Assessment Run H19 2021

HER2 (BRISH or FISH)

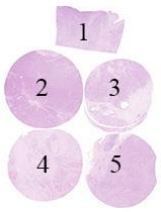
Purpose

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 H19**

	HER2 IHC*	**Dual - BRISH****	**Dual-BRISH****	**FISH*****	**FISH*****
	IHC score	**HER2/chr17 ratio¤**	**HER2 copies**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	2+	1.2	<4	1.4	<4
2. Breast carcinoma	0	0.6	<4	0.6	<4
3. Breast carcinoma	2+	1.1	<4	1.0	<4
4. Breast carcinoma	3+	2.5	≥4 and <6	2.3	≥4 and <6
5. Breast carcinoma	3+	3.2	>6	4.3	>6



* PATHWAY® (Ventana/Roche), data from two reference labs.
** Ventana HER2 Dual ISH DNA Probe Cocktail, 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 24-72 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 and BRISH data from reference laboratories to analyze scoring consensus.

Consensus scores from the NordiQC BRISH/FISH reference laboratories

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

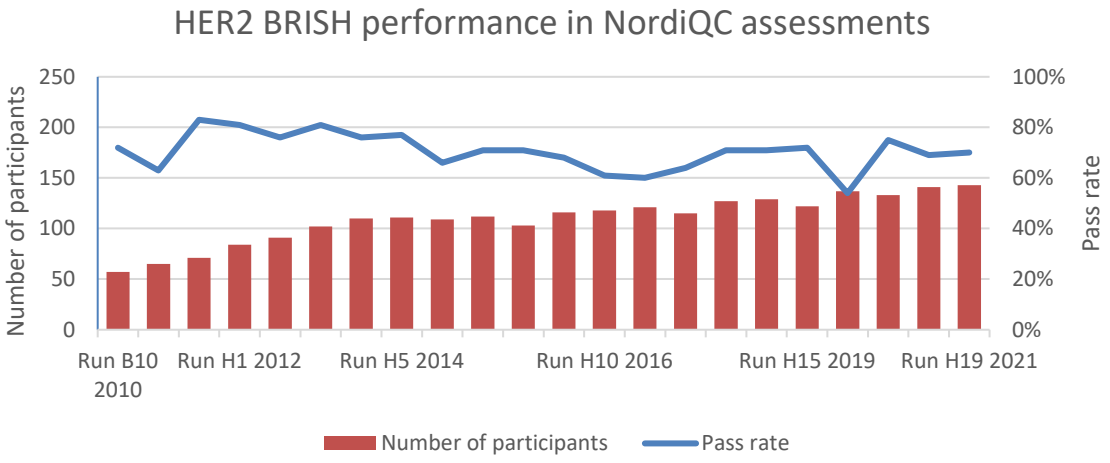
Participation

Number of laboratories registered for HER2 BRISH	156
Number of laboratories returning slides	143 (92%)
Number of laboratories returning scoring sheet	126 (88%)
Number of laboratories registered for HER2 FISH	68
Number of laboratories returning scoring sheet	65 (96%)

The number of laboratories returning slides has decreased in this run H19 compared to previous assessments, due to the Covid-19 pandemic and associated postal delays. All slides returned after the assessment were assessed, and received advice if the result being insufficient, but data is not included in this report.

Performance history
This was the twenty-fifth assessment of HER2 BRISH in NordiQC and a relatively consistent pass rate around 70% has been observed in the last seven assessments (except for run H16). Overall data and pass rates from the latest runs are shown in Graph 1.

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



Results BRISH, technical assessment
In total, 143 laboratories participated in this assessment. 100 laboratories (70%) 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 H19.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **800-4422/780-4422**	22	Ventana/Roche	8	6	6	2	64%	36%
INFORM™ HER2 Dual ISH + IHC **800-4422 + HER2 IHC (GPA)***	7	Ventana/Roche	4	0	2	1	57%	57%
VENTANA HER2 Dual ISH **800-6043**	94	Ventana/Roche	43	27	19	5	75%	46%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)***	13	Ventana/Roche	4	4	4	1	62%	31%
Zyto*Dot*® 2C **C-3022 / C-3032**	4	ZytoVision	0	3	1	0	-	-
One colour HER2 systems								
Zyto*Dot*® **C-3003**	3	ZytoVision	0	1	1	1	-	-
Total	143		59	41	33	10	100	-
Proportion			41%	29%	23%	7%	70%	

1) Proportion of Sufficient Results (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
* GPA; Gene Protein Assay (HER2 BRISH + PATHWAY HER2 IHC).

Comments
In this run and concordance with the latest assessments, the vast majority of participants (95%) used BRISH HER2 systems from Ventana/Roche. 75% (107 of 143) used the newly launched VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) and mainly on the expense of the INFORM™ HER2 Dual ISH assay (800-4422/780-4422) being used by 20% of the participants (29 of 143). A slightly increased proportion of participants (15%) used the Ventana/Roche BRISH HER2 systems in combination with HER2 IHC providing a Gene Protein Assay (GPA). In the evaluation of the technical assessment, only the HER2 BRISH results were addressed.

In contrast to most previous assessments a technically optimal 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 was only obtained by the two Ventana/Roche dual-colour BRISH systems as shown in Table 2.
The insufficient results were most frequently characterized by large negative areas in one or more of the breast carcinoma samples, but also caused by silver precipitates, impaired morphology, generally weak or missing signals for either HER2 and/or chr17.
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). 67% (29 of 43) of the insufficient results were characterized by large negative areas covering more than 25% of one or more of the breast carcinomas. In 12% of the insufficient results (5 of 44) missing signals were observed and in the remaining 21% (9 of 43) different artefacts as impaired morphology, silver precipitates and negative areas were seen 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), has as described above gained popularity and was found to be more successful and provided both a higher pass rate and proportion of optimal results compared to the INFORM™ HER2 Dual ISH assay (800-4422/780-4422), as shown in Table 2. In contrast to most previous assessments, it was observed that inclusion of HER2 IHC and hereby application of a HER2 GPA was less successful compared to a traditional HER2 BRISH system. This was observed for both the INFORM™ Dual ISH system (800-4422) and the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043).

The breast carcinoma, tissue core no 1, was found to be technically more challenging compared to the four other samples included in the TMA used for this run. In general, the signals demonstrated were slightly reduced in size and in areas being less distinct and in addition accompanied by an impaired nuclear

morphology. Due to these observations, no result was downgraded despite not fulfilling acceptance criteria as described by ASCO/CAP. The sample was handled and processed accordingly to guidelines from ASCO/CAP but fixed for 72 hours in 10% NBF being maximum time recommended, whereas the four other samples were fixed for 24-48 hours.

Optimal protocol settings: Two-colour HER2 systems
94 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche).
Optimal demonstration of HER2 BRISH using this assay was typically based on the vendor recommended protocol settings based on a 2-step Heat Induced Epitope Retrieval (HIER) procedure using Cell Conditioning 1 (CC1) at 84°C followed by Cell Conditioning 2 (CC2) at 82°C and subsequent proteolysis in ISH Protease 3 or Protease 3 for 12-20 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.
Among the laboratories reporting these protocol settings a pass rate of 74% (43 of 58) was obtained, 48% optimal. Protocols submitted indicating single HIER in either CC1 or CC2 was excluded. These data entry most likely were incorrect, as the assay is locked and based on the combined HIER in CC1 and CC2. Same pass rates and proportion of optimal results were observed for protocols registered and based on either the combined or single HIER method.

13 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY®** (Ventana/Roche). Optimal demonstration of HER2 BRISH using this assay was typically based on the 2-step HIER procedure in CC1 followed by CC2 and subsequent proteolysis in ISH Protease 3 for 20 min. at 36°C. 3 of 3 laboratories reporting these protocol settings all obtained an optimal result

22 laboratories used the **INFORM™ Dual ISH system 800-4422** (Ventana/Roche).
Optimal demonstration of HER2 BRISH was typically based on HIER in CC2 for 24-28 min. or CC1 for 16-28 min. at 74-90°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 50% of the submitted protocols (5 of 10).

7 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 CC2 for 32 min. at 90°C and subsequent proteolysis in ISH Protease 2 or Protease 2 for 4-16 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. Using these protocol settings, sufficient results were seen in 60% of the submitted protocols (3 of 5).

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	1.4	<4	Non-amplified
2. Breast carcinoma	0.6	<4	Non-amplified
3. Breast carcinoma	1.0	<4	Non-amplified
4. Breast carcinoma	2.3	≥4 and <6	Amplified
5. Breast carcinoma	4.3	>6	Amplified

* data from one NordiQC reference laboratory.

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, H19***

BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **800-4422/780-4422**	20	Ventana/Roche	15	5	75%
INFORM™ HER2 Dual ISH + IHC **800-4422 + HER2 IHC (GPA)**	6	Ventana/Roche	6	0	100%
VENTANA HER2 Dual ISH **800-6043**	82	Ventana/Roche	76	6	93%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	12	Ventana/Roche	10	2	83%
Zyto*Dot*® 2C **C-3022 / C-3032**	3	ZytoVision	3	0	-
Zyto*Dot*® **C-3003**	3	ZytoVision	2	1	-
FISH					
Pathvysion HER-2 DNA **6N4630 / 30-161060**	16	Abbott	15	1	94%
HER2 IQFISH **GM333**	5	Dako/Agilent	5	0	100%
HER2 IQFISH **K5731**	14	Dako/Agilent	13	1	83%
BOND HER2 FISH system **TA9217**	4	Leica	4	0	-
HER2/CEN17 FISH probe **MF2001**	2	Maixin	2	0	-
FISH Kit **MAD-FISH-PTK + CT-PA / MDS**	1	Master Diagnostica	1	0	-
Rembrandt Her-2-C17 probe **C801P.5206**	2	PanPath	2	0	-
ZytoLight **Z-2015 / Z-2020/ Z-2077**	15	ZytoVision	15	0	100%
ZytoMation ERBB2/CEN17 Dual Color FISH Probe **Z-2292**	4	ZytoVision	4	0	-
ERBB2/CCP17 FISH Probe kit **CT-PAC001**	2	CytoTest	2	0	-
Total	191		175	16	
Proportion			81%	19%	

*The number varies from Table 2. Not all participants have submitted a scoring sheet.

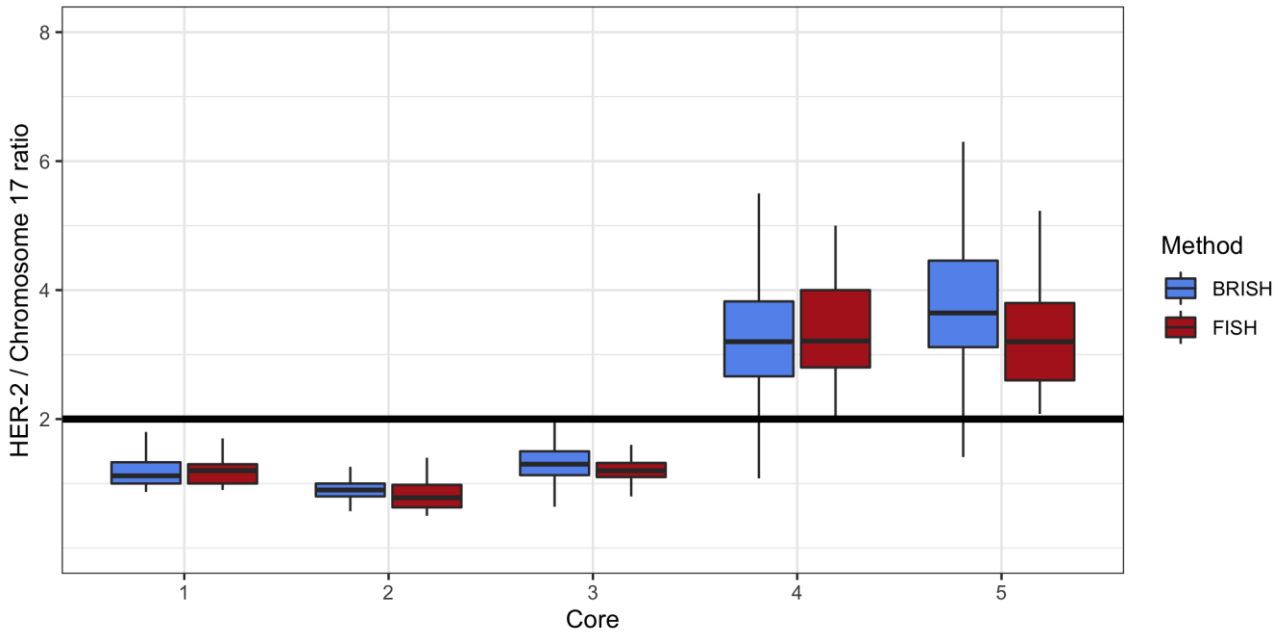
191 of the 211 (91%) participating laboratories completed scoring sheets on the NordiQC homepage. These evaluations were compared to the HER2 ISH amplification status obtained by the NordiQC reference laboratories, summarized in Graph 2 and 3. For the laboratories performing FISH, the consensus rate was 97%, and 89% for laboratories using BRISH. This was a significant increase for both laboratories that used FISH and BRISH compared to the latest runs and the highest level obtained in this HERs ISH module.

Similar to the latest assessments, participants using FISH had in the HER2 ISH run H19 a marginally higher level of consensus in the individual cores than participants using BRISH.
It was observed that the consensus rates for interpretation of the individual cores were identical for laboratories that produced a staining reaction assessed as technically sufficient (BRISH only) and laboratories with an insufficient mark (90% and 89%, respectively). Despite a result evaluated as insufficient by the NordiQC assessor group, laboratories were still able to correctly evaluate the slide. The ISH rejection criteria as outlined by the 2013/2018 ASCO/CAP HER2 guidelines and being applied by NordiQC indicate retest is required 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 HER2 expression or HER2 evaluation in specific "hot-spot areas" identified by HER2 IHC.

As mentioned above, the breast carcinoma, tissue core no. 1 was fixed in 10% NBF for 72 hours and was found to be technically more challenging to give distinct signals. This was in particular observed for laboratories performing BRISH and submitting scoring sheets as 13% (n=16) within this group were unable to reliably score this sample. In comparison, this problem or challenge was only registered for 2% of laboratories performing FISH (n=1).

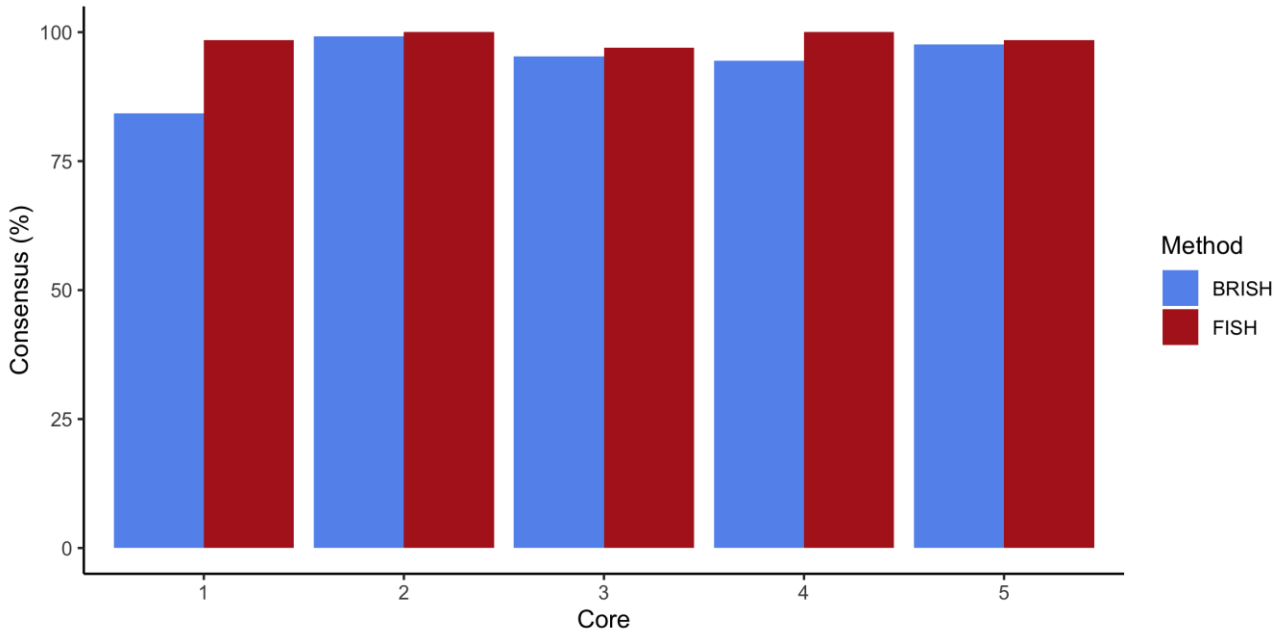
Participants overall interpretation of amplification ratios and consensus rates are shown in Graph 2 and 3.

Graph 2



NordiQC HER2 ISH run H19: Participant interpretation of amplification status

Graph 3



NordiQC HER2 ISH run H19: Consensus depending on method

Conclusion
In this assessment a technical optimal demonstration of HER2 BRISH could only be obtained by the Ventana/Roche two-colour HER2 systems **VENTANA HER2 Dual ISH 800-6043** and **INFORM™ HER2 Dual ISH 800-4422** / **780-4422**.
The recently released **VENTANA HER2 Dual ISH 800-6043** assay was most successful with an overall pass rate of 75%.
Insufficient results were mainly caused by large negative areas in one or more of the included tissue cores. In addition, also impaired morphology, excessive background and more artefacts in combination characterized insufficient results.
For all systems, retrieval settings – HIER and proteolysis - must be carefully balanced to provide sufficient demonstration of HER2 (and chr17 signals) and preserve morphology.
Despite an assay harmonization and application of best practice protocols have been accomplished, the overall pass rate is still at a moderate level.
Laboratories performing FISH achieved a slightly higher consensus rate for the interpretation of HER2 amplification status compared to laboratories performing BRISH and in addition also more successful to demonstrate and evaluate the HER2 status in all 5 tissue cores.

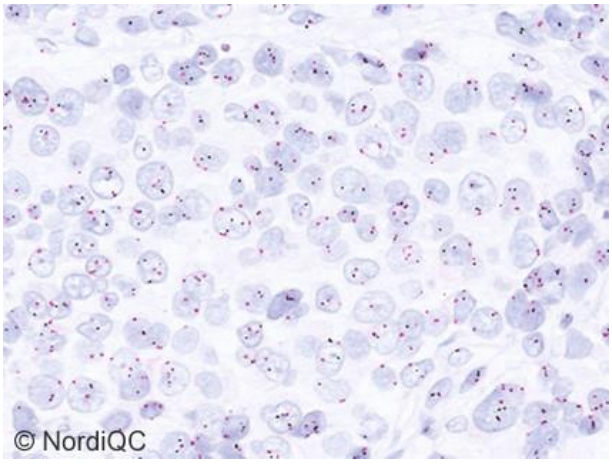


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification: HER2/chr17 ratio 0.6*.
The HER2 genes are stained black and chr17 red. The morphology is preserved, and signals distinctively demonstrated.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

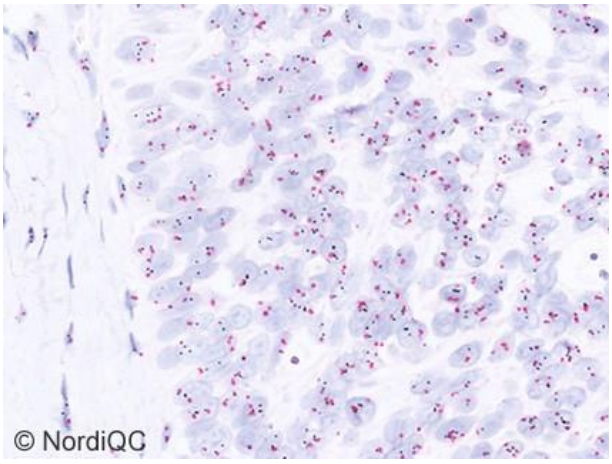


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.0 – 1.1*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in both the neoplastic and stromal cells.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

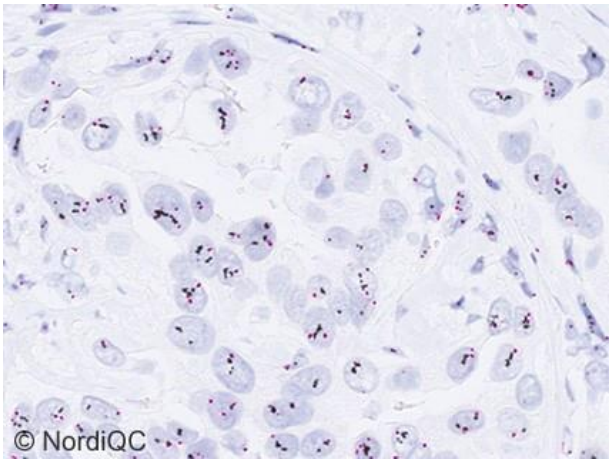


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 2.3-2.5*. The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated, and the HER2 signals are in some cells located in large clusters.
NordiQC and virtually all participants interpreted this tumour as amplified.

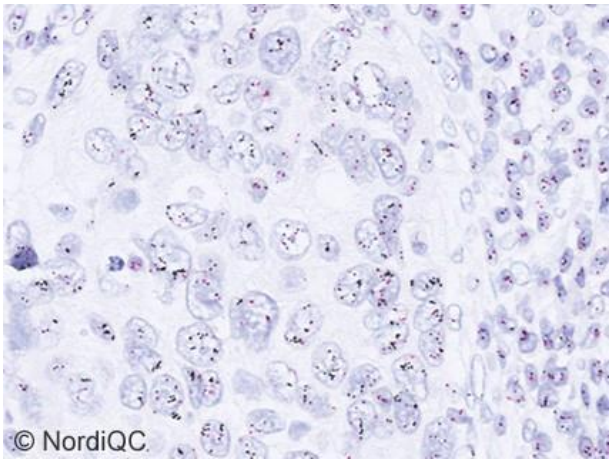


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 3.2-4.3*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in both the neoplastic and stromal cells.
NordiQC and virtually all participants interpreted this tumour as amplified.

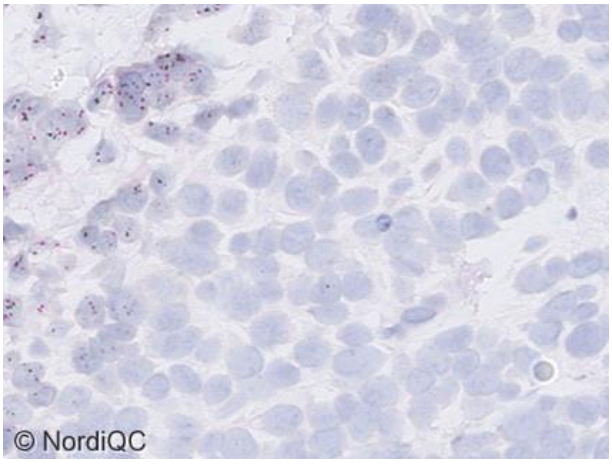


Fig. 3a
Insufficient staining result for the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.0-1.1*.
The HER2 genes are stained black, chr17 red.
Large areas (> 25% of areas with neoplastic cells) are totally negative. This aberrant staining reaction / "negative spot artefact" was most likely caused by a technical issue during the staining process in the BenchMark instrument.

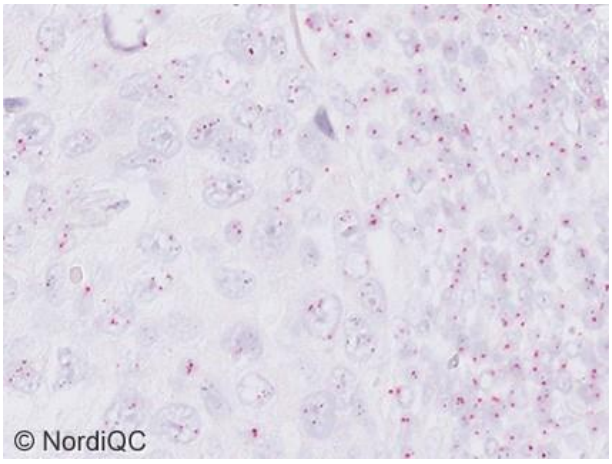


Fig. 3b
Insufficient staining result for the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 3.2-4.3*.
The HER2 genes are stained black, chr17 red.
Virtually only chr17 signals are identified and it is not possible to determine HER2 gene status. The participant interpreted this tumour as non-amplified.
NordiQC and virtually all participants interpreted this tumour as amplified.

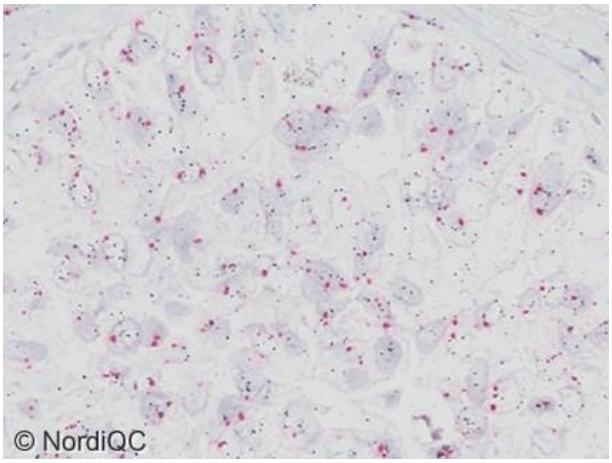


Fig. 4a
Insufficient staining of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio > 1.0-1.1*. The HER2 genes are stained black, chr17 red.
Silver precipitates outside the cells are seen in large areas (> 25% of areas with neoplastic cells) and in addition impaired nuclear morphology is seen compromising the interpretation. The excessive and aberrant precipitation was most likely caused by a technical problem during the staining process in the BenchMark instrument.

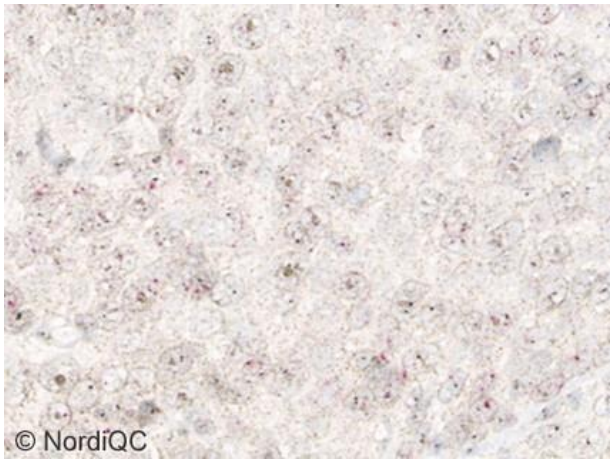


Fig. 4b
Insufficient staining of the HER2 gene status using the INFORM™ Dual ISH kit cat. no. 800-4422, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio > 1.0-1.1*. The HER2 genes are stained black, chr17 red.
An excessive background staining is seen and the diffuse brown precipitation obscures the scoring and establishment of HER2 gene status.

* Range of data from two NordiQC reference laboratories.

SN/LE 16.04.2021
Table 4 updated 27.04, SN