Assessment Run H24 2023
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 hybridization (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 H24[#]**

	**HER2 IHC***	**Dual - BRISH****	**FISH*****	**FISH*****
	IHC score	**HER2/chr17 ratio[¤]**	**HER2/chr17 ratio[¤]**	**HER2 copies**
1. Breast carcinoma	0	0.6 - 0.9	0.6 - 1.0	1.3 - 1.7
2. Breast carcinoma	3+	3.4	5.8	9.6
3. Breast carcinoma	1+	1.5 - 1.7	1.3 - 1.4	2.5 - 2.6
4. Breast carcinoma	3+	2.5 - 3.3	2.3 - 3.4	4.3 - 6.3
5. Breast carcinoma	2+	1.1 - 1.2	1.0 - 1.1	1.9 - 2.6

* 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 block as used in run H23

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.

Staining was assessed as **optimal**, if the HER2/chr17 ratios could be evaluated in all five tissues and no technical artefacts compromising the interpretation being observed.

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 large negative areas with no signals (>25% of the core)

Staining was assessed as **borderline** if one of the tissues could not be evaluated properly e.g. due to weak or missing signals, a low signal-to-noise ratio, excessive background staining or impaired morphology.

Staining was assessed as **poor** if two or more of the tissue cores could not be evaluated properly e.g. due to weak or missing signals, a low signal-to-noise ratio, excessive background staining or impaired morphology

Note that the assessment criteria were modified in this run compared to the latest assessments. In this run large negative areas of > 25% of the individual tissue cores were accepted providing the HER2 gene amplification level still reliably could be evaluated. However, a slide with large negative areas was not compatible with an optimal assessment mark and was downgraded to good providing an otherwise optimal result being observed.

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

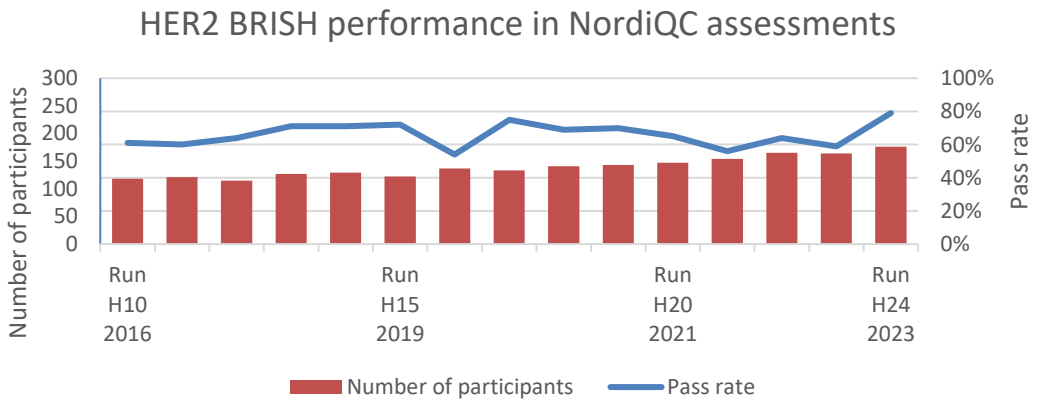
Participation

Number of laboratories registered for HER2 BRISH	185
Number of laboratories returning slides	176 (95%)
Number of laboratories returning scoring sheet	161
Number of laboratories registered for HER2 FISH	69
Number of laboratories returning scoring sheet	64

At the date of technical assessment meeting, 95% 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 H24 the overall pass rate of 79% was significantly improved compared to the levels obtained in the latest assessment runs as illustrated in Graph 1. The improvement is mainly caused by new modified assessment criteria applied in this run allowing large negative areas of >25% in one of the tissue cores providing an evaluation of the HER2/chr 17 ratio still adequately could be obtained.

Graph 1. **Proportion of sufficient results for HER2 BRISH in NordiQC assessments, 2016 – 2023**



Results BRISH, technical assessment
In total, 176 laboratories participated in this assessment. 140 laboratories (79%) 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 H24.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **780-4422/ 800-4422**	10	Ventana/Roche	5	4	1	0	90%	50%
VENTANA HER2 Dual ISH **800-6043**	137	Ventana/Roche	57	61	16	3	86%	42%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA*)**	20	Ventana/Roche	2	8	9	1	50%	10%
Zyto*Dot*® 2C **C-3022 / C-3032**	7	ZytoVision	0	3	2	2	43%	0%
One colour HER2 systems								
Zyto*Dot*® **C-3003**	2	ZytoVision	0	0	1	1	-	-
Total	176		64	76	29	7		
Proportion			36%	43%	17%	4%	79%	

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. 89% (157 of 176 participants) used the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) and 6% (10 of 176) the INFORM™ HER2 Dual ISH assay (780-4422/800-4422). 5% (9 of 176) used HER2 BRISH systems, ZytoDot® from Zytovision.
11% of participants (20 of 176) used the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) 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 by both 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 compromising the evaluation of HER2/chr 17 ratio, but also caused by impaired morphology, generally weak or missing signals for either HER2 and/or chr17.
In this assessment 83% (30 of 36) of the insufficient results were characterized by too weak or completely false negative signals for HER2/chr 17 in one or more tissue cores either as single feature or combined with other artefacts as impaired morphology and/or weak counterstaining. In the remaining 17% of the insufficient results these were mainly caused by impaired morphology, excessive counterstaining and/or silver precipitates compromising the read-out of HER2/chr 17 ratio.

As described in the assessment report for run H23 2023 and illustrated in Graph 1, no significant improvement in pass rates had been obtained for HER2 BRISH in the period from 2016-2023 and a cumulated average level of 64% has been obtained in these NordiQC assessment runs H10-H23. In all these 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 in the sample evaluated. However, by internal discussions within the NordiQC assessor panel and from correspondences with participants and Ventana/Roche, it was decided to modify and relent the assessment criteria accepting larger negative areas in the individual tissue cores providing these still reliably could be scored concerning HER2/chr17 ratio. However, a slide with large negative areas was not compatible with an optimal assessment mark and was downgraded to good providing an otherwise optimal result being observed. The negative areas observed are random artefacts especially observed for the Ventana/Roche HER2 BRISH systems and an artefact recognized by both Ventana/Roche, NordiQC and the participants. In daily practice the end-user decides if samples with false negative areas can be scored or needs to be retested. The decision to relent the criteria was also based on the fact, that virtually all participants now use same or similar protocol settings for HER2 BRISH being locked by the vendor and thus not possible to optimize these further.

In both this and previous assessment run H23, the combined GPA assay (VENTANA HER2 Dual ISH 800-6043 + HER2 IHC) was found less successful giving a pass rate of 43%, 10 % optimal. The insufficient results were typically characterized by a successful IHC test for HER2 and as such showing a distinct and strong 3+ IHC membranous reaction of the neoplastic cells in the tumour tissue core no. 2, but only

scattered cells displaying HER2 gene signals despite being highly amplified (Her2/Chr17 ratio of 3.4-5.8 and > 6 HER2 signals pr cell) and likewise also in the IHC 2+ tissue core no. 5 without gene amplification being identified as 2+ IHC but only few cells having HER2/Chr 17 signals. The central protocol settings e.g. HIER time/temp., HIER buffers and proteolysis reported for the GPA assay were similar to the settings reported for the single use of the VENTANA HER2 Dual ISH 800-6043 assay and thus not possible to identify any protocol parameters causing the very low pass rate in these two runs. However, one plausible explanation might be related to the strong 3+ HER2 IHC reaction in the tumour cells obscuring the penetration of HER2/chr 17 BRISH probes and/or enzymatic visualization of these.

Optimal protocol settings: Two-colour HER2 systems
137 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 for a total of 40 min. 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 85% (84 of 99) was obtained, 40% being optimal.

20 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY®** (Ventana/Roche). The optimal result using this GPA assay, was based on HIER in CC1 and CC2 for 32 and 24 min., respectively and a subsequent proteolysis in ISH Protease 3 for 20 min. at 36°C. Among the laboratories using the GPA assay a pass rate of 50% was obtained, 10% optimal.

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	0.6 - 1.0	<4	Non-amplified
2. Breast carcinoma	5.8	>6	Amplified
3. Breast carcinoma	1.3 - 1.4	<4	Non-amplified
4. Breast carcinoma	2.3 - 3.4	>4	Amplified
5. Breast carcinoma	1.0 - 1.1	<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, H24**

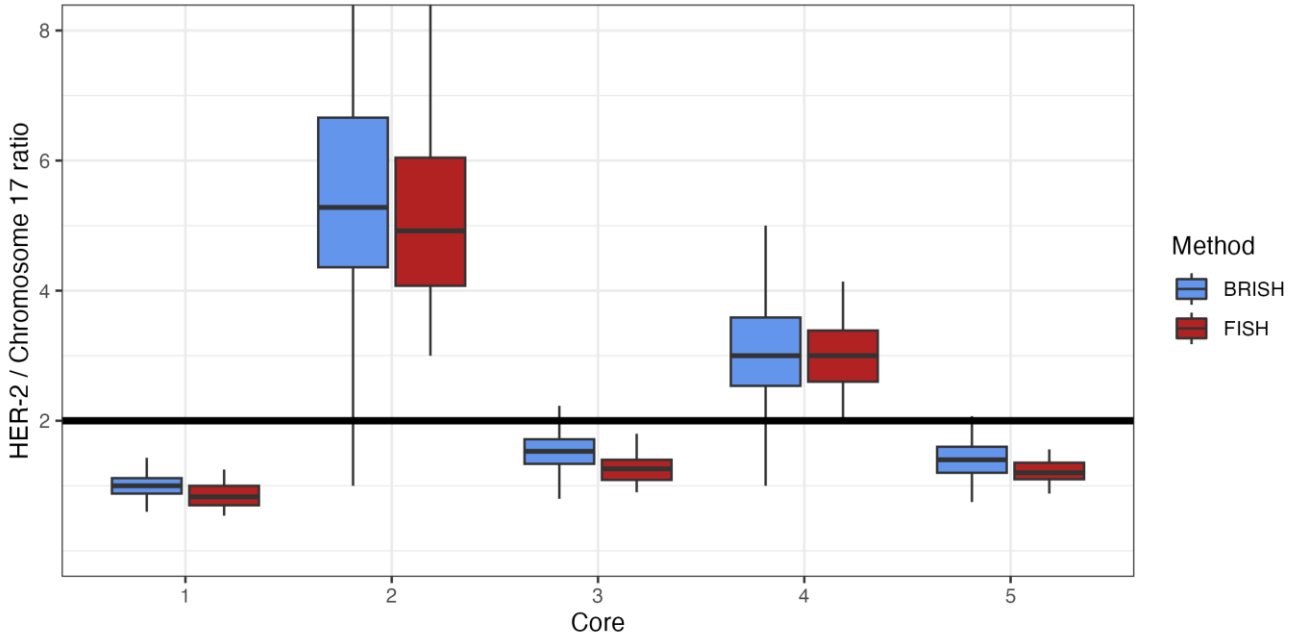
BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **780-4422/ 800-4422**	9	Ventana/Roche	7	2	78%
VENTANA HER2 Dual ISH **800-6043**	125	Ventana/Roche	96	29	77%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	18	Ventana/Roche	13	5	72%
Zyto*Dot*® 2C **C-3022 / C-3032**	7	ZytoVision	6	1	86%
Zyto*Dot*® **C-3003**	2	ZytoVision	1	1	-
FISH					
PathVysion HER-2 DNA **6N4630 / 30-161060**	12	Abbott	11	1	92%
HER2 IQFISH **GM333**	14	Dako/Agilent	14	0	100%
HER2 IQFISH **K5731**	4	Dako/Agilent	4	0	-
SureFISH **G110144G-8**	1	Dako/Agilent	1	0	-
BOND HER2 FISH system **TA9217**	8	Leica Biosystems	8	0	100%
HER2/CEN17 FISH probe **MF2001**	1	Maixin	1	0	
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**	1	PanPath	1	0	
CytoTest **CT-PAC001**	1	CytoTest Inc	1	0	
ZytoLight **Z-2015 / Z-2020/ Z-2077**	16	ZytoVision	14	2	88%
ZytoMation ERBB2/CEN17 Dual Color FISH Probe **Z-2292**	4	ZytoVision	4	0	-
Total	225		183	42	
Proportion			81%	19%	

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

225 of the 240 (94%) 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 overall consensus rate was 94%, and 76% for laboratories using BRISH, which was comparable to the levels obtained in last run H23 (98% and 80% for FISH and BRISH, respectively). Cumulated data hereby indicate that FISH assays provide a higher interlaboratory agreement for HER2/chr17 status for compared to BRISH assays. In this context, the reduced interlaboratory disagreement for HER2/chr17 status most likely also is impacted by the technical challenges as negative areas, impaired morphology, excessive background/nuclear staining being reflected in the relatively low pass rates seen for BRISH assays in runs H23 and H24.

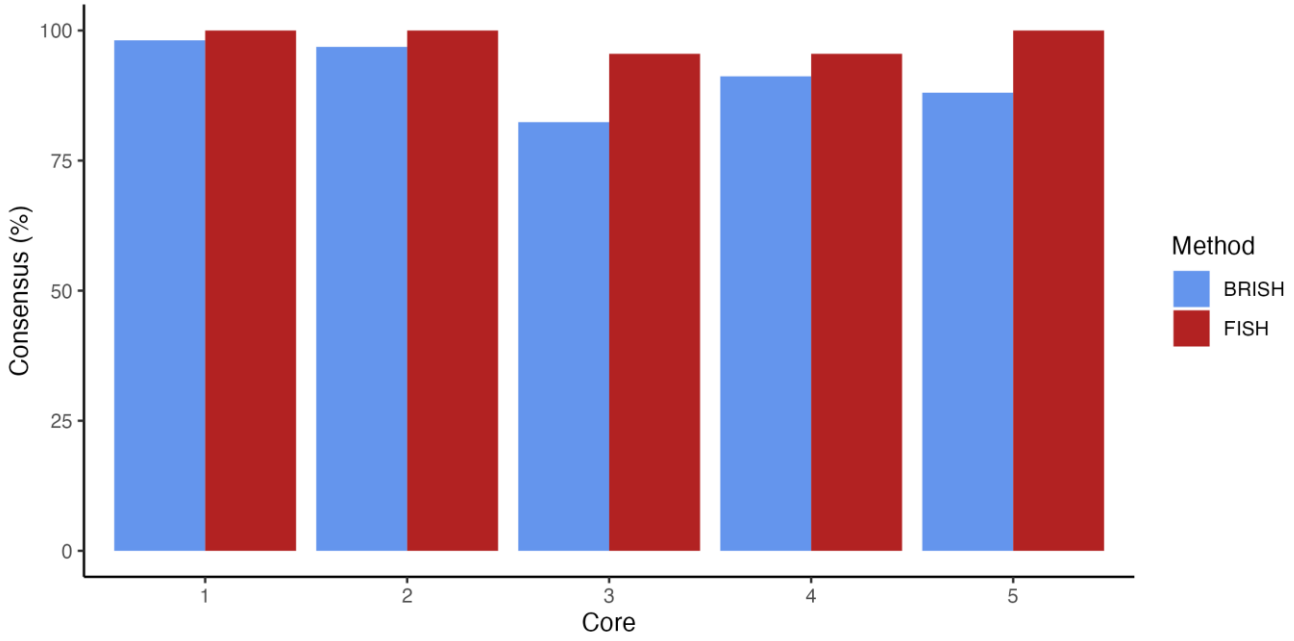
The discrepancies for read-out among participants were mostly related to tissue core no. 3. This tissue was by NordiQC and 61/64 (95%) of the participants performing FISH scored as non-amplified, but by 25/161 (16%) of the participants performing BRISH classified as HER2 amplified or equivocal. The breast carcinoma, tissue core no. 3 was by the NordiQC reference ISH methods characterized as HER2 negative (IHC 1+) with a HER2/Chr17 ratio of 1.3-1.7 and HER2 copy number of 2.5-2.6.

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



Graph 2

NordiQC HER2 ISH run H24: Participant interpretation of amplification status



Graph 3

NordiQC HER2 ISH run H24: Consensus depending on method

Conclusion

In this assessment a technical optimal demonstration of HER2 BRISH could be obtained by the widely used Ventana/Roche two-colour HER2 systems **VENTANA HER2 Dual ISH** and **INFORM™ HER2 Dual ISH.** . Focusing on the technical quality of the HER2 BRISH assays an improved pass rate of 79% was obtained being significantly superior to the level of 59% seen in run H23. The improvement was primarily related to the changed assessment criteria, allowing large negative areas of >25% providing the individual tissue cores still could be evaluated with confidence.

For the most commonly used assay, the **VENTANA HER2 Dual ISH 800-6043** assay, being used by 137 participants the overall pass rate was 86% and 42% optimal. The proportion of optimal results was comparable to the level observed in run H23 (where 43% were optimal).

The insufficient results were mainly caused by generally too weak or completely false negative results 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.

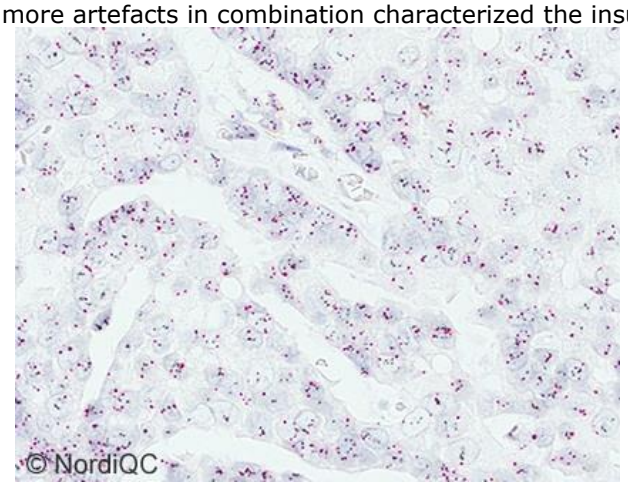


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 without HER2 gene amplification:
HER2/chr17 ratio 1.3 - 1.7, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The morphology is well preserved, and signals distinctively demonstrated.
NordiQC and most participants interpreted this tumour as non-amplified.

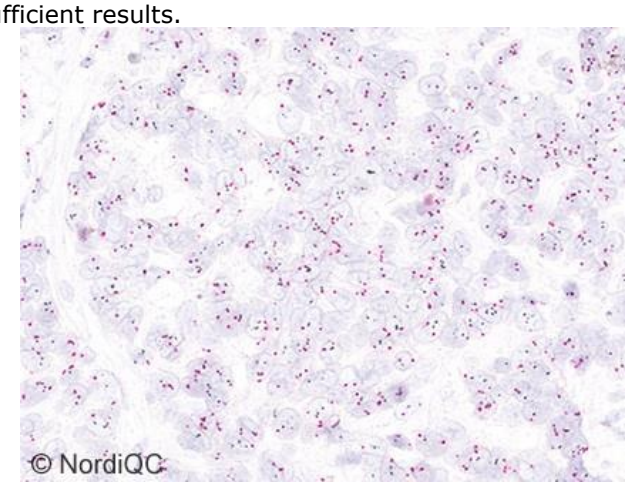


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.0 – 1.2, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

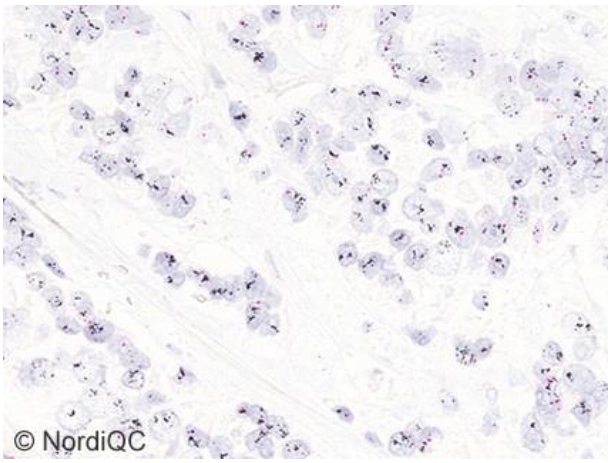


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 with HER2 gene amplification:
HER2/chr17 ratio 3.4 – 5.8, >6 HER2 copies *.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as amplified.

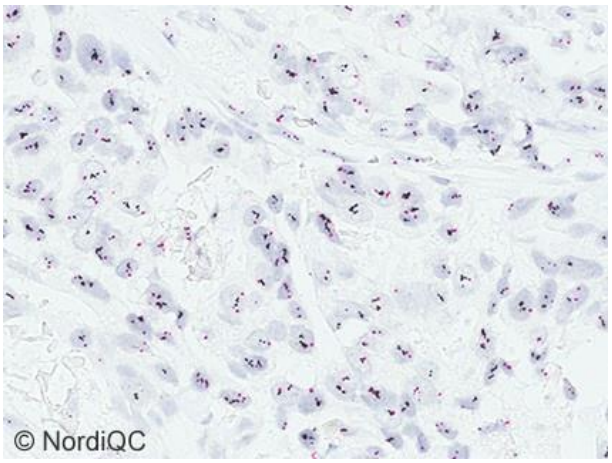


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification:
HER2/chr17 ratio 2.3-3.4, ≥4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in all the neoplastic cells.
NordiQC and virtually all participants interpreted this tumour as amplified.

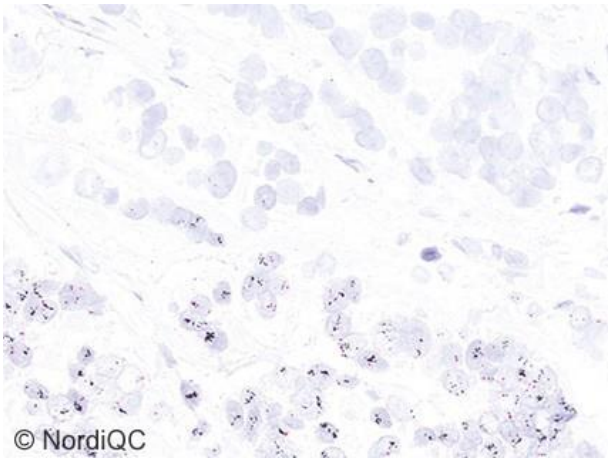


Fig. 3a
Sufficient result assessed as good using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 with HER2 gene amplification:
HER2/chr17 ratio 3.4 – 5.8, >6 HER2 copies*.
The HER2 genes are stained black and chr17 red. Large areas with neoplastic cells (>25% of tumour areas) are totally negative, but the tumour can still be evaluated with confidence. This aberrant staining reaction / "negative spot artefact" was most likely caused by a technical issue during the staining process in the BenchMark instrument.

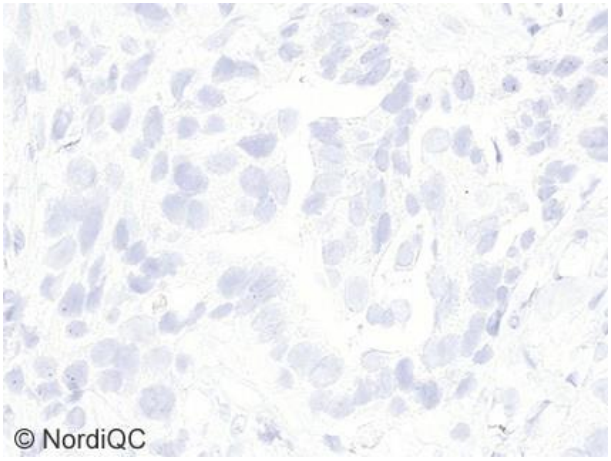


Fig. 3b
Insufficient result of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 with HER2 gene amplification:
HER2/chr17 ratio 3.4 – 5.8, >6 HER2 copies*.
Virtually all cells are negative for both HER2 and chr17 signals and no HER2 gene status can be determined. 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 and 3a – same tumour.

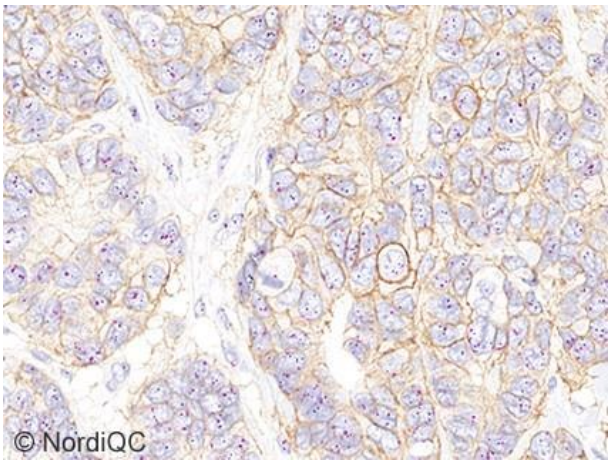


Fig. 4a
Optimal staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.0 – 1.2, <4 HER2 copies*.
The gene protein 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/chr17 gene status simultaneously.
The participant interpreted this tumour as non-amplified being concordant to the status determined by NordiQC and virtually all participants
Compare with Fig. 1b. – same tumour.

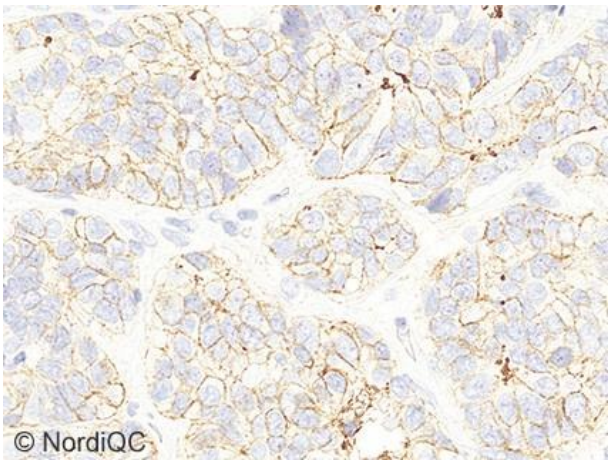


Fig. 4b
Insufficient staining of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 5 without HER2 gene amplification:
HER2/chr17 ratio 1.0 – 1.2, <4 HER2 copies*.
The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown.
The IHC level is interpreted as 2+ but the vast majority of neoplastic cells are totally negative concerning HER2 and Chr 17 signals and thus cannot reliably be scored. This aberrant staining reaction / "negative spot artefact" was most likely caused by a technical issue during the staining process in the BenchMark instrument.

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

SN/LE 28.11.2023