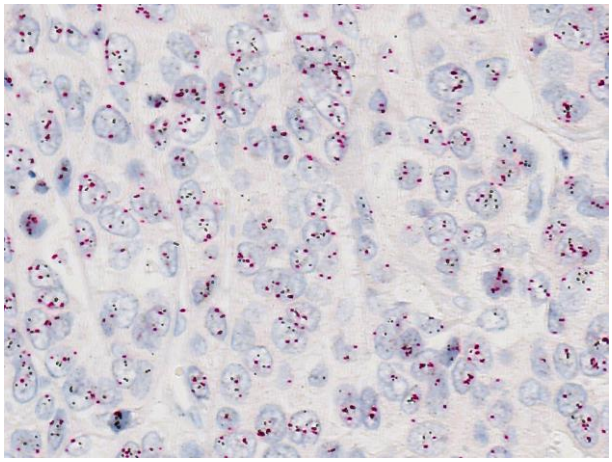


Fig. 1b (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification: HER2/chr17 ratio > 1.0-1.2*. The HER2 genes are stained black and chr17 red. Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER2 gene copies are identified. NordiQC and the majority of participants interpreted this tumour as non-amplified or equivocal.

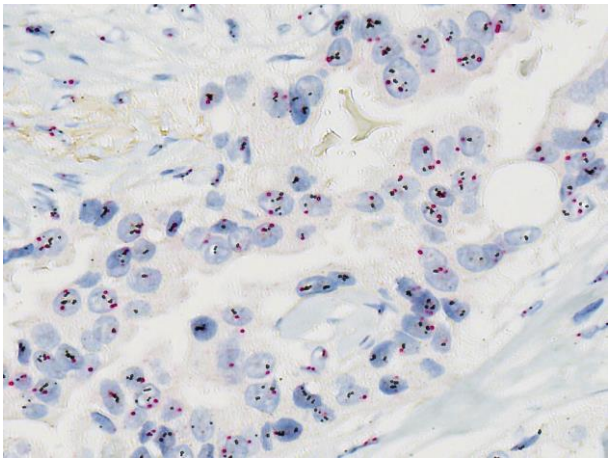


Fig. 2a (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 2.8-3.3*. The HER2 genes are stained black and chr17 red. The HER2 signals are distinctively demonstrated. NordiQC and the vast majority of participants interpreted this tumour as amplified.

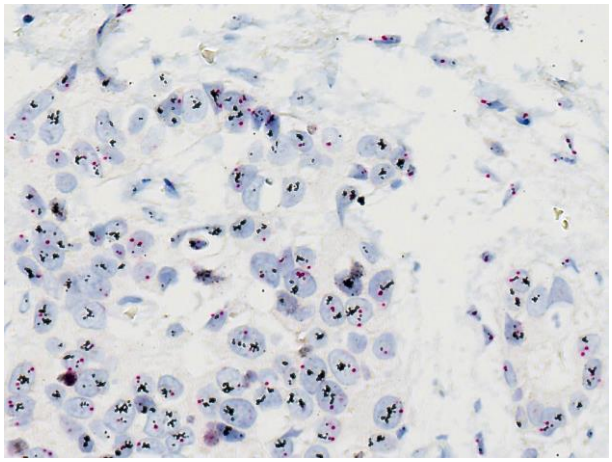


Fig. 2b (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 5 with high level HER2 gene amplification: HER2/chr17 ratio > 6.5-8.5*. The HER2 genes are stained black and chr17 red. The signals are distinctively demonstrated, and many HER2 signals are located in large clusters. NordiQC and all but one participant interpreted this tumour as positive, highly amplified.

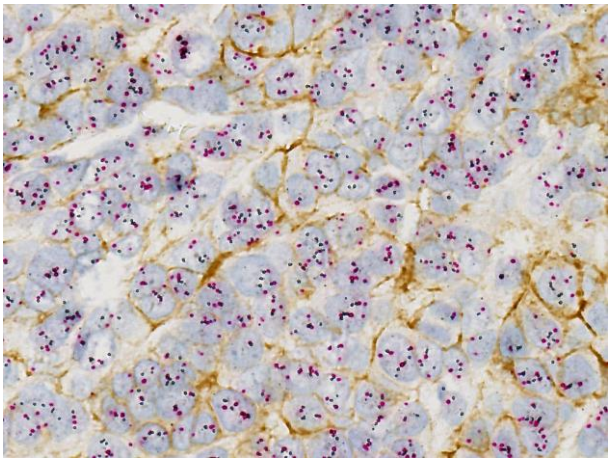


Fig. 3a (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification: HER2/chr17 ratio 1.0-1.2*. The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown. The IHC level is interpreted as 2+ and the GPA assay visualizes IHC hot-spots to evaluate the HER2 gene status precisely. Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER2 gene copies are identified. The participant interpreted this tumour as non-amplified. NordiQC and the majority of participants interpreted this tumour as non-amplified or equivocal.

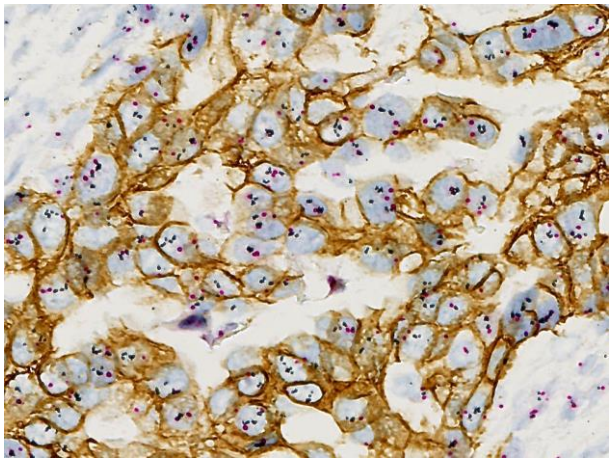


Fig. 3b (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio > 2.8-3.3*. The gene protein assay (GPA) l
0abels the HER2 genes black, chr17 red and HER2 protein brown. The IHC level is interpreted as 2+ and the GPA assay visualizes the HER2 IHC overexpression and the HER2 gene status simultaneously. The participant interpreted this tumour as positive, low level amplified. NordiQC and the majority of participants interpreted also this tumour as positive, low to moderately amplified.

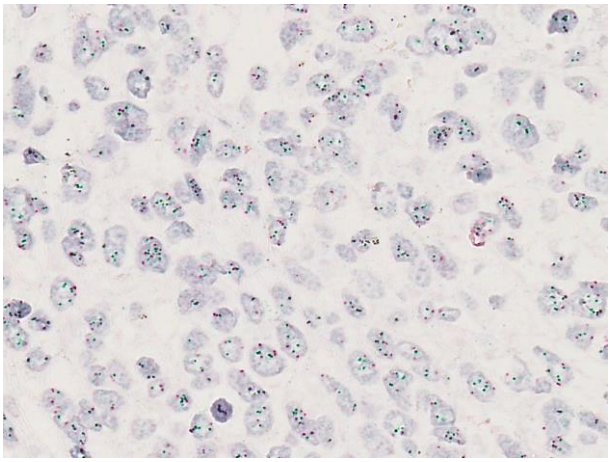


Fig. 4a (x400)
Optimal demonstration of the HER2 gene status using the ZytoDot® 2C C-3022/C-3032, ZytoVision, of the breast carcinoma no. 2 without HER2 gene amplification: HER2/chr17 ratio > 1.0-1.2*. The HER2 genes are stained green and chr17 red. HER2 and chr17 signals are distinctively demonstrated. Many cells show polysomia and in areas a level of ≥ 4 but less < 6 HER2 gene copies are identified. NordiQC and the majority of participants interpreted this tumour as non-amplified or equivocal. Compare with Figs. 1b and 3a – same tumour.

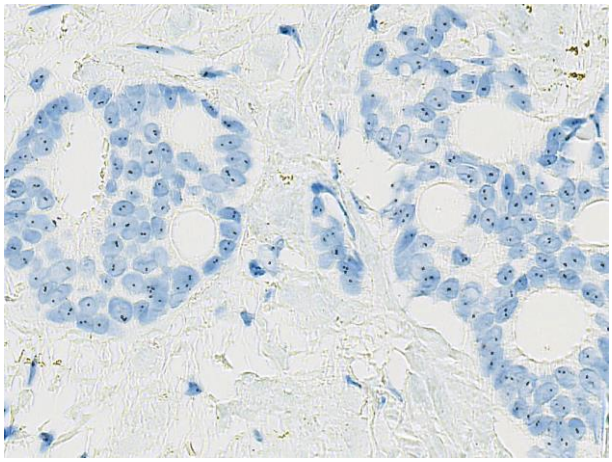


Fig. 4b (x400)
Optimal demonstration of the HER2 gene status using the INFORM™ SISH system 780-4332, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.2*. The HER2 genes are stained black and signals are distinctively demonstrated. The participant interpreted this tumour as non-amplified. NordiQC and the vast majority of participants interpreted also this tumour as non-amplified. Compare with Fig. 1a – same tumour.

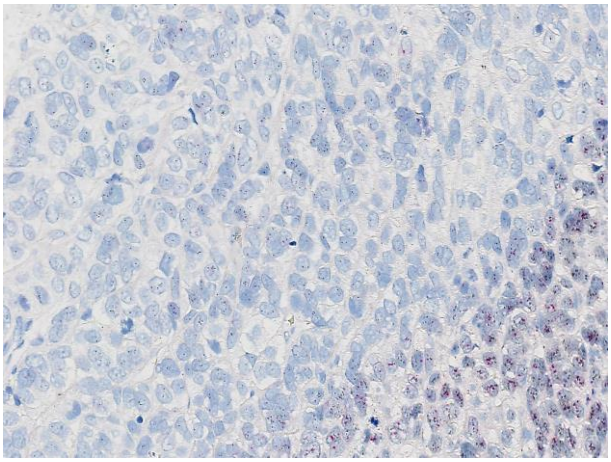


Fig. 5a (x200)
Insufficient staining for the HER2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 2 without gene amplification: HER2/chr17 ratio > 1.0-1.2*. Large areas (> 25% of the neoplastic cells) of core no. 2 are totally negative. This aberrant staining reaction / "negative spot artefact" was most likely caused by a technical problem during the staining process in the BenchMark instrument. Similar protocol settings were applied as used in Fig. 1, Fig. 2 and Fig. 5b.

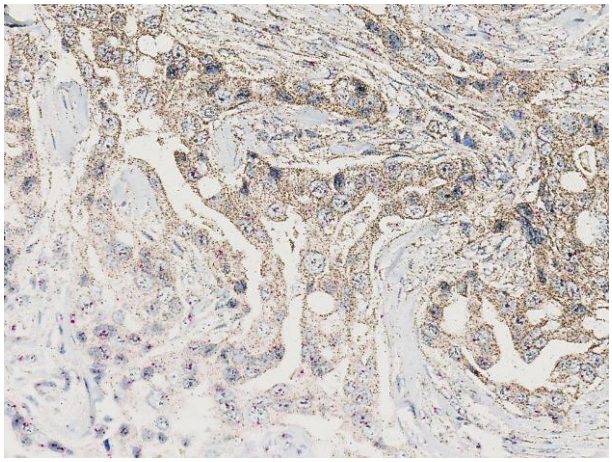


Fig. 5b (x200)
Insufficient staining for the HER2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 2.8-3.3*. The HER2 genes are stained black and chr17 red. Silver precipitates are seen in large areas (> 25% of the neoplastic cells. This aberrant staining reaction was most likely caused by a technical problem during the staining process in the BenchMark instrument. Compare with Fig. 2a. Similar protocol settings were applied as used in Fig. 1, Fig 2 and Fig 5a.

* HER2 FISH, Zytovision (range of data from one reference lab.)

ON/LE/MV/RR 10.12.18