Assessment Run H17 2020

HER2 (BRISH or FISH)

(Updated 29.04.2020 with Table 4, Interpretation and ISH assays)

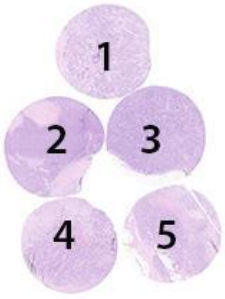
Purpose

The primary focus of this assessment is evaluation of the technical performance of HER2 Brightfield in-situ hybridization (BRISH) tests performed by the NordiQC participants for demonstration and establishment of the HER2 gene amplification level in breast carcinomas. In addition, the participants are asked to interpret and score the amplification status in the breast carcinomas and submit these to NordiQC in order to evaluate the inter-observer variability. The evaluation of inter-observer concordance is applicable for participants using either BRISH based tests or Fluorescent in-situ hybridisation (FISH) based tests. The obtained assessment marks in NordiQC is indicative of the performance of the tests but due to the limited number and composition of samples, internal validation and extended quality control, e.g. regularly measuring the HER2 results, is necessary.

Material

Table 1. **Content of the multi-block used for the NordiQC HER2 ISH assessment, run H17**

	HER2 IHC*	**Dual - SISH****	**FISH*****	**FISH*****
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	1+	1.6	1.3	<4
2. Breast carcinoma	0	0.8	0.8	<4
3. Breast carcinoma	3+	4.7	4.0	>6
4. Breast carcinoma	2+	4.0	3.0	>6
5. Breast carcinoma	2+	1.8	1.7	<4



* 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), 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 2013/2018 guidelines for tissue preparation of breast tissue for HER2 ISH analysis.

HER2 BRISH, Technical assessment

The NordiQC assessors evaluate the technical quality of the BRISH tests and at this point do not conduct a precise estimation of the HER2 amplification status. The main criteria for the technical evaluation are as listed below.

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.

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

The ASCO/CAP 2018 guidelines were applied for the interpretation of the HER2 status:

Amplified: HER2/chr17 ratio ≥ 2.0 using a dual probe assay with an average ≥ 4 HER2 copies per cell/nucleus. Using a single probe assay an average of ≥ 6 HER2 copies per cell/nucleus. (Group 1)

Equivocal (Additional work-up required):

HER2/chr17 ratio of ≥ 2.0 using a dual probe assay with an average of < 4 HER2 gene copies per cell/nucleus (Group 2)

HER2/chr17 ratio of < 2.0 using a dual probe assay with an average of ≥ 6 HER2 gene copies per cell/nucleus (Group 3)

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) (Group 4)

Unamplified: HER2/chr17 ratio < 2.0 using a dual probe assay with an average < 4 HER2 gene copies per cell/nucleus (both dual and single probe assay) (Group 5)

Participation

Number of laboratories registered for HER2 BRISH	150
Number of laboratories returning slides	133 (89%)
Number of laboratories returning scoring sheet	120 (90%)
Number of laboratories registered for HER2 FISH	68
Number of laboratories returning scoring sheet	62 (91%)

Results BRISH, technical assessment
In total, 133 laboratories participated in this assessment. 100 laboratories (75%) 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 H17.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **800-4422/780-4422**	32	Ventana/Roche	7	14	8	3	66%	22%
INFORM™ HER2 Dual ISH + IHC **800-4422 + HER2 IHC**	18	Ventana/Roche	10	6	2	0	89%	56%
VENTANA HER2 Dual ISH **800-6043**	73	Ventana/Roche	38	20	9	6	80%	52%
Zyto*Dot*® 2C **C-3022 / C-3032**	5	ZytoVision	1	3	0	1	80%	20%
One colour HER2 systems								
INFORM™ HER2 SISH **780-4332**	2	Ventana/Roche	1	0	0	1	-	-
Zyto*Dot*® **C-3003**	3	ZytoVision	0	0	2	1	-	-
Total	133		57	43	21	12	100	-
Proportion			43%	32%	16%	9%	75%	

1) Proportion of Sufficient Results (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Comments
In this assessment, an optimal technical performance for the demonstration of HER2 / Chr17 signals permitting an adequate evaluation of the HER2 gene amplification status in the five breast carcinomas included in the multi-tissue block could be obtained by all the applied dual-colour BRISH systems as shown in Table 2.

The vast majority of participants used BRISH systems from Ventana/Roche. 55% (73 of 133) used the newly launched VENTANA HER2 Dual ISH DNA Probe Cocktail, 800-6043, whereas 38% (50 of 133) used the INFORM™ HER2 Dual ISH assay, 800-4422/780-4422.
The insufficient results were typically characterized by excessive background staining including silver precipitates, impaired morphology, generally weak or missing signals or large negative areas in one or more of the breast carcinoma samples. In this run, and in concordance with the previous NordiQC runs, the ISH rejection criteria defined in the 2013/2018 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 thus rated as insufficient (poor or borderline). 48% (16 of 33) of the insufficient results were characterized by large negative areas covering more than 25% of one or more of the breast carcinomas, 30% (10 of 33) were caused by impaired morphology and in 24% (8 of 33) excessive background / silver precipitates were observed – some insufficient results showed different artefacts at the same time. Minor focal staining artefacts were accepted if they did not compromise the overall interpretation in each of the five individual tissue cores.
The VENTANA HER2 Dual ISH DNA Probe Cocktail, 800-6043 was found to be more successful compared to the INFORM™ HER2 Dual ISH assay, 800-4422/780-4422, both from Roche/Ventana as seen in Table 2, which was also seen in the previous NordiQC run H17. The newly launched assay thus provided both a general higher pass rate of 80% and also an increased proportion of optimal results of 52% compared to 66% and 22%, respectively, for the "classic" INFORM™ Dual ISH system 800-4422.
In this assessment, an increased pass rate especially compared to the latest run H16, but also to the recent runs was seen, as shown in Graph 1. One of the contributing factors seems to be related to the expanded use of the VENTANA HER2 Dual ISH DNA Probe Cocktail, 800-6043 on the expense of the less successful INFORM™ HER2 Dual ISH assay, 800-4422/780-4422. In this context, it has to be emphasized that the INFORM™ HER2 Dual ISH assay, 800-4422/780-4422, used in combination with IHC for HER2 (PATHWAY®) showed a superior performance and pass rates similar to the newly launched ISH assay.

Optimal protocol settings: Two-colour HER2 systems

73 laboratories used the **Ventana Dual ISH system 800-6043** (Ventana/Roche). Optimal demonstration of HER2 BRISH using this assay was typically based on a 2-step Heat Induced Epitope Retrieval (HIER) procedure using Cell Conditioning 1 (CC1) for 16 min. at 84°C followed by Cell Conditioning 2 (CC2) for 24 min. at 82°C and subsequent proteolysis in ISH Protease 3 or Protease 3 for 16-20 min. at 36-37°C. The HER2 and chr17 probe cocktail being applied for 60 min. at 44°C following a denaturation step at 80°C for 8 min. – both steps and parameters are fixed by the vendor.
The NordiQC homepage for protocol submission at present does not precisely support data entry for a combined 2-step HIER procedure, which complicates the subsequent data analysis to elucidate on the protocol robustness and pass rates using the range of "optimal protocol settings" listed by the participants. This feature is expected to be improved for the next HER2 ISH assessment, run H18. Overall, a pass rate of 80% was obtained, 52% being optimal.

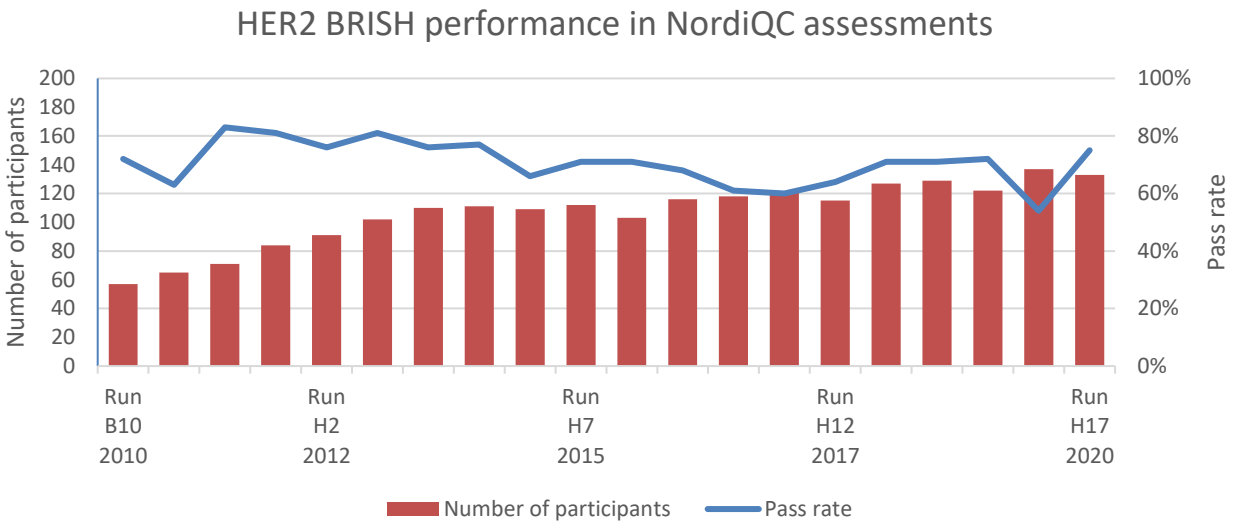
32 laboratories used the **INFORM™ Dual ISH system 800-4422** (Ventana/Roche). Optimal demonstration of HER2 BRISH was typically based on HIER in CC2 for 20-64 min. or CC1 for 16 min. at 84-90°C and subsequent proteolysis in ISH Protease 3 or Protease 3 for 16-20 min. at 36-37°C. The HER2 and chr17 probe 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) were seen in 76% of the submitted protocols (16 of 21).

18 laboratories used the **INFORM™ Dual ISH system 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 CC1 or CC2 for 24-32 min. at 90-95°C and subsequent proteolysis in ISH Protease 2 or 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 min. HER2 PATHWAY® was typically performed with iVIEW or UltraView as detection system. Using these protocol settings, sufficient results were seen in 87% of the submitted protocols (13 of 15).

For the **Zyto*Dot*® 2C system C-3022 / C-3032** (ZytoVision), one protocol gave an optimal result. This protocol was based on HIER in EDTA pH 8 for 15 min. at 95°C, proteolysis in pepsin for 4 min. at 20°C, hybridization at 37°C for 20 hours following a denaturation at 75°C for 5 min. and visualization with the ZytoVision detection kit C-3022. Using these or similar protocol settings, sufficient results were seen in 75% of the submitted protocols (3 of 4).

Performance history
This was the twenty-third assessment of HER2 BRISH in NordiQC and an improved pass rate was observed compared to the latest runs. Overall data and pass rates from the last twenty runs are shown in Graph 1.

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



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 carcinoma	1.3	<4	Non-amplified
2. Breast carcinoma	0.8	<4	Non-amplified
3. Breast carcinoma	4.0	>6	Amplified
4. Breast carcinoma	3.0	>6	Amplified
5. Breast carcinoma	1.7	<4	Non-amplified

* data from one NordiQC reference laboratory.

185 of the 201 (92%) participating laboratories completed scoring sheets on the NordiQC homepage. These evaluations were compared to the HER2 ISH amplification status obtained by the NordiQC reference laboratories, summarized in Graph 2 and 3. For the laboratories performing FISH, the consensus rate was 58% (36 of 62) and 49% (60 of 123) for laboratories using BRISH. This was a significant decrease for both laboratories that used FISH and BRISH compared to the last run where the consensus rate was 85% and 75%, respectively.

In general, for both BRISH and FISH, high consensus rates were observed between participants and NordiQC regarding the HER2 amplification status except for tissue core no. 5 which was challenging for many laboratories. See graphs 2 and 3.

For BRISH and FISH, disagreement of the interpretation of the HER2 amplification status between the participants and NordiQC data was related to "overrating" the HER2 status and thus an aberrant classification compared to the NordiQC reference data and the majority of other participants.

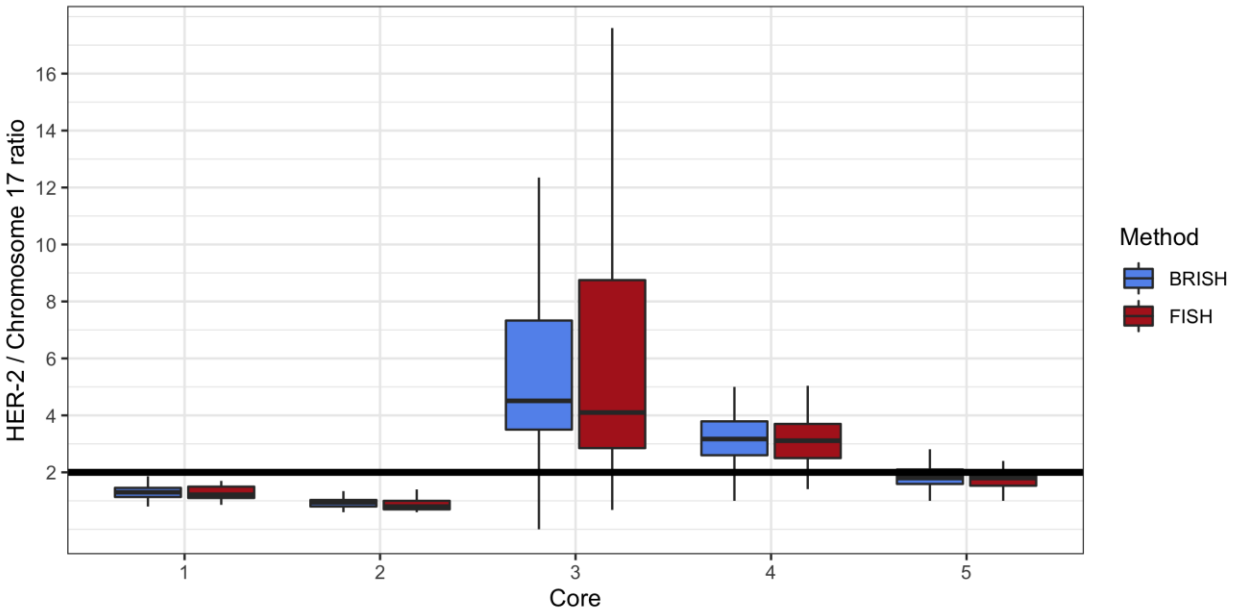
Tumour no. 5 was by the NordiQC reference laboratories characterized as non-amplified. The tumour showed HER2 ratio of 1.7-1.8 and < 4 HER2 gene copies were identified. This tumour was, by some laboratories using either FISH or BRISH classified as amplified (n=37), equivocal (n=38) or indeterminable (n=5). The tumour did have dispersed cells with ≥ 4 and < 6 HER2 gene copies, but was by the majority of laboratories and the two NordiQC reference laboratories interpreted as non-amplified – see Fig. 1b.

Similar to last assessment, participants using FISH had in HER2 ISH run H17 a marginally higher level of consensus in the individual cores than participants using BRISH.

It was observed that the consensus rates of the individual cores among laboratories that produced staining reaction assessed as technically sufficient (BRISH only) were higher than laboratories with an insufficient mark (55% and 31%, 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 2013/2018 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.

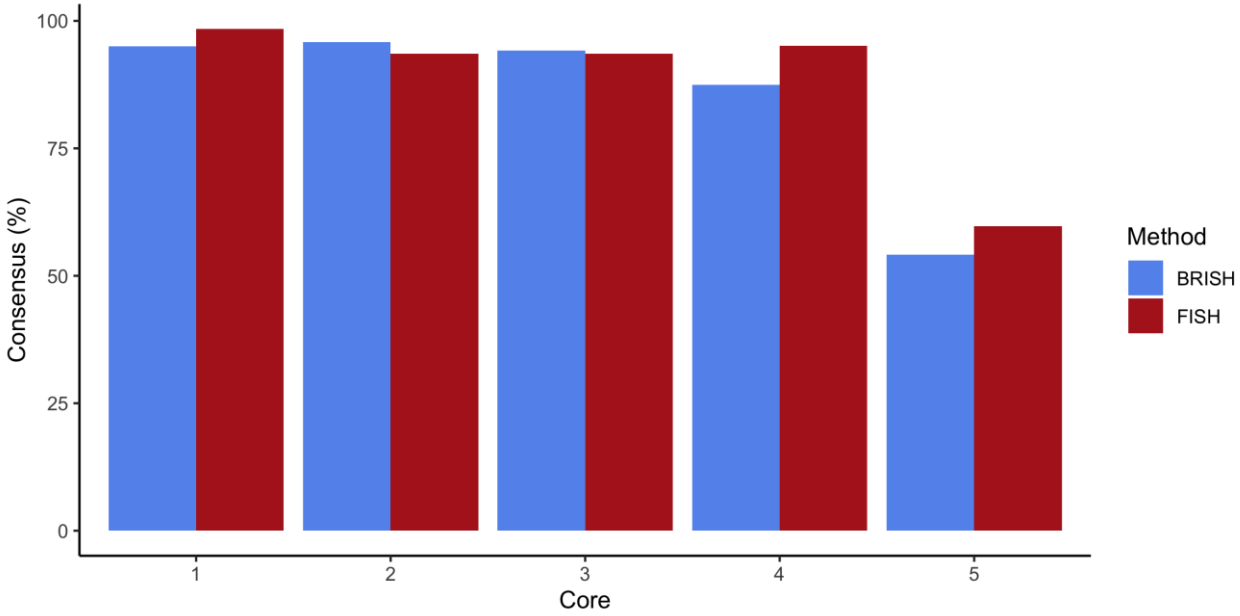
Participants overall interpretation of amplification ratios and consensus rates are shown in Graph 2 and 3.

Graph 2



NordiQC HER2 ISH run H17: Participant interpretation of amplification status

Graph 3



NordiQC HER2 ISH run H17: Consensus between participants and NordiQC

No technical evaluation of FISH protocols was performed. Table 4 shows the ISH assays used by the participants and concordance level to the NordiQC data observed. 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. **ISH assays used and level of consensus HER2 status to NordiQC reference data, H17**

BRISH	n	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **800-4422/780-4422**	27*	Ventana/Roche	14	13	52%
INFORM™ HER2 Dual ISH + IHC **800-4422 + HER2 IHC**	15*	Ventana/Roche	11	4	73%
VENTANA HER2 Dual ISH **800-6043**	72*	Ventana/Roche	32	40	44%
INFORM™ HER2 SISH **780-4332**	2	Ventana/Roche	1	1	
Zyto*Dot*® 2C **C-3022 / C-3032**	4*	ZytoVision	2	2	-
Zyto*Dot*® **C-3003**	3	ZytoVision	0	3	-
FISH					
Pathvysion HER-2 DNA **6N4630 / 30-161060**	14	Abbott	7	7	50%
HER2 IQFISH **GM333**	4	Dako/Agilent	3	1	-
HER2 IQFISH **K5731**	14	Dako/Agilent	8	6	57%
ERBB2/CCP17 FISH probe kit **CT-PAC001**	1	Cyto Test Inc	1	0	-
BOND HER2 FISH system **TA9217**	4	Leica	2	2	-
ERBB2 (17q12) / SE17 **KBI-14701**	1	Leica	0	1	-
HER2/CEN17 FISH probe **MF2001**	2	Maixin	2	0	-
FISH Kit **MAD-FISH-PTK + CT-PA**	1	Master Diagnostica	1	0	-
Rembrandt Her-2-C17 probe **C801P.5206**	2	PanPath	0	2	-
ZytoLight **Z-2015 / Z-2020/ Z-2077**	14	ZytoVision	11	3	79%
ZytoMation ERBB2/CEN17 Dual Color FISH Probe **Z-2292**	4	ZytoVision	0	4	-
F*lex*ISH® ERBB2/CEN 17 Dual Color Probe **Z-2166**	1	ZytoVision	1	0	-
Total	185		96	89	
Proportion			52%	48%	52%

*The number varies from Table 1. Not all participants have submitted a scoring sheet.

Conclusion

In this assessment and in concordance with previous NordiQC HER2 ISH runs, technical optimal demonstration of HER2 BRISH could be obtained by all the commercially available two-colour HER2 systems used by the participants being **INFORM™ HER2 Dual ISH 800-4422** (Ventana/Roche), **Ventana HER2 Dual ISH 800-6043** (Ventana/Roche) and **ZytoDot® 2C** (ZytoVision).
The combined **INFORM™ HER2 Dual ISH** assay with **HER2 IHC PATHWAY®** (Ventana/Roche) and the newly released **Ventana HER2 Dual ISH 800-6043** assay (Ventana/Roche) were most successful with pass rates of 89% and 80%, respectively.
For all systems, retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient demonstration of HER2 (and chr17 signals) and preserve morphology.

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

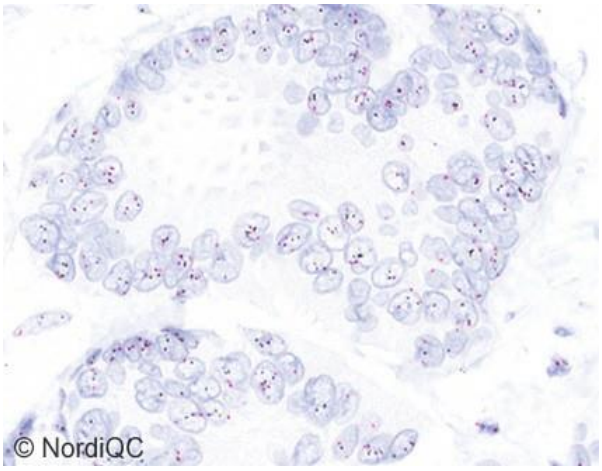


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 1 without HER2 gene amplification: HER2/chr17 ratio > 1.3-1.6*. The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

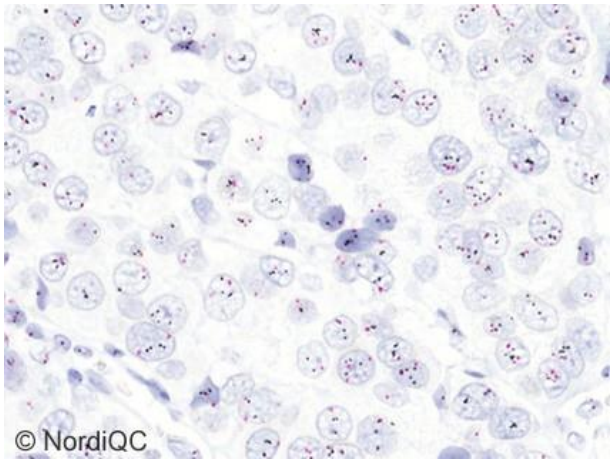


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification: HER2/chr17 ratio 1.7-1.8*. The HER2 genes are stained black and chr17 red. The signals are distinctively demonstrated.
The participant interpreted this tumour as non-amplified.
NordiQC and the majority of participants interpreted this tumour as non-amplified, but it was by 21% and 20% interpreted as equivocal or amplified, respectively.

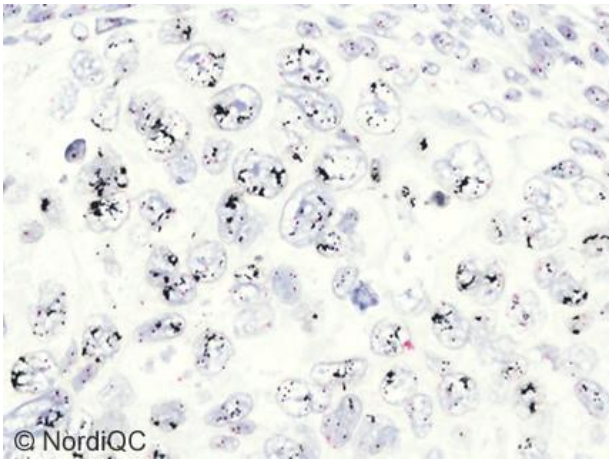


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification: HER2/chr17 ratio 4.0-4.7*. The HER2 genes are stained black and chr17 red. The HER2 signals are distinctively demonstrated, and the majority of HER2 signals are located in large clusters.
NordiQC and virtually all participants interpreted this tumour as amplified.

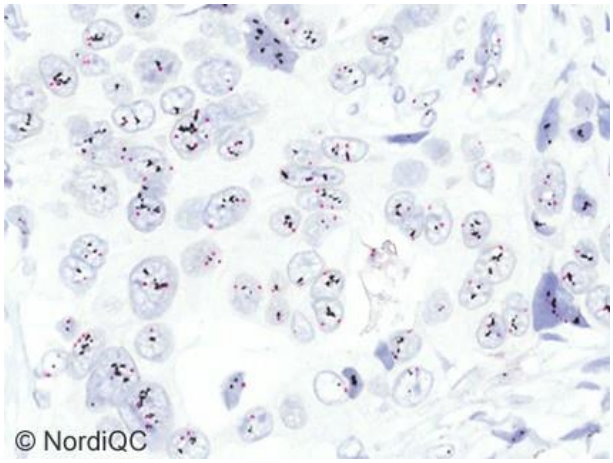


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 3.0-4.0*. The HER2 genes are stained black and chr17 red. The signals are distinctively demonstrated.
NordiQC and virtually all participants interpreted this tumour as amplified.

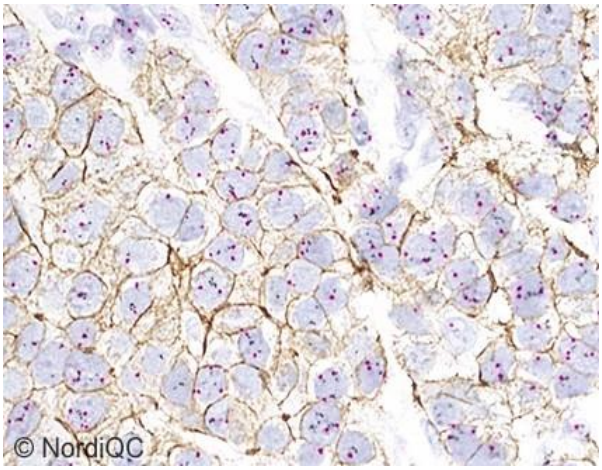


Fig. 3a
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification: HER2/chr17 ratio 1.7-1.8*. The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown. The IHC level is interpreted as 2+ and the GPA assay visualizes IHC hotspots to evaluate the HER2 gene status precisely. The participant interpreted this tumour as non-amplified. NordiQC and the majority of participants interpreted this tumour as non-amplified, but it was by 21% and 20% interpreted as equivocal or amplified, respectively.

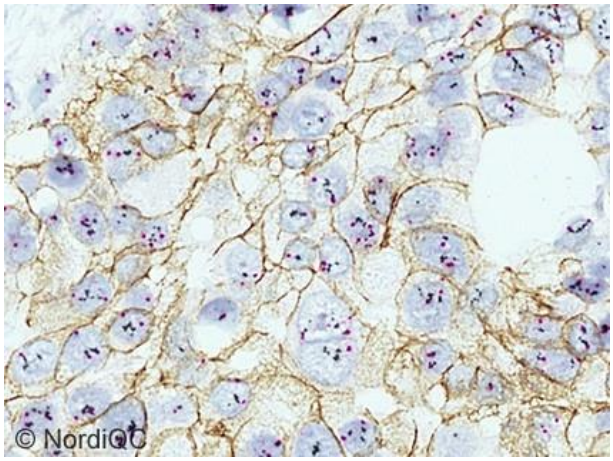


Fig. 3b
Optimal demonstration of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 3.0-4.0 *. The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown. The IHC level is interpreted as 2+ and the GPA assay visualizes the HER2 protein expression and the HER2 gene status simultaneously.
The participant interpreted this tumour as amplified. NordiQC and virtually all participants interpreted this tumour as amplified.

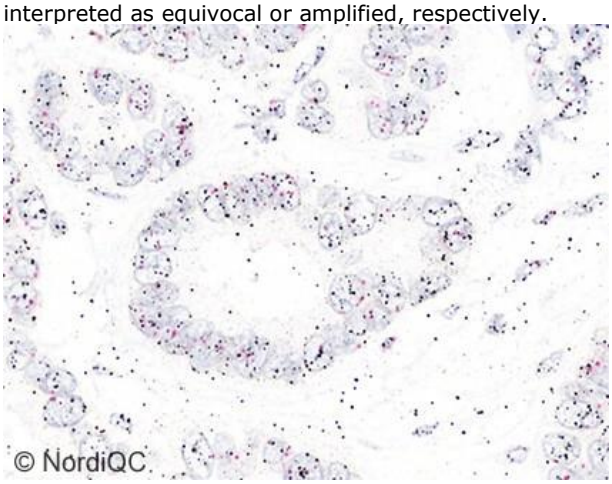


Fig. 4a
Insufficient staining of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 1 without HER2 gene amplification: HER2/chr17 ratio > 1.3-1.6*. HER2 genes are stained black, chr17 red.
Silver precipitates are seen in large areas (> 25% of areas with neoplastic cells) and interpretation is compromised. The excessive and aberrant precipitation was most likely caused by a technical problem during the staining process in the BenchMark instrument.
Vendor recommended protocol settings were applied.
Compare with Fig. 1a. – same tumour.

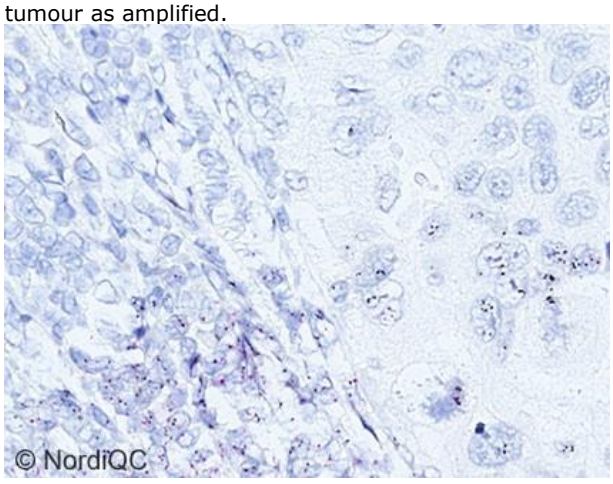


Fig. 4b
Insufficient staining of the HER2 gene using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification: HER2/chr17 ratio > 4.0-4.7*. HER2 genes are stained black, chr17 red. Large areas (> 25% of areas with neoplastic cells) are totally negative. This aberrant staining reaction / "negative spot artefact" was most likely caused by a technical problem during the staining process in the BenchMark instrument.
Vendor recommended protocol settings were applied.
Compare with Fig. 2a. – same tumour.

* Range of data from two NordiQC reference laboratories.

SN/LE/HLK/RR 29.04.2020