Assessment Run H22 2022

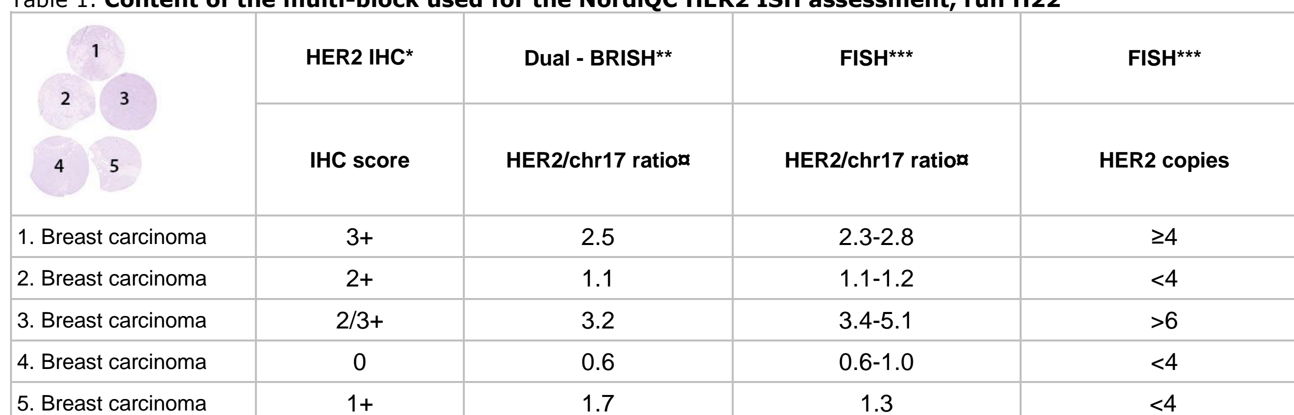
HER2 (BRISH or FISH)

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 H22#**



	HER2 IHC*	Dual - BRISH**	FISH***	FISH***
	IHC score	HER2/chr17 ratio¤	HER2/chr17 ratio¤	HER2 copies
1. Breast carcinoma	3+	2.5	2.3-2.8	≥4
2. Breast carcinoma	2+	1.1	1.1-1.2	<4
3. Breast carcinoma	2/3+	3.2	3.4-5.1	>6
4. Breast carcinoma	0	0.6	0.6-1.0	<4
5. Breast carcinoma	1+	1.7	1.3	<4

* PATHWAY® (Ventana/Roche), data from two reference labs.
** Ventana HER2 Dual ISH DNA Probe Cocktail, data from one reference lab.
*** HER2 FISH (Zytovision), range of data from two tests from one reference lab.
¤HER2/chr17: HER2 gene/chromosome 17 ratio.
Same tissues as used in run H21

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

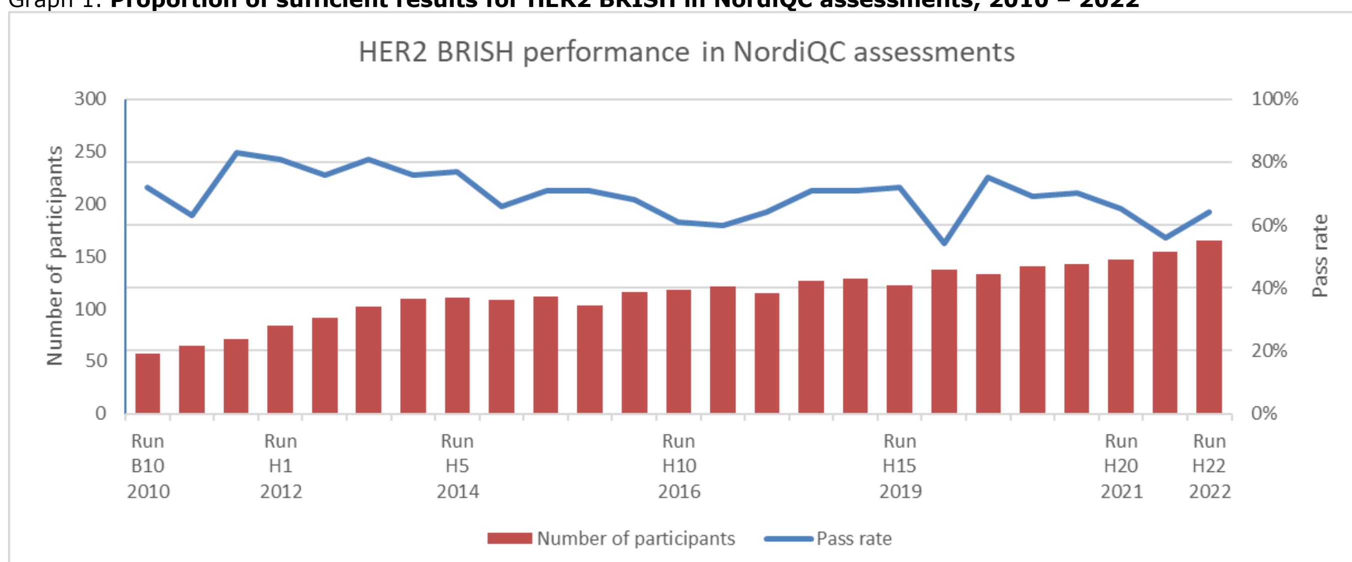
Participation

Number of laboratories registered for HER2 BRISH	177
Number of laboratories returning slides	165 (94%)
Number of laboratories returning scoring sheet	148
Number of laboratories registered for HER2 FISH	63
Number of laboratories returning scoring sheet	59

At the date of technical assessment meeting, 94% 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
In this assessment run H22 the overall pass rate was improved compared to the pass rate seen in the latest run H21 as illustrated in Graph 1, but still at a low level and inferior to the cumulated average level of 69% obtained in the 25 NordiQC assessment runs performed for HER2 BRISH from 2010-2022.

Graph 1. **Proportion of sufficient results for HER2 BRISH in NordiQC assessments, 2010 – 2022**



Results BRISH, technical assessment
In total, 165 laboratories participated in this assessment. 106 laboratories (64%) 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 H22.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **780-4422/ 800-4422**	5	Ventana/Roche	2	2	0	1	80%	40%
INFORM™ HER2 Dual ISH **780-4422/ 800-4422 (GPA)***	1	Ventana/Roche	0	1	0	0	-	-
VENTANA HER2 Dual ISH **800-6043**	132	Ventana/Roche	42	47	34	9	68%	31%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	18	Ventana/Roche	8	2	6	2	56%	38%
Zyto*Dot*® 2C **C-3022 / C-3032**	7	ZytoVision	1	1	4	1	29%	14%
One colour HER2 systems								
Zyto*Dot*® **C-3003**	2	ZytoVision	0	0	1	1	-	-
Total	165		53	53	45	14		
Proportion			32%	32%	27%	9%	64%	

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 in concordance with the latest assessments, the vast majority of participants (95%) used BRISH HER2 systems from Ventana/Roche. 92% (151 of 165 participants) used the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) and 3% (5 of 165) the INFORM™ HER2 Dual ISH assay (800-4422/780-4422). 12% of participants (19 of 156) using one of the two Ventana/Roche BRISH HER2 systems applied these 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, 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 obtained both by the two Ventana/Roche dual-colour BRISH systems and the ZytoVision ZytoDot® 2C system.
The insufficient results were most frequently characterized by large negative areas in one or more of the breast carcinoma samples, but also caused by 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 artefacts as listed above. In these cases, the staining results were thus rated as insufficient (poor or borderline). 70% (41 of 59) of the insufficient results were characterized by large negative areas covering more than 25% of one or more of the breast carcinomas. In the remaining 30% of the insufficient results these were caused by different artefacts as impaired morphology, excessive counterstaining, weak signals, silver precipitates and in more cases also 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 this context it has to be emphasized that focal negative areas <25% was accepted and did not impact the assessment mark and consequently also observed for results evaluated as optimal.

In this assessment the overall pass rate was improved to the level seen in the latest run H21 as illustrated in Graph 1, but still at a low level and inferior to the cumulated average level of 69% obtained in the 25 NordiQC assessment runs performed for HER2 BRISH from 2010-2022.
As indicated from Table 2, a consolidation and harmonization of methods used for HER2 BRISH has been effectuated, but despite this consolidation it has not been possible to improve the pass rates being more or less stable at the same level for more than five years. It is difficult to identify the exact root cause for the relatively low and disappointing pass rate and it can be impacted by many parameters and at different sites as both the laboratories, NordiQC and BRISH system vendors. From the laboratory perspective, the consolidation of both the choice of BRISH systems and also a certain harmonization of the protocols applied for these systems have been implemented. From NordiQC, the same assessment criteria have been applied, same requirements for tissue processing procedures for the samples used for the

assessments (ASCO/CAP guidelines) but the samples originating from different donors. From the BRISH vendor site, precise and validated guidelines concerning protocol set-up for the recently launched VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) are provided. However, these fundamentals have not been successful to improve and maintain a stable pass rate at a satisfactory level in the NordiQC HER2 BRISH assessment runs. An assessment evaluation by external quality programs gives an input to the performance of a specific analysis but cannot be used isolated to judge the quality (precision and accuracy) of this analysis and internal quality measurement must always be conducted in combination with the external quality assessment. In this aspect, for the laboratories receiving an insufficient mark as borderline or poor and caused by e.g. large negative areas >25% in the samples, they are encouraged to perform an internal analysis of retests needed on daily basis, general quality observed and if needed take contact to the vendor of the BRISH system to make a plan how to improve the reproducibility of the analysis.

Optimal protocol settings: Two-colour HER2 systems
132 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 64% (57 of 89) was obtained, 30% being optimal.

18 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY®** (Ventana/Roche). The optimal results using this GPA assay, were typically reported to be based on HIER in either CC1 (n=2), CC2 (n=3) or a combination of CC1 and CC2 (n=3) and for all with a subsequent proteolysis in ISH Protease 3 for 16-20 min. at 36°C.

7 laboratories used the **ZytoDot® 2C system C-3022 / C-3032** (ZytoVision)
One protocol provided an optimal demonstration of HER2 BRISH and was based on HIER in EDTA, PT-0002-500 (ZytoVision) for 15 min. at 95°C, proteolysis in pepsin for 6 min. at 37°C, hybridization at 37°C for 16 hours following a denaturation at 75°C for 5 min. and visualization with the ZytoVision detection kit C-3022.

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	2.3-2.8	≥4	Amplified
2. Breast carcinoma	1.1-1.2	<4	Non-amplified
3. Breast carcinoma	3.4-5.1	>6	Amplified
4. Breast carcinoma	0.6-1.0	<4	Non-amplified
5. Breast carcinoma	1.3	<4	Non-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, H22**

BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **780-4422/800-4422**	4	Ventana/Roche	2	2	-
INFORM™ HER2 Dual ISH + IHC **780-4422 + HER2 IHC (GPA)**	1	Ventana/Roche	0	1	-
VENTANA HER2 Dual ISH **800-6043**	121	Ventana/Roche	78	43	65%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	13	Ventana/Roche	11	2	85%
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	12	2	86%
HER2 IQFISH **GM333**	6	Dako/Agilent	6	0	100%
HER2 IQFISH **K5731**	8	Dako/Agilent	7	1	88%
SureFISH **G110144G-8**	1	Dako/Agilent	1	0	-
BOND HER2 FISH system **TA9217**	8	Leica Biosystems	6	2	75%
HER2/CEN17 FISH probe **MF2001**	2	Maixin	2	0	-
FISH Kit **MAD-FISH-MDS**	2	Master Diagnostica	1	1	-
FISH ERB2 probe **KBI-10701**	1	Kreatech	1	0	
Rembrandt Her-2-C17 probe **C801K.5206**	1	PanPath	1	0	-
ZytoLight **Z-2015 / Z-2020/ Z-2077**	11	ZytoVision	11	0	100%
ZytoMation ERBB2/CEN17 Dual Color FISH Probe **Z-2292**	4	ZytoVision	4	0	-
ERBB2/CCP17 FISH Probe kit **CT-PAC001**	1	CytoTest	1	0	-
Total	207		151	56	
Proportion			73%	27%	

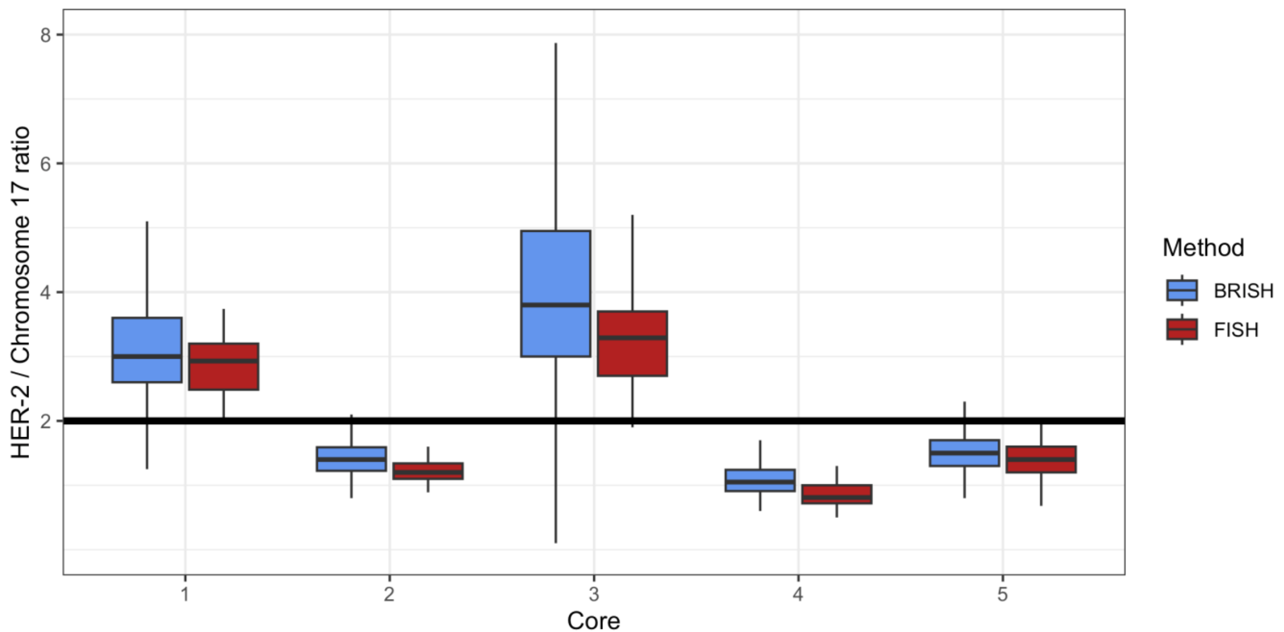
*The number varies from Table 2. Not all participants have submitted a scoring sheet.

207 of the 228 (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 90%, and 66% for laboratories using BRISH. For FISH, this was a slightly increased level compared to the last run H21, whereas the level observed for BRISH was significantly reduced compared to the last two runs showing consensus rate of 86% in both run H20 and H21.

For BRISH, 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 (Optimal and Good) and for laboratories with an insufficient mark (Borderline and Poor) being 67% and 69%, respectively. Despite a result evaluated as insufficient by the NordiQC assessor group, laboratories typically still were 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

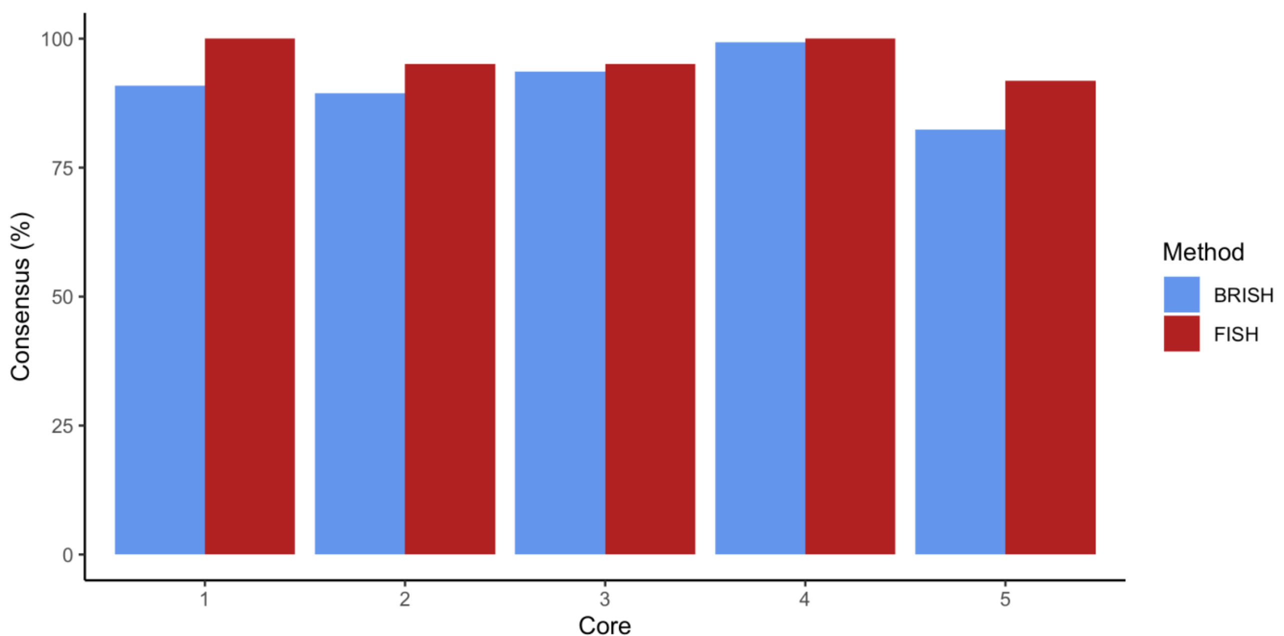
expression, biopsy material with limited tumor mass or HER2 evaluation in specific "hot-spot areas" identified by HER2 IHC.
The discrepancies for read-out were mostly related to tissue samples no. 1, 3 and 5. Tissue core no. 1 was by NordiQC and 100% of the participants performing FISH scored as amplified, but by 9 participants performing BRISH classified as HER2 negative and non-amplified. Same tendency was seen for tissue core no. 3, being classified as highly amplified by NordiQC and >90% of the participants performing FISH, but by 7 BRISH results scored as non-amplified. Finally, the tissue core no. 5 was expected to be HER2 non-amplifed as verified by the NordiQC data and 98% of the participants performing FISH, but scored as amplified by 11 participants performing BRISH.

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



Graph 2

NordiQC HER2 ISH run H22: Participant interpretation of amplification status



Graph 3

NordiQC HER2 ISH run H22: Consensus depending on method

Conclusion
In this assessment a technical optimal demonstration of HER2 BRISH could be obtained by both the two Ventana/Roche two-colour HER2 systems **VENTANA HER2 Dual ISH** and **INFORM™ HER2 Dual ISH** and also by the **ZytoVision ZytoDot® 2C** system .
Overall focusing on the technical quality of the HER2 BRISH assays a relatively low pass rate of only 64% was obtained. This level has almost been consistent for the last runs.
For the most commonly used assay, the **VENTANA HER2 Dual ISH 800-6043** assay, being used by 132 participants the pass rate was 68% and 31% optimal.
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 the insufficient results.
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.

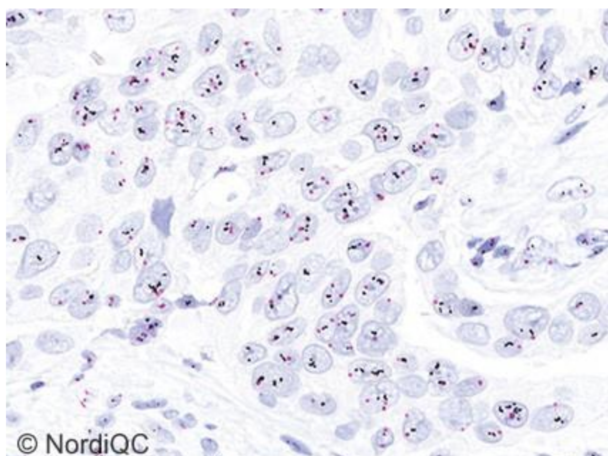


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 1 with HER2 gene amplification:
HER2/chr17 ratio 2.3 - 2.8, ≥4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The morphology is well preserved, and signals distinctively demonstrated.
NordiQC and most participants interpreted this tumour as amplified.

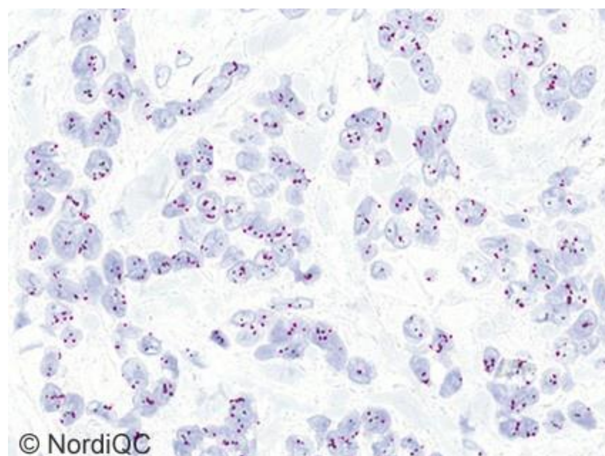


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification:
HER2/chr17 ratio 1.1 – 1.2, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

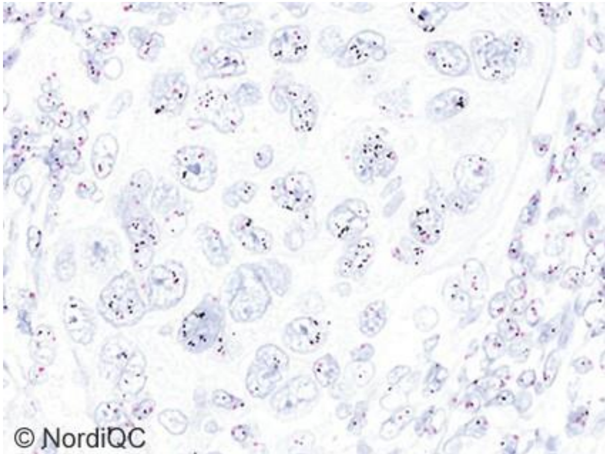


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification:
HER2/chr17 ratio 3.2 – 5.1, >6 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated, and the HER2 signals are in some cells located in large clusters.
NordiQC and virtually all participants interpreted this tumour as amplified.

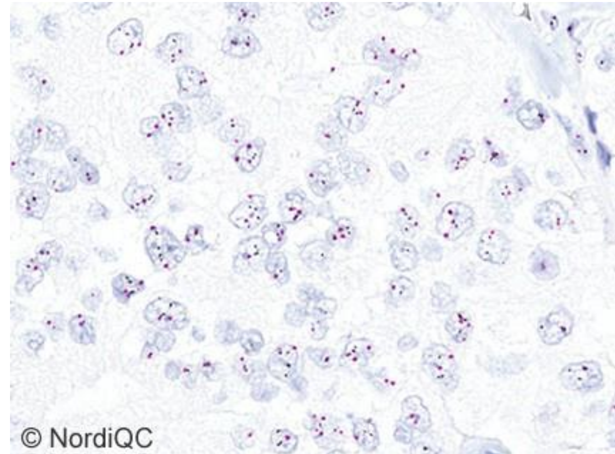


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.3-1.7, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in all the neoplastic cells.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

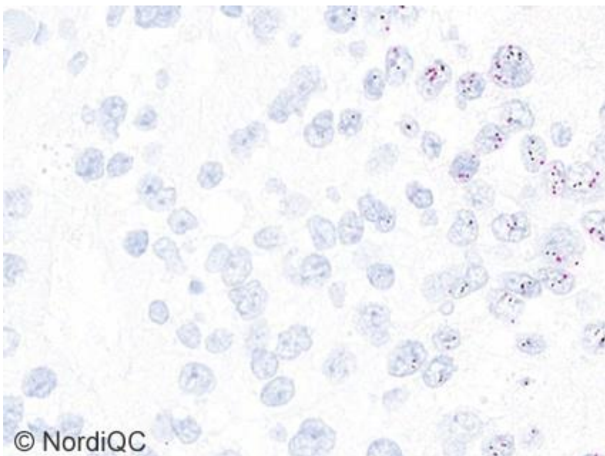


Fig. 3a
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.3-1.7, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The vast majority of cells 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.

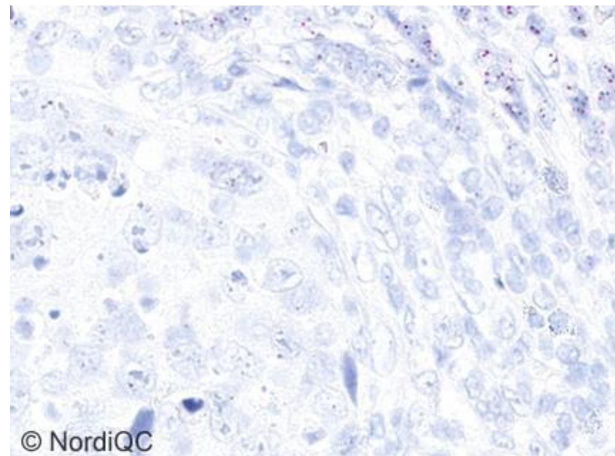


Fig. 3b
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification:
HER2/chr17 ratio 3.2 – 5.1, >6 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The vast majority of cells and large areas (>25% of areas with neoplastic cells) are totally negative – in this field only normal cells show signals while the large neoplastic cells are 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. 2a – same tumour.

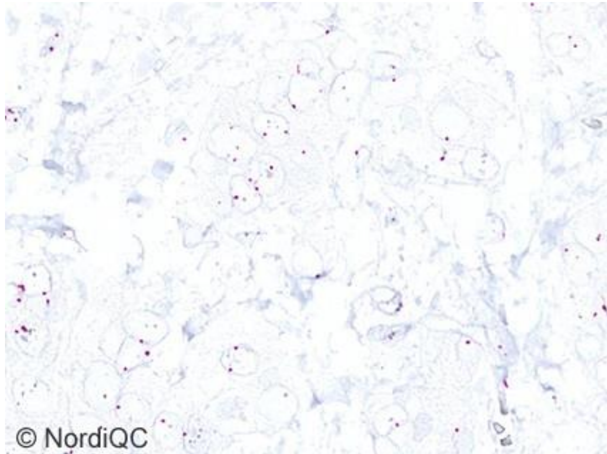


Fig. 4a
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.3-1.7, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
An extensive impaired morphology is seen characterized by "empty" nuclei and only the nuclear membranes are left. Excessive retrieval can cause this pattern, however the protocol reported is identical to the protocol used in Figs. 1 - 2 giving optimal results.

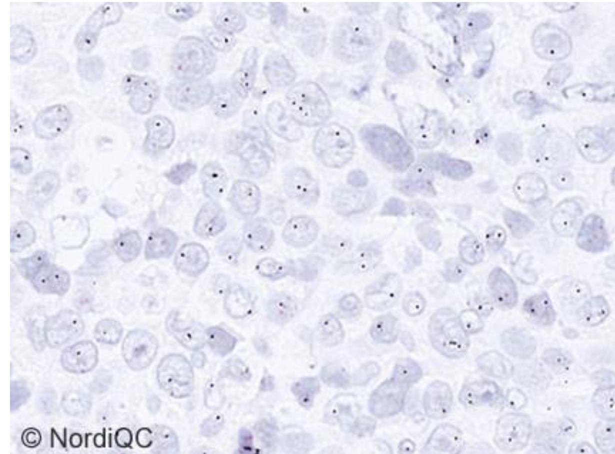


Fig. 4b
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no.4 without HER2 gene amplification:
HER2/chr17 ratio 0.6-1.0, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The vast majority only show HER2 copies (black), while the chr17 signals are only seen in few cells and ratio cannot be established. The protocol settings being identical to the protocol providing optimal results as shown in Figs. 1 – 2.

* Range of data from FISH and BRISH performed in two NordiQC reference laboratories.

SN/LE/RR 13.12.2022