Assessment Run H20 2021

HER2 (BRISH or FISH)

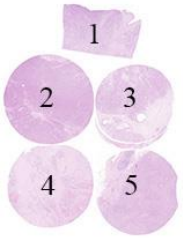
Purpose

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 H20#**

	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.
Same tissue materials as in run H19

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)

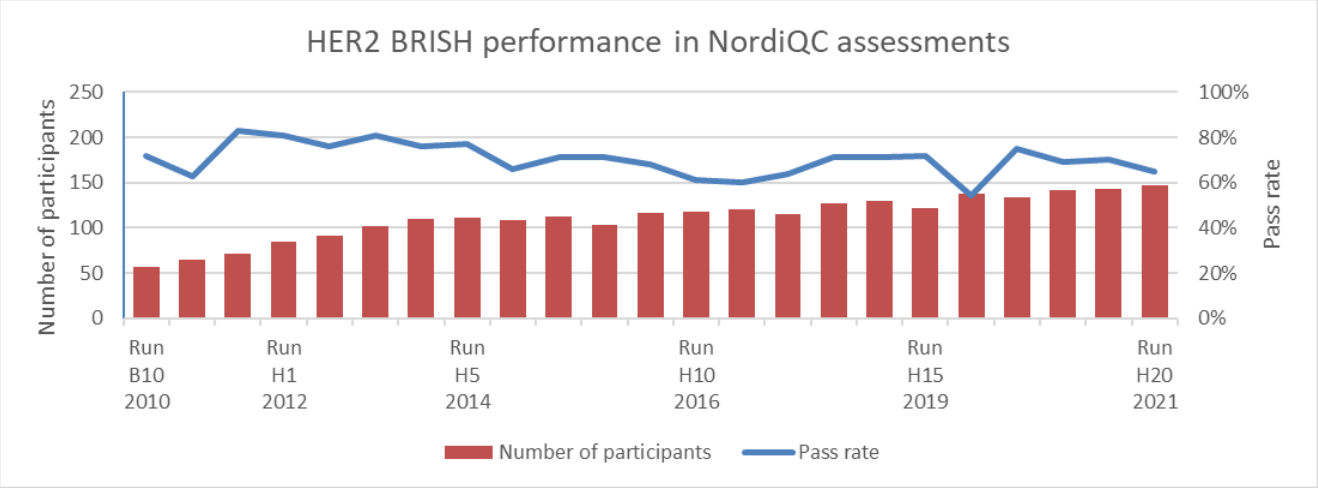
Participation

Number of laboratories registered for HER2 BRISH	160
Number of laboratories returning slides	147 (92%)
Number of laboratories returning scoring sheet	132
Number of laboratories registered for HER2 FISH	63
Number of laboratories returning scoring sheet	63

At the date of technical assessment meeting, 92% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment meeting were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

Performance history
This was the twenty-sixth assessment of HER2 BRISH in NordiQC and a pass rate of 65% was obtained, which was slightly reduced compared to the level seen in the last assessment using same tissue material circulated. 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, 147 laboratories participated in this assessment. 95 laboratories (65%) 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 H20.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **780-4422/ 800-4422**	13	Ventana/Roche	8	1	3	1	69%	62%
INFORM™ HER2 Dual ISH + IHC **780-4422 + HER2 IHC (GPA)***	2	Ventana/Roche	0	0	2	0	-	-
VENTANA HER2 Dual ISH **800-6043**	106	Ventana/Roche	63	12	25	6	71%	60%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)***	17	Ventana/Roche	2	8	5	2	59%	12%
Zyto*Dot*® 2C **C-3022 / C-3032**	7	ZytoVision	0	1	4	2	-	-
One colour HER2 systems								
Zyto*Dot*® **C-3003**	2	ZytoVision	0	0	1	1	-	-
Total	147		73	22	40	12	95	-
Proportion			50%	15%	27%	8%	65%	

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 (94%) used BRISH HER2 systems from Ventana/Roche. 84% (123 of 147 participants) used the relatively 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 10% of the participants (15 of 147). 13% of participants (19 of 147) 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.

As shown in Table 2 and in concordance to the results observed in the last run H19, 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.
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 line 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% (35 of 52) of the insufficient results were characterized by large negative areas covering more than 25% of one or more of the breast carcinomas. In 10% of the insufficient results (5 of 52) missing or weak signals were observed and in the remaining 23% (10 of 52) 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.

In contrast to the previous assessments, the recently launched VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) being used by the majority of participants and the "established" INFORM™ HER2 Dual ISH assay (780-4422 / 800-4422) provided virtually same overall pass rate and proportion of optimal results (see Table 2). For both assays, 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. For the participants using a GPA assay based on one of the two Ventana Dual ISH assays (n=19), an accumulated pass rate of 53%, 11% optimal was obtained. For the participants using the Ventana Dual ISH assays as traditional BRISH assays without IHC (n=119), an accumulated pass rate of 71%, 60% optimal was observed.

Similar to the results observed in run H19 (same tissue material being applied for the TMAs used in H19 and H20) 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 for breast carcinoma 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
106 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 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.
Among the laboratories reporting these protocol settings a pass rate of 72% (46 of 64) was obtained, 64% 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.

17 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY®** (Ventana/Roche). The two optimal results using this GPA assay, were reported to be based on HIER in CC2 for 16 and 24 min. and subsequent proteolysis in ISH Protease 3 for 20 and 28 min. at 36°C.

13 laboratories used the **INFORM™ Dual ISH system 780-4422/800-4422** (Ventana/Roche).
Optimal demonstration of HER2 BRISH was typically based on HIER in CC2 for 24 min. or CC1 for 16 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 89% of the submitted protocols (8 of 9).

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, H20**

BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **780-4422/800-4422**	12	Ventana/Roche	11	1	92%
INFORM™ HER2 Dual ISH + IHC **780-4422 + HER2 IHC (GPA)**	2	Ventana/Roche	2	0	-
VENTANA HER2 Dual ISH **800-6043**	93	Ventana/Roche	80	13	86%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	12	Ventana/Roche	10	2	83%
Zyto*Dot*® 2C **C-3022 / C-3032**	7	ZytoVision	6	1	86%
Zyto*Dot*® **C-3003**	2	ZytoVision	1	1	-
FISH					
PathVysion HER-2 DNA **6N4630 / 30-161060**	14	Abbott	14	0	100%
HER2 IQFISH **GM333**	5	Dako/Agilent	5	0	100%
HER2 IQFISH **K5731**	15	Dako/Agilent	13	2	87%
BOND HER2 FISH system **TA9217**	4	Leica Biosystems	4	0	-
HER2/CEN17 FISH probe **MF2001**	2	Maixin	1	1	-
FISH Kit **MAD-FISH-MDS**	1	Master Diagnostica	1	0	-
FISH ERB2 probe **KBI-10701**	1	Kreatech	0	1	
Rembrandt Her-2-C17 probe **C801K.5206**	2	PanPath	2	0	-
ZytoLight **Z-2015 / Z-2020/ Z-2077**	13	ZytoVision	11	2	85%
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		167	24	
Proportion			87%	13%	

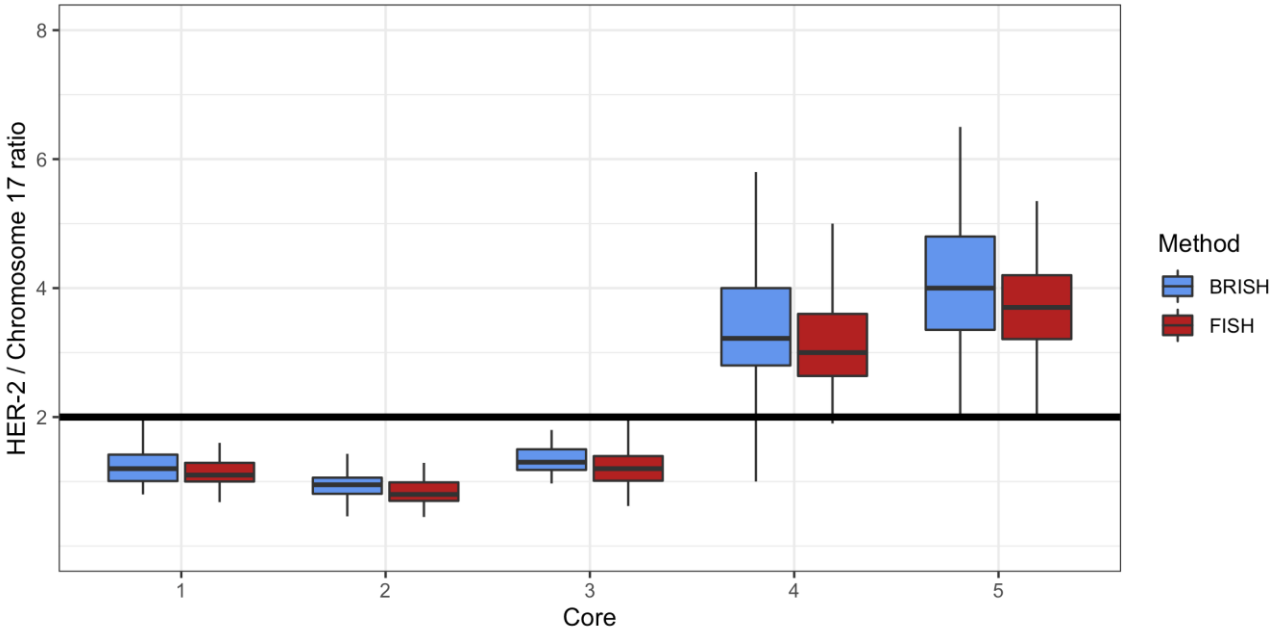
*The number varies from Table 2. Not all participants have submitted a scoring sheet.

191 of the 210 (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 (see page 6). For the laboratories performing FISH, the consensus rate was 91%, and 86% for laboratories using BRISH. This was a slightly reduced level compared to the last run H19, but still at relatively high level and superior to previous runs.

It was observed that the consensus rates for interpretation of the individual cores were virtually identical for laboratories that produced a staining reaction assessed as technically sufficient (BRISH only) and laboratories with an insufficient mark (86% and 87%, 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 without gene signals. 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.

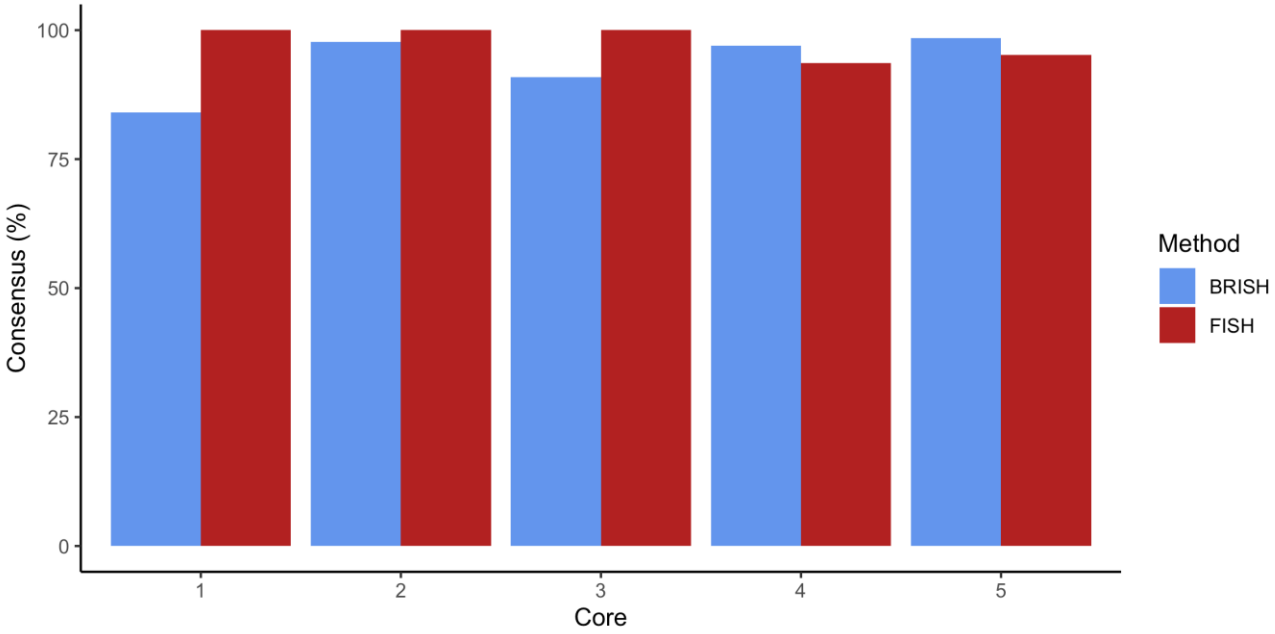
As observed in run H19, the breast carcinoma, tissue core no. 1 being fixed in 10% NBF for 72 hours was found to be technically more challenging to give distinct signals compared to the four other tissue samples being fixed for 24-48 hours in 10% NBF. This was in particular observed for laboratories performing BRISH and submitting scoring sheets as 10% (n=13) within this group were unable to reliably score this sample. In comparison, this problem or challenge was only registered for 3% of laboratories performing FISH (n=2). No plausible explanation for the differences observed could be identified form the protocol settings registered.

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



Graph 2

NordiQC HER2 ISH run H20: Participant interpretation of amplification status



Graph 3

NordiQC HER2 ISH run H20: 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 780-4422** / **800-4422**.

Despite that the assays were applied within optimal protocol settings a relatively low pass rate was obtained. As such, for the most commonly used assay, the **VENTANA HER2 Dual ISH 800-6043** assay, gave a pass rate of 72% and 64% optimal when used within appropriate protocol settings.

The 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 in the latest runs for HER2 BRISH, the overall pass rate is still only 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.

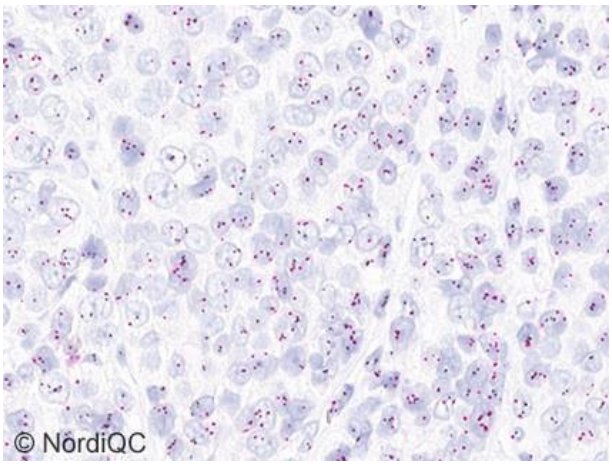


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification: HER2/chr17 ratio 0.6*.
The HER2 genes are stained black and chr17 red. The morphology is preserved, and signals distinctively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

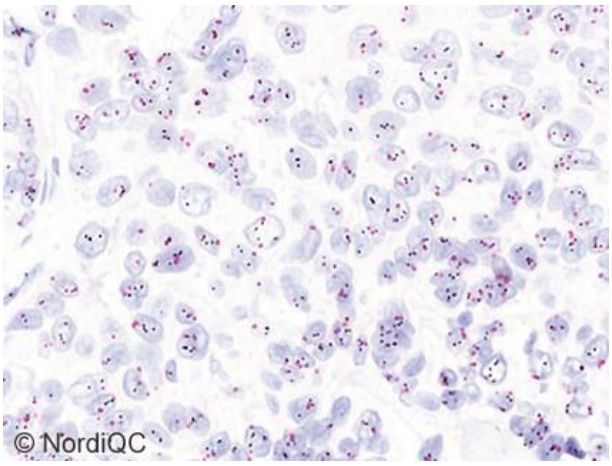


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.0 – 1.1*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated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

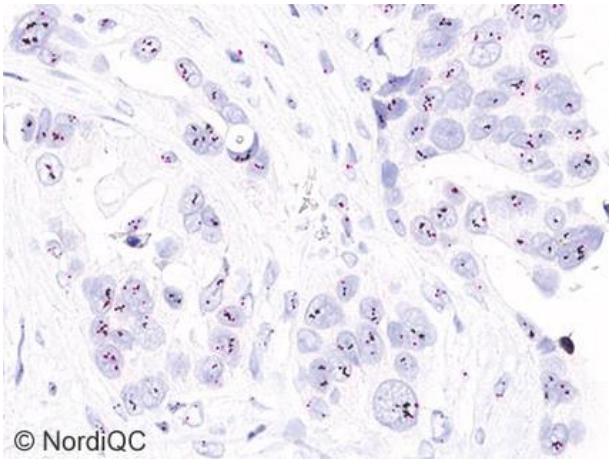


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 2.3-2.5*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated,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

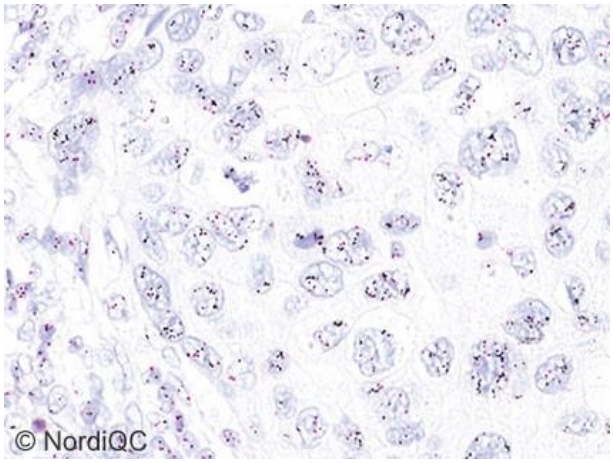


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 3.2-4.3*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated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

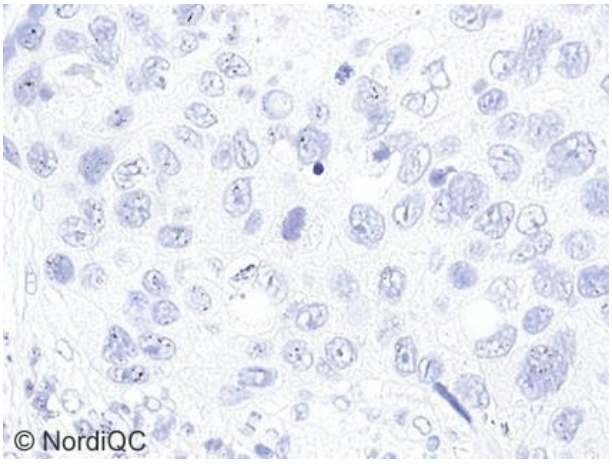


Fig. 3a
Insufficient staining result for the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 3.2-4.3*.
The HER2 genes are stained black, chr17 red.
Virtually all cells (few with HER2 signals) and 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.
Compare with Fig. 2b. – same tumour.

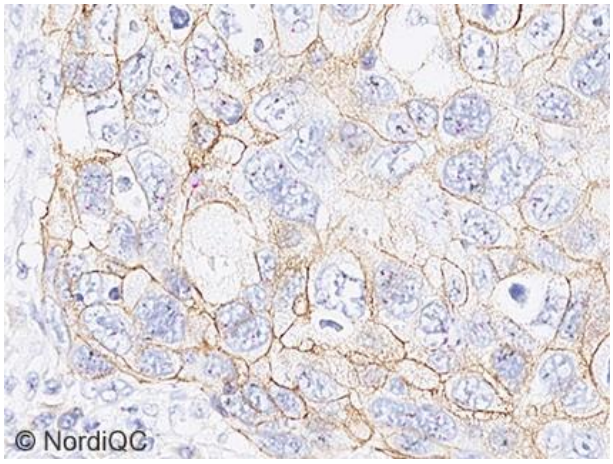


Fig. 3b
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 3.2-4.3*.
The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown.
The IHC result for HER2 is as expected but only few and weak HER2 genes and chr17 signals can be identified.
This aberrant staining reaction was most likely caused by a technical issue during the staining process in the BenchMark instrument.
Compare with Fig. 2b. – same tumour.

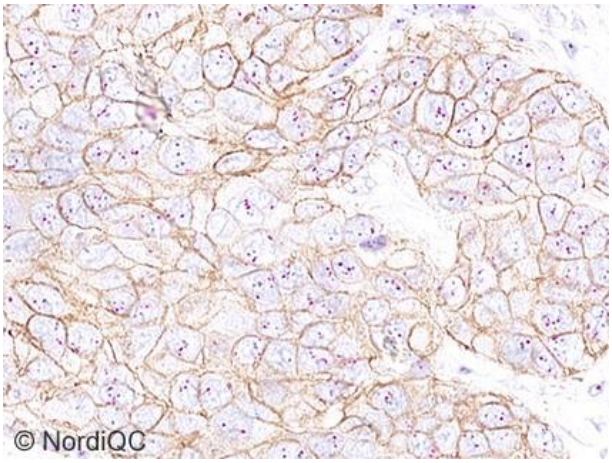


Fig. 4a
Optimal staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification: HER2/chr17 ratio 1.0 – 1.1*.
The gene protein assay (GPA) labels 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 non-amplified.
NordiQC and virtually all participants interpreted this tumour as non-amplified.
Compare with Fig. 1b. – same tumour.

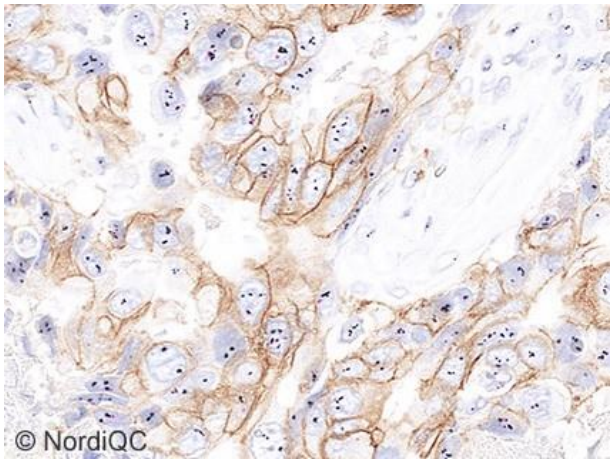


Fig. 4b
Optimal staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification: HER2/chr17 ratio 2.3-2.5*.
The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown.
The participant interpreted this tumour as amplified.
NordiQC and virtually all participants interpreted this tumour as amplified.
Compare with Fig. 2a. – same tumour

* Range of data from two NordiQC reference laboratories.

SN/LE 05.12 2021