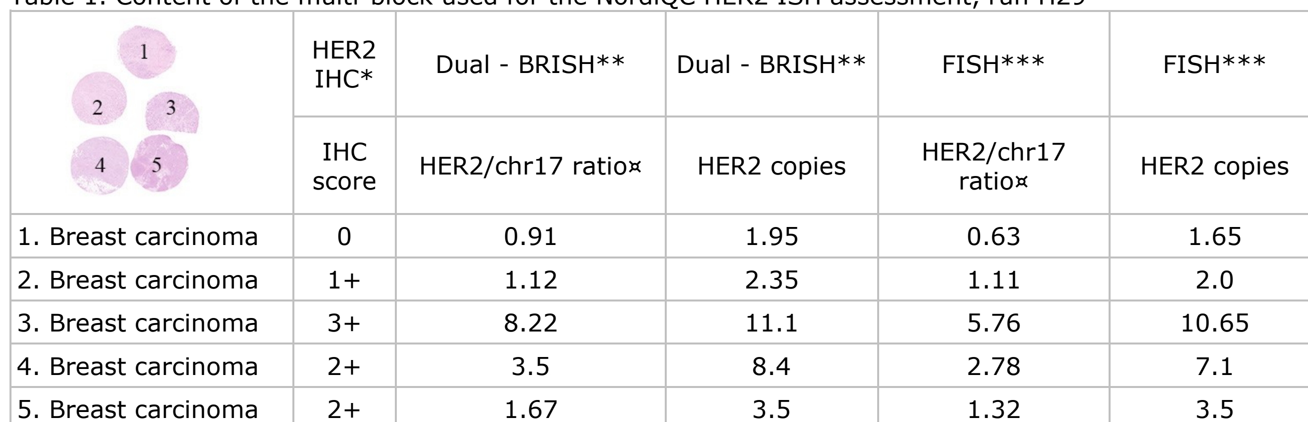
Assessment Run H29 2026

HER2 (BRISH or FISH)

Purpose
The primary focus of this assessment was the evaluation of the technical performance of HER2 Brightfield in-situ hybridization (BRISH) tests performed by the NordiQC participants to demonstrate and establish the HER2 gene amplification level in breast carcinomas. In addition, the participants were asked to interpret and score the amplification status in the breast carcinomas and submit their results to NordiQC to evaluate the inter-observer variability. The evaluation of inter-observer concordance was applicable for participants using either BRISH or Fluorescent in-situ hybridization (FISH) based tests. The assessment marks obtained in NordiQC are indicative of test performance but due to the composition and limited number 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 H29



	HER2 IHC*	Dual - BRISH**	Dual - BRISH**	FISH***	FISH***
	IHC score	HER2/chr17 ratio¤	HER2 copies	HER2/chr17 ratio¤	HER2 copies
1. Breast carcinoma	0	0.91	1.95	0.63	1.65
2. Breast carcinoma	1+	1.12	2.35	1.11	2.0
3. Breast carcinoma	3+	8.22	11.1	5.76	10.65
4. Breast carcinoma	2+	3.5	8.4	2.78	7.1
5. Breast carcinoma	2+	1.67	3.5	1.32	3.5

* VENTANA® HER2 (4B5), 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.

All tissues were fixed for 24-48 hours in 10% neutral buffered formalin according to the ASCO/CAP 2023 guidelines for tissue preparation of breast tissue for HER2 ISH analysis.

Method - HER2 BRISH, Technical assessment
The NordiQC assessors evaluate the technical quality of the BRISH tests and at this point do not conduct a precise estimate of the HER2 amplification status. The main criteria for the technical evaluation are 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 were observed. Small blank spots <25% of the core were 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.

We observed that core 5 was challenging to interpret, primarily due to impaired morphology. However, it was still included in the assessment, as it contained sufficiently preserved areas that allowed evaluation. Notably, approximately 10 participants classified this core as "not suitable for scoring" in their assessments.

Note that the assessment criteria were modified in run H24 compared to previous assessments. Large negative areas (> 25% of individual tissue cores) were accepted, provided that the HER2 gene amplification level still reliably could be evaluated. However, slides containing such large negative areas were not eligible

for an optimal assessment and were instead downgraded to "good," assuming all other aspects of the evaluation were optimal.

HER2 BRISH and FISH interpretation
For both BRISH and FISH, participating laboratories were asked to submit their scores including interpretation of the HER2/chr17 gene status for each tissue. Results were compared with NordiQC reference data from FISH and BRISH analyses to assess scoring concordance.

Consensus scores from the NordiQC BRISH/FISH reference laboratories
- Breast carcinoma, no. 1, 2 and 5: non-amplified
- Breast carcinoma, no. 3 and 4: amplified

The ASCO/CAP 2023 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)

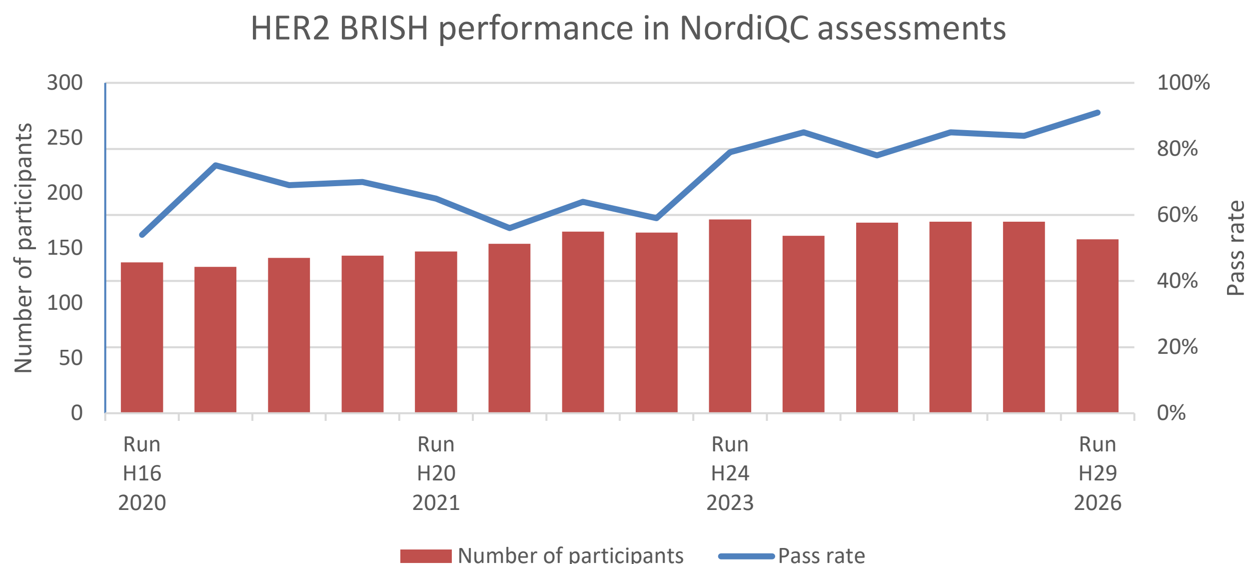
Participation

Number of laboratories registered for HER2 BRISH	206
Number of laboratories returning BRISH slides	158 (77%)
Number of laboratories registered for HER2 FISH	68
Number of laboratories returning scoring sheet (BRISH and FISH)	230 (84%)

At the time of the technical assessment, 77% of the participants registered for BRISH had returned the circulated NordiQC slides. Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Performance history
In this assessment run (H29), the overall pass rate of 91% showed an increase compared to the previous assessment run H28 (84% pass rate), as illustrated in Graph 1. The improvement seen in the latest runs is mainly caused by the new modified assessment criteria introduced in run H24, which allow large negative areas (>25% in one or more of the tissue cores) provided that adequate evaluation of the HER2/chr17 ratio still could be obtained.

Graph 1. Proportion of sufficient results for HER2 BRISH in NordiQC assessments, 2020 – 2026



Results BRISH, technical assessment
In total, 158 laboratories participated in this assessment. 143 laboratories (91%) 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 H29.

Two colour HER2 systems	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
VENTANA HER2 Dual ISH 800-6043	145	Ventana/Roche	57	73	13	2	90%	39%
VENTANA HER2 Dual ISH + IHC 800-6043 + HER2 IHC (GPA*)	10	Ventana/Roche	3	7	0	0	100%	30%
Zyto*Dot*® 2C C-3022 / C-3032	2	ZytoVision	1	1	0	0	-	-
One colour HER2 systems								
Zyto*Dot*® C-3003	1	ZytoVision	0	1	0	0	-	-
Total	158		61	82	13	2		
Proportion			39%	52%	8%	1%	91%	

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, virtually all participants (98%) used BRISH HER2 systems from Ventana/Roche. Of the participants, 92% (145 of 158) used the VENTANA HER2 Dual ISH DNA Probe Cocktail (800-6043) and 2% (3 of 158) used the HER2 BRISH system ZytoDot®.
6% of participants (10 of 158) 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 assessed.

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 Zyto*Dot*® 2C systems.
Insufficient results were most frequently characterized by large negative areas (>25%) in one or more breast carcinoma samples, compromising evaluation of the HER2/chr17 ratio. Additional causes included impaired morphology and generally weak or absent signals for HER2 and/or chr17 or weak counterstaining compromising the identification of tumour cells for scoring.
These artefacts were typically observed as single features or combined with other artefacts and were seen in 93% (14 of 15) of the insufficient results.

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 2019-2023. A cumulated average pass rate of 65% was obtained in the NordiQC assessment runs H15-H23. In all these runs, the ISH rejection criteria defined in the 2013/2018 ASCO/CAP HER2 guidelines were applied. In brief, a repeated test must be performed if more than 25% of the signals/cells cannot be interpreted in the sample evaluated.
However, based on internal discussions within the NordiQC assessor panel and correspondence with participants and Ventana/Roche, it was decided to modify the assessment criteria to accept larger negative areas within individual tissue cores, provided that the HER2/chr17 ratio could still be reliably assessed.

Slides with large negative areas were not compatible with an optimal assessment and were downgraded to "good," even if the remaining evaluation was otherwise optimal.
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 modify the criteria was also based on the fact that virtually all participants now use the same or similar protocol settings for HER2 BRISH, which are locked by the vendor and therefore cannot be further optimized.

In contrast to the three previous assessments (runs H26-H28), the combined GPA assay (VENTANA HER2 Dual ISH 800-6043 + HER2 IHC) performed better in the present run, achieving a pass rate of 100% compared with 58% in run H28.
In this run, the combined GPA assay (VENTANA HER2 Dual ISH 800-6043 + HER2 IHC) was more successful compared to the "standard" VENTANA HER2 Dual ISH assay. The "standard" VENTANA HER2 Dual ISH assay provided a pass rate of 90% (39% being optimal), whereas the GPA assay gave a pass rate of 100% (30% being optimal).

Optimal protocol settings: Two-colour HER2 systems
A temporary data entry error affecting the registration of HIER settings (CC1 and CC2) on the NordiQC homepage influenced the validity of the numbers used in the subsequent data analysis. In our calculations, we included all protocols with correct entries indicating HIER in CC1 and CC2 for 40 minutes. We also included protocols that reported only CC1 or only CC2 for 40 minutes, as well as protocols in which two values had been entered under HIER. In addition, we performed a comparison with laboratories that used CC1 and CC2 in run H27 and H28.

145 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 82% (39 of 47) was obtained, 36% (17 of 47) being optimal.

10 laboratories used the **VENTANA Dual ISH system 800-6043** (Ventana/Roche) in combination with immunohistochemical demonstration for **HER2 PATHWAY**® (Ventana/Roche). Optimal results using this GPA assay were typically based on HIER in CC1 followed by HIER in CC2 for a total of 56 min. and subsequent proteolysis in ISH Protease 3 for 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 40% (4 of 10) was obtained, 33% (1 of 3) being optimal.

HER2 ISH interpretation and scoring consensus
Table 3. NordiQC ISH amplification data*

	NordiQC ISH HER2/chr17 ratio	NordiQC ISH HER2 copies	NordiQC HER2 amplification status
1. Breast carcinoma	0.63-0.91	<2 (1.65-1.95)	Non-amplified
2. Breast carcinoma	1.11-1.12	<3 (2.0-2.35)	Non-amplified
3. Breast carcinoma	5.76-8.22	>10 (10.65-11.1)	Amplified
4. Breast carcinoma	2.78-3.5	>7 (7.1-8.4)	Amplified
5. Breast carcinoma	1.32-1.67	<4 (3.5)	Non-amplified

* Data from two NordiQC reference laboratories

Table 4 shows the ISH assays used by the participants and their concordance with the NordiQC-assessed amplification status.
No technical evaluation of FISH protocols was performed. It must be emphasized that it was not possible to identify the cause of an aberrant interpretation of the HER2 status, and whether this was related to the technical performance of the FISH assay or the interpretation by the observer(s).

Table 4. Level of consensus to the NordiQC HER2 amplification status, H29

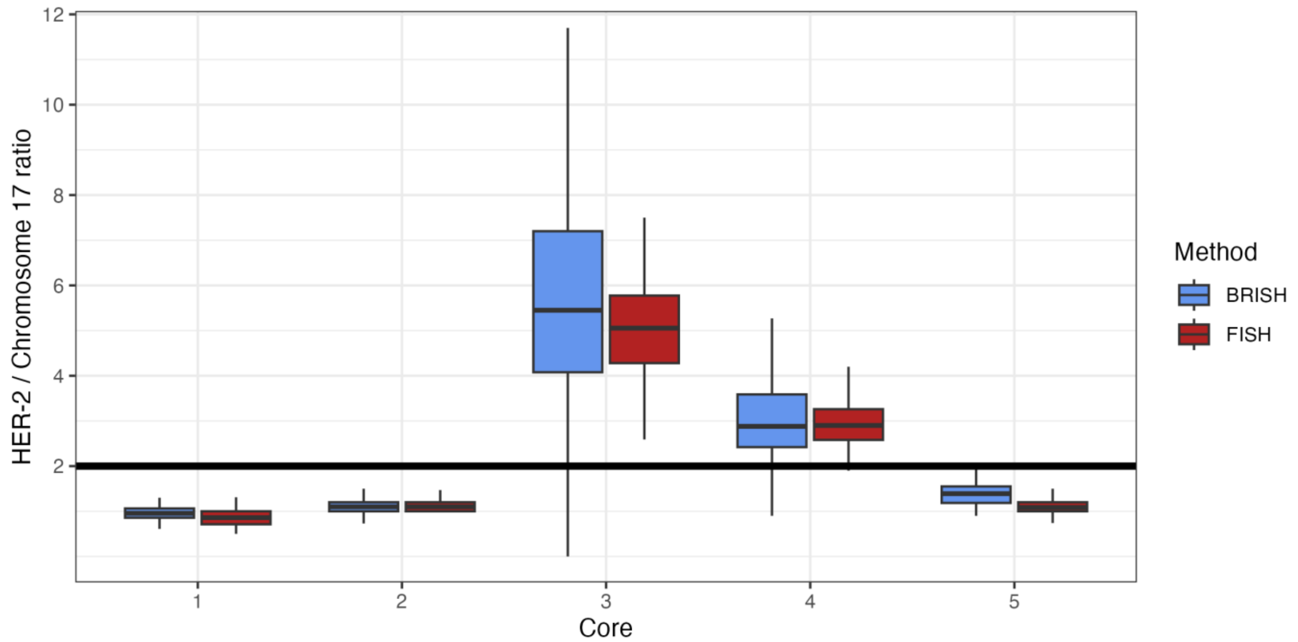
BRISH	n*	Vendor	Consensus	No consensus	Consensus rate
VENTANA HER2 Dual ISH 800-6043	157	Ventana/Roche	117	40	75%
VENTANA HER2 Dual ISH + IHC 800-6043 + HER2 IHC (GPA)	8	Ventana/Roche	6	2	75%
ZytoDot® 2C C-3022 / C-3032	4	ZytoVision	3	1	-
FISH					
PathVysion HER-2 DNA 6N4630 / 30-161060	7	Abbott	7	0	100%
CytoTest CT-PAC001	1	CytoTest Inc	1	0	-
HER2 IQFISH K5731	13	Dako/Agilent	10	3	77%
HER2 IQFISH GM333	4	Dako/Agilent	4	0	-
SureFISH G110144G-8	2	Dako/Agilent	2	0	-
BOND HER2 FISH system TA9217	7	Leica Biosystems	5	2	71%
HER2/CEN17 FISH probe MF2001	1	Fuzhou Maixin	1	0	-
FISH Kit MAD-FISH-MDS	1	Master Diagnostica	1	0	-
Rembrandt Her-2-C17 probe C801K.5206	1	PanPath	0	1	-
ZytoLight Z-2015 / Z-2020/ Z-2077	21	ZytoVision	21	0	100%
XL ERBB2 (HER2/NEU) D-6010-100-OG 10	1	MetaSystems Probes	1	0	-
HER2/CEN17 FISH probe MAD-001FA	1	Vitro	1	0	-
Fast Fluorescence in Situ Hybrid ization Probe Kit FP-001	1	Wuhan Healthcare Bio	1	0	-
Total	230		181	49	
Proportion			79%	21%	

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

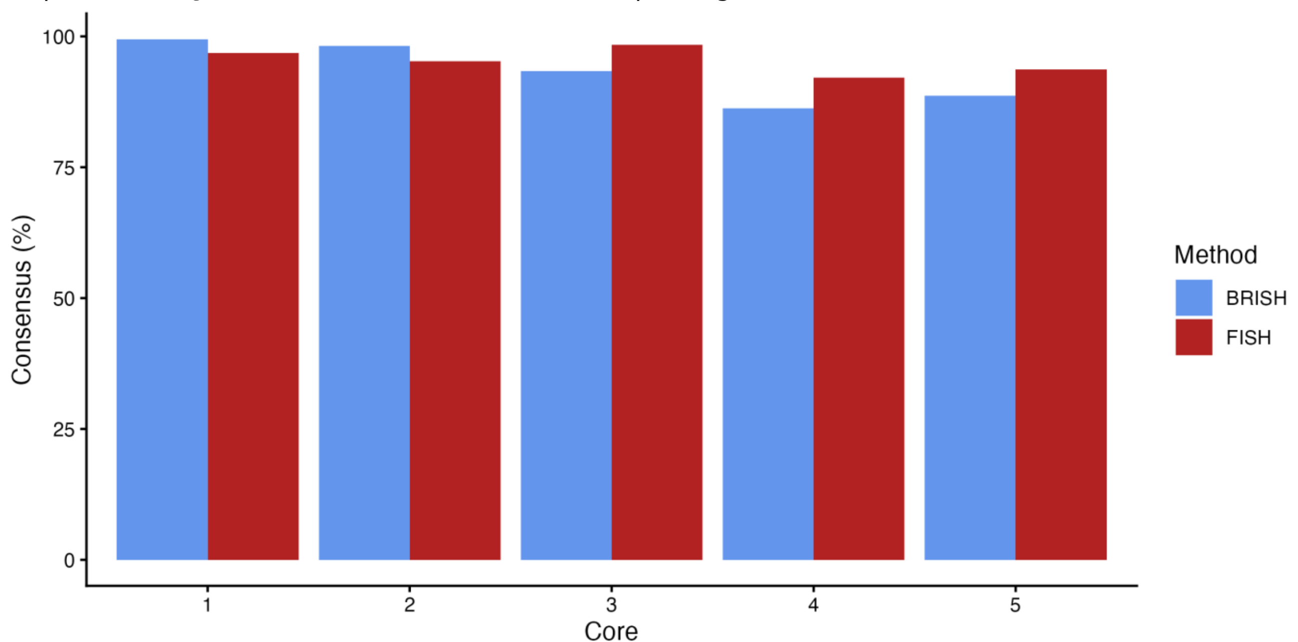
230 of the 274 (84%) participating laboratories completed scoring sheets on the NordiQC website. These evaluations were compared to the HER2 ISH amplification status obtained by the NordiQC reference laboratories, summarized in Graph 2 and 3 (see page 6). For all laboratories performing either FISH or BRISH, the overall consensus rate was 79%. For the laboratories performing FISH, the overall consensus rate was 90% (55/61), and 75% (126/169) for laboratories using BRISH. The level for BRISH was lower than run H28 (86%), whereas the consensus rate for FISH was comparable to run H28 (91%).

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

Graph 2: NordiQC HER2 ISH run H29: Participant interpretation of amplification status



Graph 3: NordiQC HER2 ISH run H29: Consensus depending on method



Conclusion

In this assessment, a technically optimal demonstration of HER2 BRISH could be obtained by the widely used Ventana/Roche two-colour **HER2 system VENTANA HER2 Dual ISH 800-6043** and the modified GPA version of the system for Ventana BenchMark platforms. Focusing on the technical quality of these HER2 BRISH assays, a pass rate of 90% was observed to be slightly better than the level of 84% in the last run H28. In this assessment the modified GPA version of the **VENTANA HER2 Dual ISH** system performed with an improved pass rate at 100%, which was significantly better than the 58% pass rate seen in H28. The insufficient results were mainly caused by generally negative areas (>25%) or completely false

negative HER2/chr17 signals in one or more of the included tissue cores and typically observed in combination with artefacts as impaired morphology and weak counterstaining.

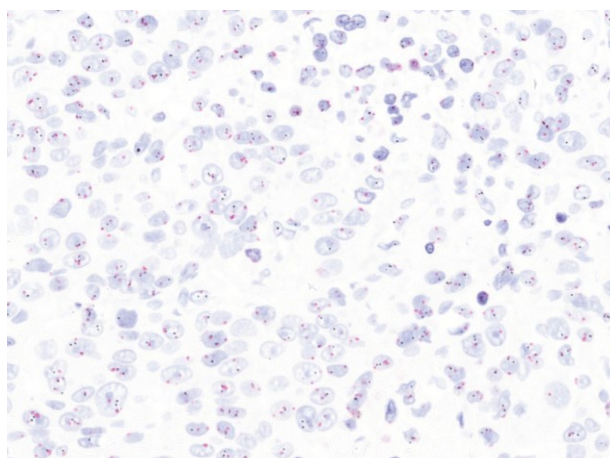


Fig. 1a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 1 without HER2 gene amplification:
HER2/chr17 ratio 0.63 – 0.91, <2 HER2 copies*.
The HER2 genes are stained black and chr17 red.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

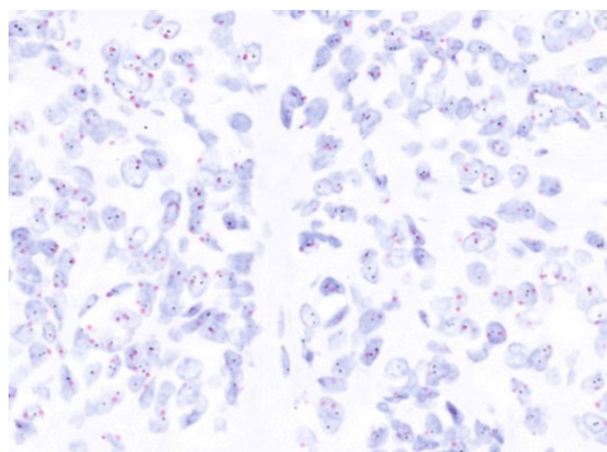


Fig. 1b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification:
HER2/chr17 ratio 1.11 – 1.12, <3 HER2 copies *.
The HER2 genes are stained black and chr17 red.
The morphology is well preserved, and signals distinctively demonstrated.
NordiQC and virtually all participants interpreted this tumour as non-amplified.

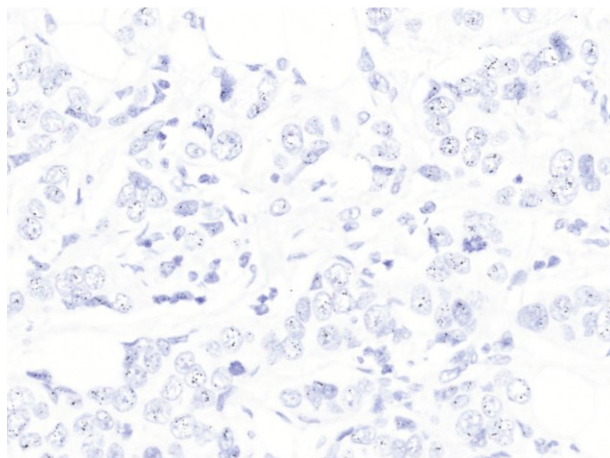


Fig. 2a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification:
HER2/chr17 ratio 5.76 – 8.22, >10 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The signals are distinctively demonstrated in all the neoplastic cells.
NordiQC and virtually all participants interpreted this tumour as amplified.

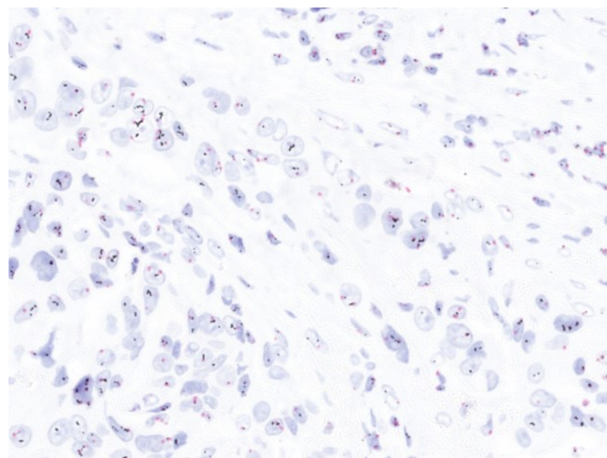


Fig. 2b
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 4 with HER2 gene amplification:
HER2/chr17 ratio 2.78 – 3.5, >7 HER2 copies*.
The HER2 genes are stained black and chr17 red.
NordiQC and most participants interpreted this tumour as amplified.

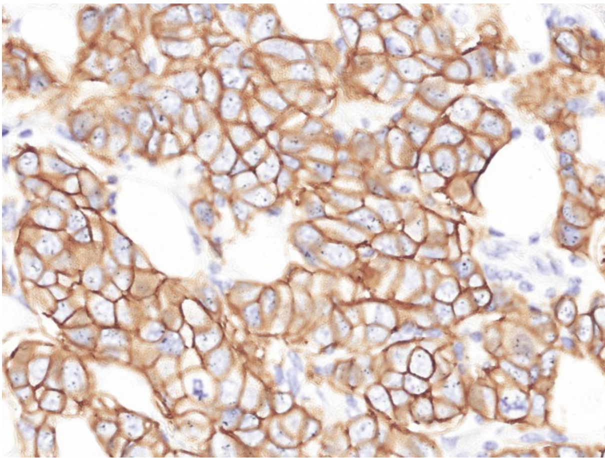


Fig. 3a
Optimal demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification:
HER2/chr17 ratio 5.76 – 8.22, >10 HER2 copies*.
The HER2 genes are stained black and chr17 red.
The IHC level is interpreted as 3+ and the GPA assay visualizes the HER2 protein expression and the HER2/chr17 gene status simultaneously.
Although some cells lack signals, the HER2 gene status can be established.
The participant interpreted this tumour as amplified being concordant to the status determined by NordiQC and virtually all participants.

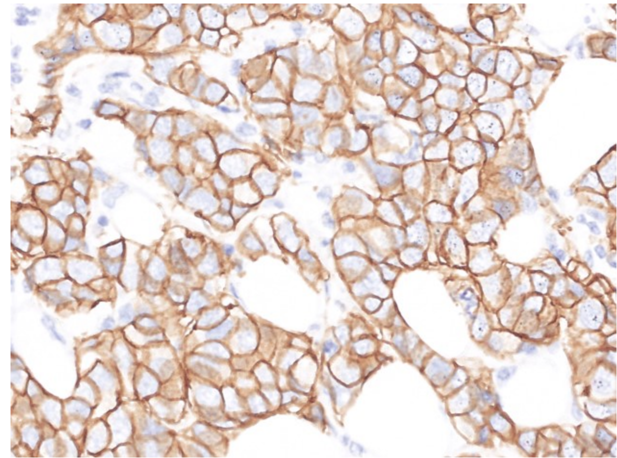


Fig. 3b
Sufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, in combination with HER2 IHC using PATHWAY, Ventana/Roche, of the breast carcinoma no. 3 with HER2 gene amplification:
HER2/chr17 ratio 5.76 – 8.22, >10 HER2 copies*.
The gene protein assay (GPA) labels the HER2 genes black, chr17 red, and the HER2 protein brown.
The IHC level is interpreted as 3+, and the GPA assay visualizes the HER2 protein expression and the HER2/chr17 gene status simultaneously. Areas with negative staining >25% are observed; however, this does not affect the evaluation of the remaining core, and HER2 and chr17 signals can still be assessed.
The applied ISH protocol was similar to Optimal ISH protocols, and the lack of staining reaction is most likely caused by DAB chromogen deposition from the IHC in the GPA assay, hindering probe penetration into the cells, or caused by a technical issue during the staining process in the BenchMark instrument.

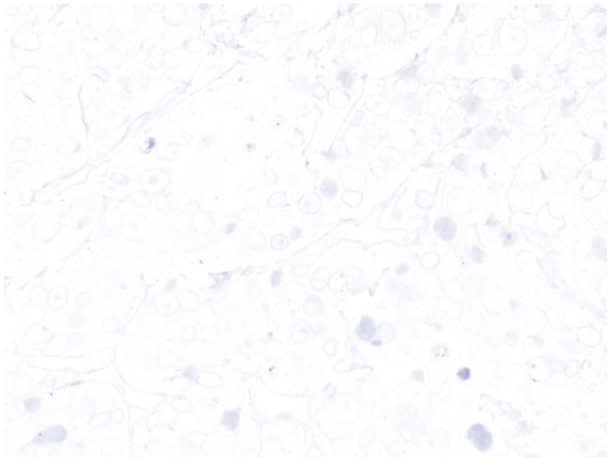


Fig. 4a
Insufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 1 without HER2 gene amplification:
HER2/chr17 ratio 0.63 – 0.91, <2 HER2 copies*.
The HER2 genes are expected to be stained black and chr17 red.
Extensively 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. (Compare with Fig. 1a of the same tumour). In addition, most HER2 signals are absent and mainly chr 17 signals are identified and HER2 status cannot be determined.

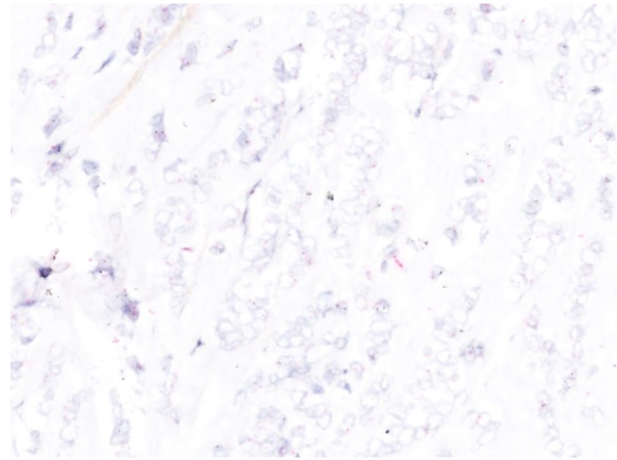


Fig. 4b
Insufficient demonstration of the HER2 gene status using the VENTANA HER2 Dual ISH kit cat. no. 800-6043, Ventana/Roche, of the breast carcinoma no. 2 without HER2 gene amplification:
HER2/chr17 ratio 1.11 – 1.12, <3 HER2 copies *.
The HER2 genes are expected to be stained black and chr17 red.
Areas with impaired morphology and negative areas are observed. "Negative artefact" was most likely caused by a technical issue during the staining process in the BenchMark instrument. Compare with Fig. 1b – same tumour and protocol, with an optimal result.

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

TB/LE/RR/KBA 11.04.2026