Assessment Run H26 2024

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 H26[#]**

	**HER2 IHC***	**Dual - BRISH****	**FISH*****	**FISH*****
	IHC score	**HER2/chr17 ratio¤**	**HER2/chr17 ratio¤**	**HER2 copies**
1. Breast carcinoma	1+	1.29	1.28	1.6
2. Breast carcinoma	3+	3.15	4.59	6.65
3. Breast carcinoma	0	0.86	0.45	1.25
4. Breast carcinoma	2+	1.2	1.07	3.1
5. Breast carcinoma	3+	5.06	6.52	10.1

* PATHWAY® (Ventana), 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 ratios.
[#] Same block as used in H25.

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. Small blank spots <25% of the core was accepted.

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 run H24 compared to previous assessments. 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 gene status. 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 4: non-amplified
- Breast carcinoma, no. 2 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)

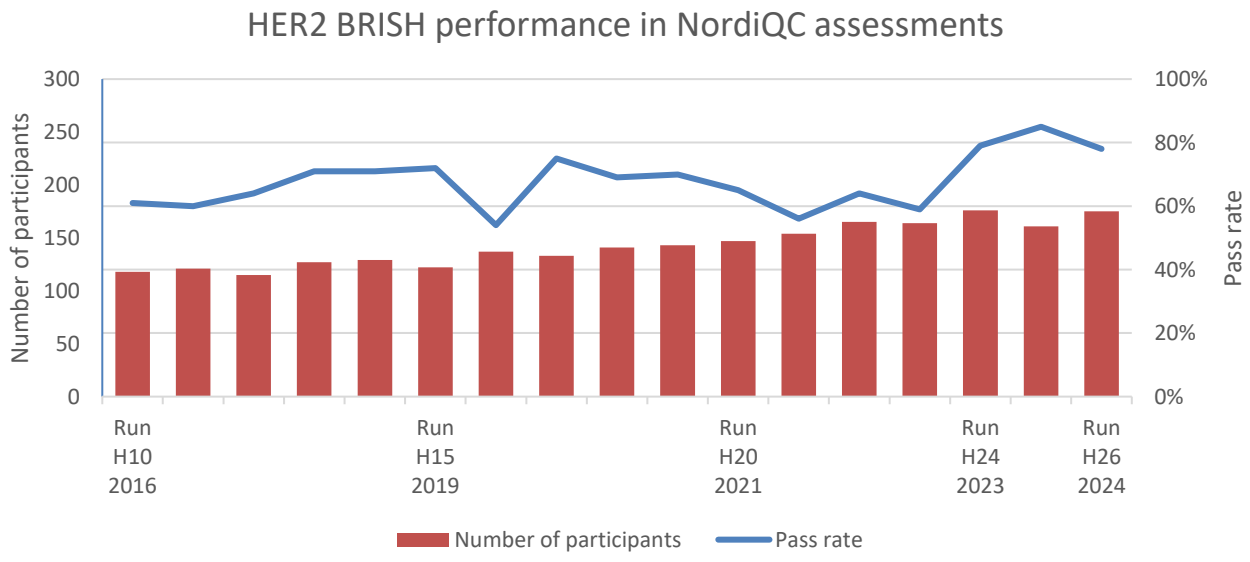
Participation

Number of laboratories registered for HER2 BRISH	182
Number of laboratories returning slides	175 (96%)
Number of laboratories returning scoring sheet	153 (87%)
Number of laboratories registered for HER2 FISH	70
Number of laboratories returning scoring sheet	61 (87%)

At the date of the technical assessment, 96% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment 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 H26 the overall pass rate of 78% was slightly reduced compared to run H25 (85% pass rate), but still improved compared to the levels obtained in the previous assessment runs as illustrated in Graph 1. The improvement seen in the latest runs is mainly caused by new modified assessment criteria applied in run H24 allowing large negative areas of >25% in one or more 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 – 2024**



Results BRISH, technical assessment
In total, 175 laboratories participated in this assessment. 137 laboratories (78%) 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 H26.**

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
INFORM™ HER2 Dual ISH **780-4422 / 800-4422**	7	Ventana/Roche	4	1	0	0	100%	86%
VENTANA HER2 Dual ISH **800-6043**	149	Ventana/Roche	77	49	18	5	85%	52%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA*)**	16	Ventana/Roche	0	2	13	1	13%	0%
Zyto*Dot*® 2C **C-3022 / C-3032**	3	ZytoVision	1	1	1	0	-	-
Total	175		84	53	32	6		
Proportion			48%	30%	18%	4%	78%	

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 (98%) used BRISH HER2 systems from Ventana/Roche. 94% (165 of 175 participants) used the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) and 4% (7 of 175) the INFORM™ HER2 Dual ISH assay (780-4422/800-4422). 2% (3 of 175) used the HER2 BRISH system ZytoDot® 2C (C-3022/C-3032) from Zytovision.
9% of participants (16 of 175) 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 all the five breast carcinomas included in the multi-tissue block was obtained by both 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 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 79% (30 of 38) 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 21% of the insufficient results these were mainly caused by impaired morphology, weak counterstaining and/or bacground reaction as 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% was obtained in the 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 in concordance to the three previous assessment (run H23, H24 and H25), the combined GPA assay (VENTANA HER2 Dual ISH 800-6043 + HER2 IHC) was found less successful giving an unsatisfactory pass rate of 13% - non optimal. The insufficient results were 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 cores no. 2 and 5 However, in tissue core no. 5 only scattered cells displayed HER2 gene signals despite being highly amplified (Her2/Chr17 ratio of 5.1-6.5 and > 6 HER2 signals pr cell). 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 VENTANA HER2 Dual ISH 800-6043 assay and thus not possible to identify any protocol parameters causing the very low pass rate in these three 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. The result was downgraded as the HER2/chr17 signals could neither be evaluated in the tumour cells or the intermingling normal cells as expected and possible without adding IHC to the protocol.

Optimal protocol settings: Two-colour HER2 systems

149 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 8-20 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 87% (52 of 60) was obtained, 58% (35 of 60) being optimal.

3 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 7 min. at 37°C, hybridization at 37°C for 12 hours following a denaturation at 75°C for 6 min. and visualization with the ZytoVision detection kit C-3022.

HER2 ISH interpretation and scoring consensus

Table 3. **NordiQC ISH amplification data***

	NordiQC ISH HER2/chr17 ratio	**NordiQC FISH HER2 copies**	**NordiQC HER2 amplification status**
1. Breast carcinoma	1.3	<2	Non-amplified
2. Breast carcinoma	3.2-4.6	>6	Amplified
3. Breast carcinoma	0.5-0.9	<2	Non-amplified
4. Breast carcinoma	1.1-1.2	<4	Non-amplified
5. Breast carcinoma	5.1-6.5	>6	Amplified

* data from one NordiQC reference laboratory.

Table 4 shows the ISH assays used by the participants and concordance level to the NordiQC data observed. No technical evaluation of FISH protocols was performed. 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, H26**

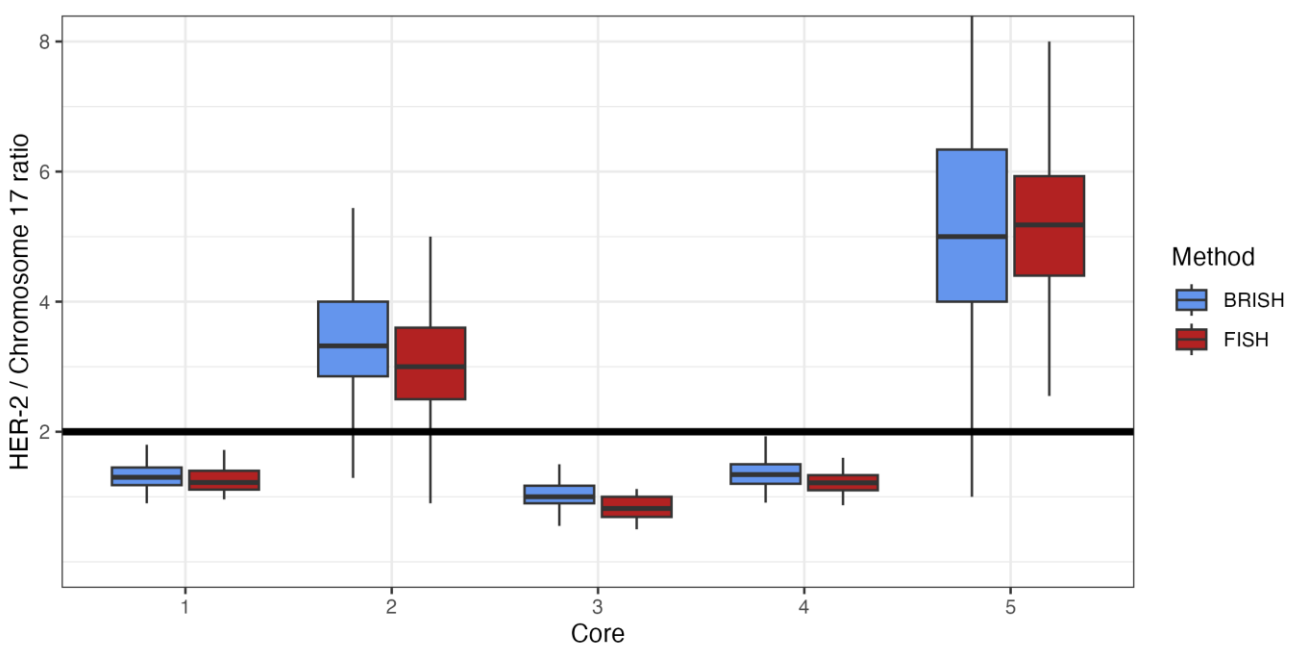
BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
INFORM™ HER2 Dual ISH **780-4422/ 800-4422**	6	Ventana/Roche	4	2	67%
VENTANA HER2 Dual ISH **800-6043**	135	Ventana/Roche	127	8	94%
VENTANA HER2 Dual ISH + IHC **800-6043 + HER2 IHC (GPA)**	9	Ventana/Roche	8	1	89%
Zyto*Dot*® 2C **C-3022 / C-3032**	3	ZytoVision	3	0	-
FISH					
PathVysion HER-2 DNA **6N4630 / 30-161060**	13	Abbott	12	1	92%
HER2 IQFISH **GM333**	5	Dako/Agilent	4	1	80%
HER2 IQFISH **K5731**	13	Dako/Agilent	12	1	92%
SureFISH **G110144G-8**	1	Dako/Agilent	1	0	-
BOND HER2 FISH system **TA9217**	6	Leica Biosystems	6	0	100%
HER2/CEN17 FISH probe **MF2001**	1	Fuzhou Maixin	1	0	-
FISH Kit **MAD-FISH-MDS**	3	Master Diagnostica	2	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**	13	ZytoVision	13	0	100%
ZytoMation ERBB2/CEN17 Dual Color FISH Probe **Z-2292**	4	ZytoVision	4	0	-
Total	214		199	15	93%
Proportion			93%	7%	

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

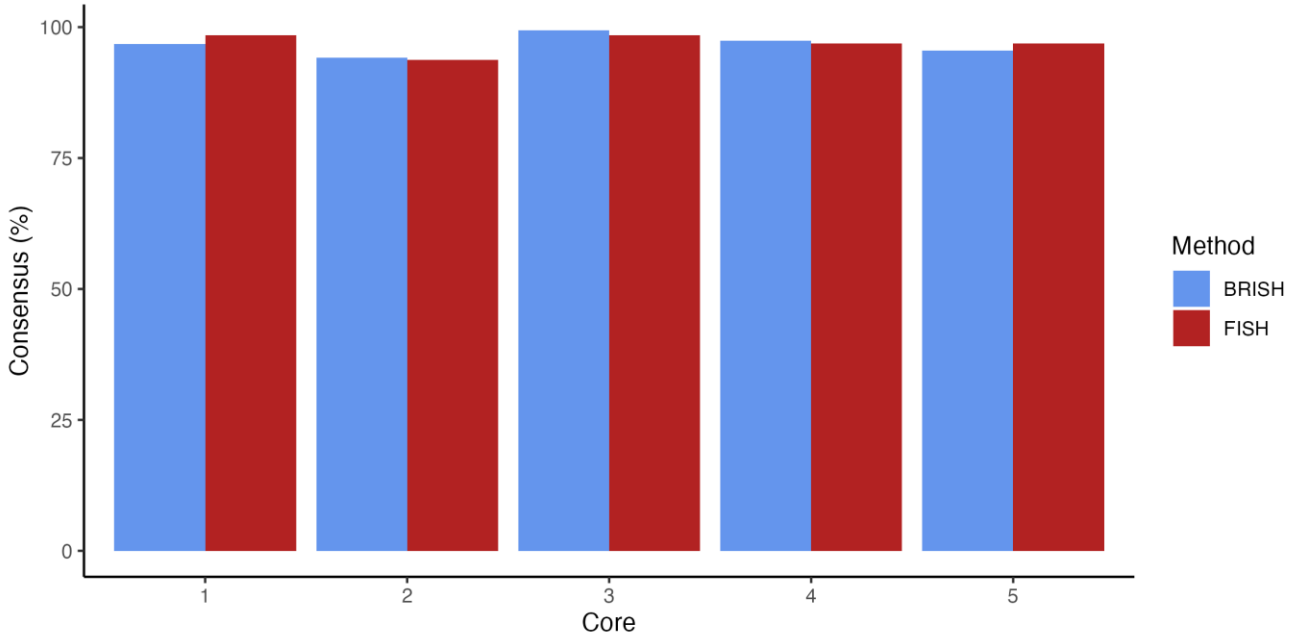
214 of the 245 (87%) participating laboratories completed scoring sheets on the NordiQC website. These evaluations were compared to the HER2 ISH amplification status obtained by the NordiQC reference laboratories, summarized in Graph 2 and 3 (see page 6). For both laboratories performing FISH and BRISH, the overall consensus rate was 93% (57/61 and 142/153, respectively). The level for FISH was comparable to run H24 (94%) and H25 (91%), whereas an increased consensus rate was obtained for BRISH especially compared with H24 (76%).

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

Graph 2: NordiQC HER2 ISH run H26: Participant interpretation of amplification status



Graph 3: NordiQC HER2 ISH run H26: 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 800-6043** and **INFORM™ HER2 Dual ISH 780-4422/800-4422** for Ventana BenchMark platforms. An optimal result could also be obtained by the **Zytovision ZytoDot® 2C C-3022/C-3032** system. Focusing on the technical quality of the HER2 BRISH assays a slightly reduced pass rate of 78% was observed compared to the level of 85% in the previous run H25. In this assessment especially the modified GPA version of the **VENTANA HER2 Dual ISH** system provided an unsatisfactory performance with a pass rate of only 13% and as such with a main impact on the overall reduced pass rate. In contrast, the **VENTANA HER2 Dual ISH** assay applied without IHC gave an overall pass rate of 85%. 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 and more artefacts in combination characterized the insufficient results.

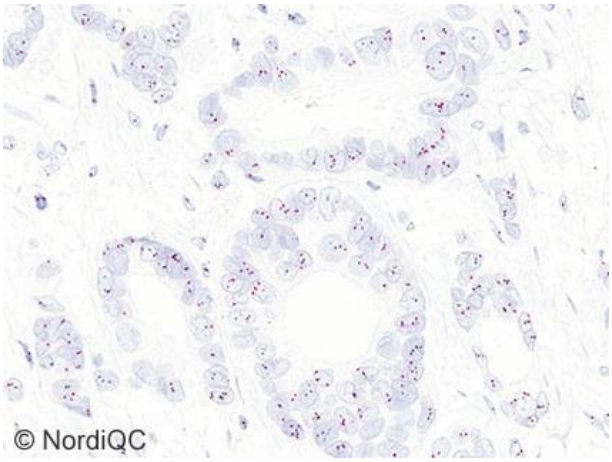


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 1 without HER2 gene amplification:
HER2/chr17 ratio 1.3, <2 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The morphology is well preserved, and signals distinctively demonstrated.
NordiQC and most participants interpreted this tumour as non-amplified.

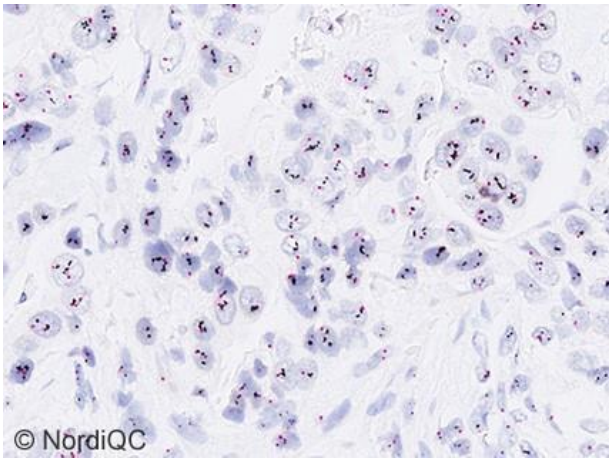


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 with HER2 gene amplification:
HER2/chr17 ratio 3.2 – 4.6, >6 HER2 copies *.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as amplified.

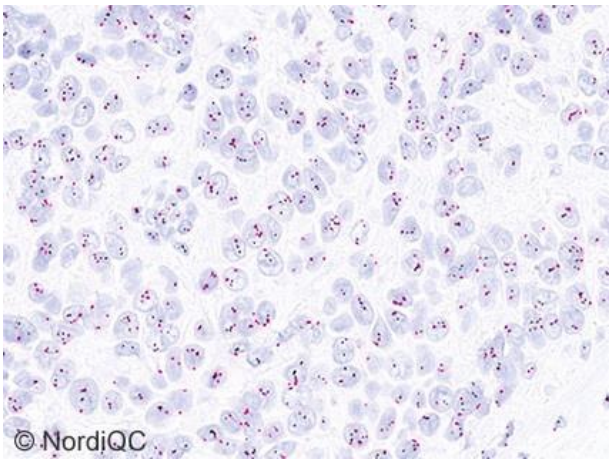


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 without HER2 gene amplification:
HER2/chr17 ratio 1.1 – 1.2, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually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

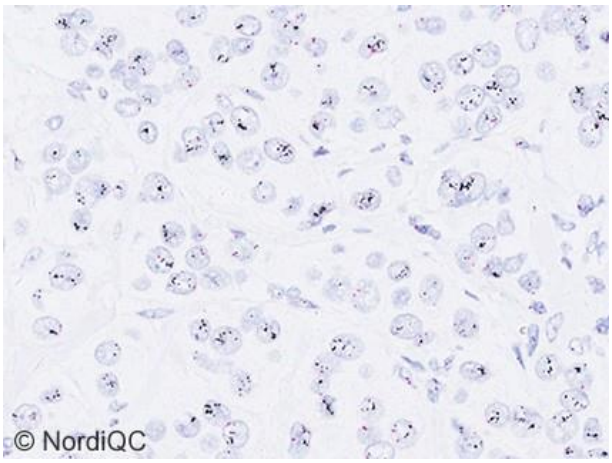


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification:
HER2/chr17 ratio 5.1-6.5, >6 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in all the neoplastic cells.
NordiQC and virtually all participants interpreted this tumour as amplified.

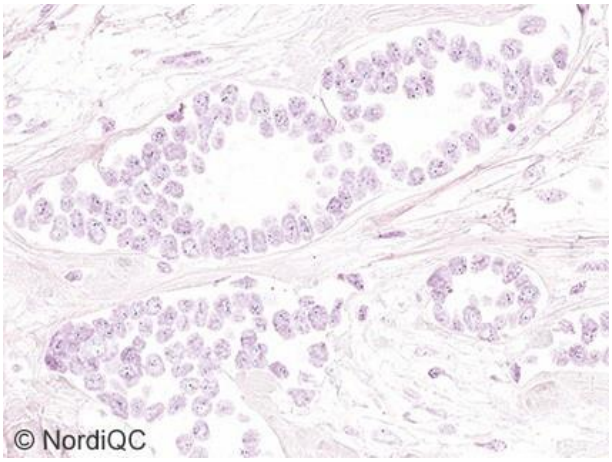


Fig. 3a
Optimal demonstration of the HER2 gene status using the ZytoDot® 2C C-3022/C-3032, ZytoVision, of the breast carcinoma no. 1 without HER2 gene amplification: HER2/chr17 ratio 1.3, <2 HER2 copies*.
The HER2 genes are stained green and chr17 red.
NordiQC and virtually all participants also interpreted this tumour as non-amplified.

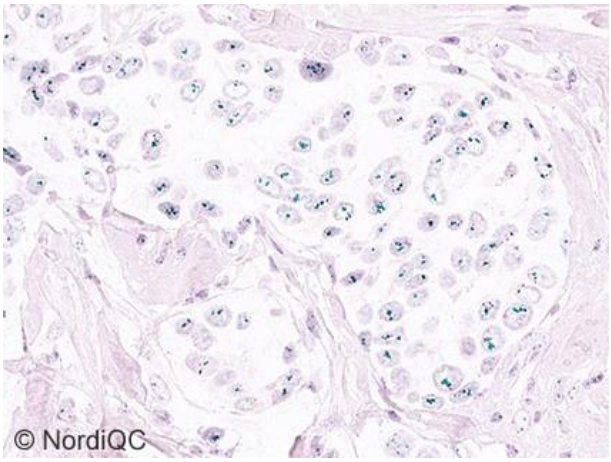


Fig. 3b
Optimal demonstration of the HER2 gene status using the ZytoDot® 2C C-3022/C-3032, ZytoVision, of the breast carcinoma no. 2 with HER2 gene amplification: HER2/chr17 ratio 3.2 – 4.6, >6 HER2 copies *.
The HER2 genes are stained green and chr17 red.
NordiQC and virtually all participants also interpreted this tumour as amplified.

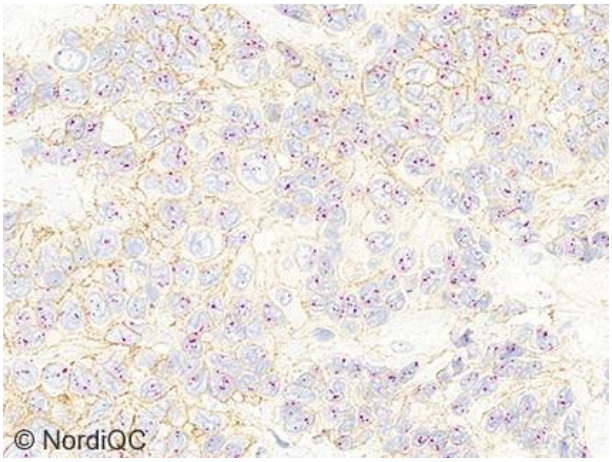


Fig. 4a
Sufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no.
800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma 4 without HER2 gene amplification: HER2/chr17 ratio 1.1 – 1.2, <4 HER2 copies*.
The gene protein 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.
Despite some cells are lacking signals, the HER2 gene status can be established.
The participant interpreted this tumour as non-amplified being concordant to the status determined by NordiQC and virtually all participants
Also compare with Fig. 4b. – same protocol.

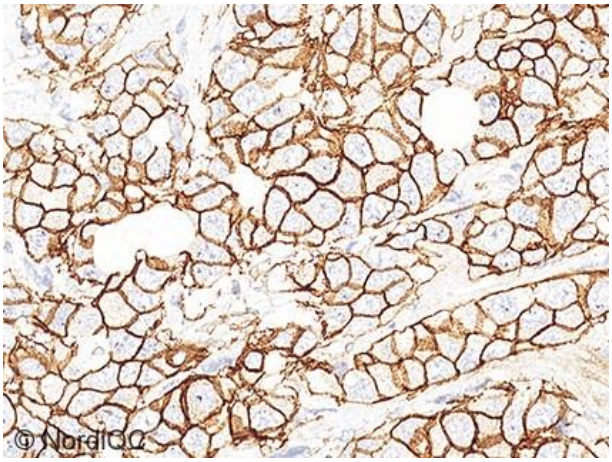


Fig. 4b
Insufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no.
800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 5 with HER2 gene amplification: HER2/chr17 ratio 5.1-6.5, >6 HER2 copies*.
The gene protein assay (GPA) labels the HER2 genes black, chr17 red and HER2 protein brown.
The IHC level is interpreted as 3+ but the vast majority of both neoplastic and stromal cells are totally negative concerning HER2 and Chr 17 signals and thus cannot reliably be scored. The ISH protocol applied was similar to successful ISH protocols and the aberrant staining reaction was most likely caused by the IHC DAB chromogen deposition in the GPA assay hindering penetration of the probes in the cells.

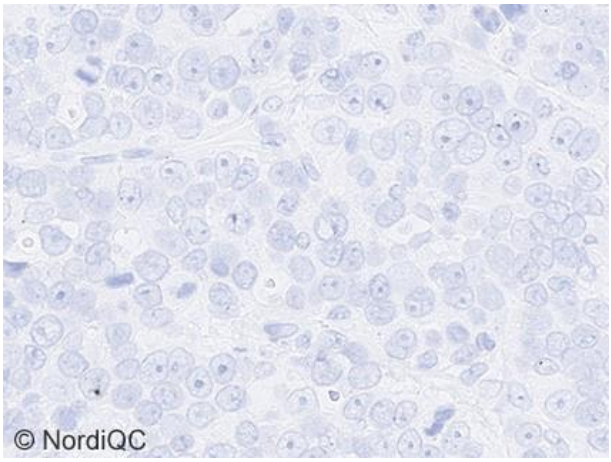


Fig. 5a
Insufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 without HER2 gene amplification:
HER2/chr17 ratio 1.1 – 1.2, <4 HER2 copies*.
Virtually all cells are negative for both HER2 and chr17 signals and the HER2 gene status cannot 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 – same tumour.

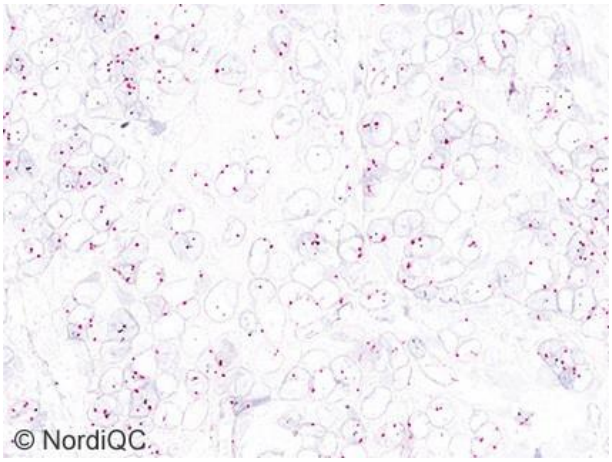


Fig. 5b
Insufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 without HER2 gene amplification:
HER2/chr17 ratio 1.1 – 1.2, <4 HER2 copies*.
The HER2 genes are stained black and chr17 red.
An extensive impaired 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.

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

SN/LE 02.12.2024