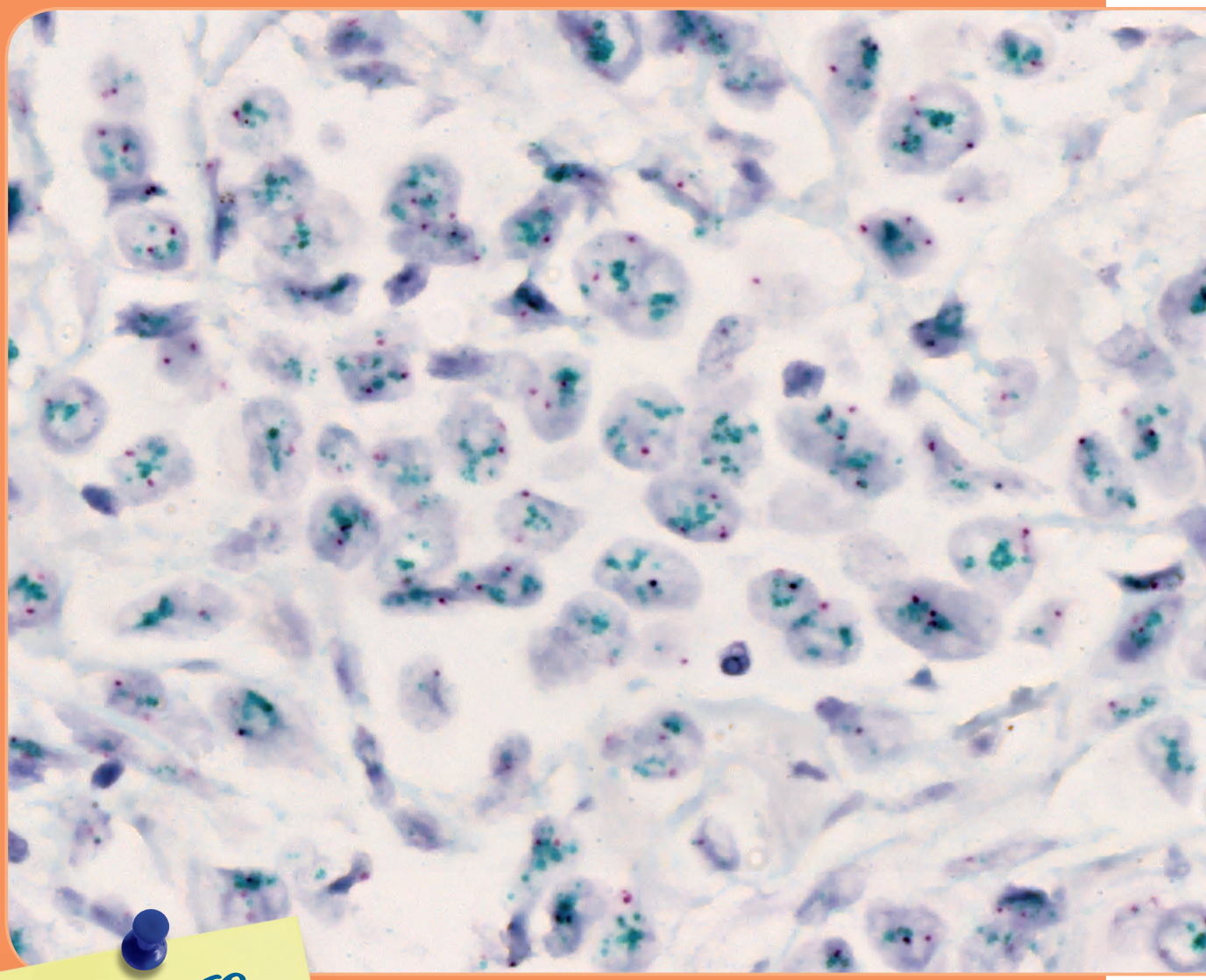
CE IVD

ZytoDot® 2C Products for CISH analysis

Breast Cancer Interpretation Guide



UPDATED

According to the NEW ASCO/CAP Guidelines 2018

ZYTOVISION

Molecular diagnostics simplified

Breast Cancer Interpretation Guide

ERBB2 (a.k.a. HER2) testing must be requested on every primary invasive breast cancer. Additionally, it is recommended to perform ERBB2 testing on metastatic sites, classified as stage IV, if tissue sample is available. This is to guide decision to pursue ERBB2-targeted therapy.
This Interpretation Guide is based on ASCO (American Society of Clinical Oncology) and CAP (College of American Pathologists) recommendations for ERBB2 testing in breast cancer (Wolff AC, *et al.* 2018*). The 2013 Guidelines Update adds bright-field ISH as an acceptable method for ERBB2 testing.
This Interpretation Guide does not claim to be complete in reference to clinical usage and appraisal of results. Moreover, it should be regarded as a practical help of ERBB2 CISH evaluation in regards to clinical decision making.

What's New?

- Revised definition of IHC 2+ (equivocal) cases as invasive breast cancer with „weak to moderate complete membrane staining observed in > 10% of tumor cells".
- If the initial ERBB2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new ERBB2 test on a surgical specimen is no longer stated as mandatory.
- Less common CISH patterns should be reviewed by IHC using the same tissue sample:
  1) ERBB2/CEN 17 ratio of ≥ 2.0 with an average ERBB2 gene copy number of < 4 signals/nucleus (CISH group 2)
  2) ERBB2/CEN 17 ratio of < 2.0 with an average ERBB2 gene copy number of ≥ 6 signals/nucleus (CISH group 3)
  3) ERBB2/CEN 17 ratio of < 2.0 with an average ERBB2 gene copy number of ≥ 4 and < 6 signals/nucleus (CISH group 4)

IHC results:
a) IHC result is 3+: ERBB2 positive
b) IHC result is 2+: Recount at least 20 cells by CISH in the IHC 2+ staining area by an additional observer. New CISH category or ERBB2 diagnosis is negative (CISH group 2+4)/positive (CISH group 3)
c) IHC result is 0 or 1+: ERBB2 negative

- The concomitant IHC review for ISH group 2 to 4 is recommended to be performed in the same institution to ensure parallel interpretation and quality of the two assays.
- The usage of a dual color ISH probe is recommended by the Expert Panel instead of using a single color ISH assay. Concomitant IHC review becomes part of the interpretation of single color ISH results.

Evaluation Procedure

1. Localize the invasive component of a breast cancer specimen.
2. Screen the entire slide prior to CISH signal counting for areas of aggregate population of ERBB2 amplified cells.
3. The area for counting should include clearly distinguishable and well distributed nuclei.
4. Count at least 20 non-overlapping cells in two separate areas of a population of tumor cells in the invasive component of the carcinoma (at least 10 cells per area). If there is a second population of tumor cells with increased ERBB2 signals per cell comprising > 10% of tumor cells on the slide, perform a separate counting of at least 20 cells within this cell population.
5. Determine the ERBB2 status according to the ERBB2 Interpretation Guide.
6. A second person should count an additional 20 non-overlapping cells if ERBB2/CEN 17 ratio is 1.8 - 2.2 and ERBB2 copy number is < 6.0.
7. Report if ERBB2 status is indeterminate due to e.g. artifacts, analytic testing failure, etc. or if ERBB2 status is discordant with other histopathologic findings and repeat test with another specimen.

The validation of CISH probes is required for each type of tissue that is intended to be tested in clinical practice since different tissue types exhibit different cell types with different nuclei diameters which may result in different cut-off values. In order to correctly interpret the results, the user must validate this product prior to use in diagnostic procedures according to national and/or international guidelines.

*Wolff AC, *et al.* (2018) J Clin Oncol 36: 2105-2122

ERBB2 Interpretation Guide

Invasive component of breast cancer specimen

ERBB2 testing by validated IHC assay

- IHC 3+ positive
- IHC 2+ equivocal
- IHC 1+ negative
- IHC 0 negative

IHC 2+ equivocal:
- New specimen (if available): New IHC Test
- New specimen (if available): CISH Test

IHC 1+ negative:
- Same specimen (Reflex ERBB2 test): CISH Test

Interpretation of ERBB2/CEN 17 ratio
Total score ERBB2: _____ / Total score CEN 17: _____ = _____

ERBB2/CEN 17 ratio ≥ 2.0

Interpretation of average ERBB2 copy number
Total score ERBB2: _____ / Number of counted nuclei: _____ = _____

- CISH group 1) Average ERBB2 copy number ≥ 4.0 → CISH positive
- CISH group 2) Average ERBB2 copy number < 4.0

ERBB2/CEN 17 ratio < 2.0

Interpretation of average ERBB2 copy number
Total score ERBB2: _____ / Number of counted nuclei: _____ = _____

- CISH group 3) Average ERBB2 copy number ≥ 6.0
- CISH group 4) Average ERBB2 copy number ≥ 4.0 and < 6.0

CISH group 5) Average ERBB2 copy number < 4.0 → CISH negative

IHC assessment

Assess IHC using sections from the same tissue sample used for CISH

- IHC 0 or 1+ → ERBB2 negative*
- IHC 2+ → Observer blinded to previous results recounts CISH, counting at least 20 cells
- IHC 3+ → ERBB2 positive

Observer blinded to previous results recounts CISH, counting at least 20 cells:
- Same CISH result (CISH group 2+4) → ERBB2 negative*
- Same CISH result (CISH group 3) → ERBB2 positive
- Other CISH result/category → Final category should be adjudicated per internal procedures

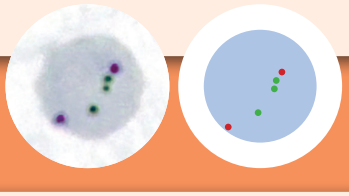
* Evidence is limited on the efficacy of ERBB2-targeted therapy

Signal Interpretation Guide

ERBB2 non-amplified cell

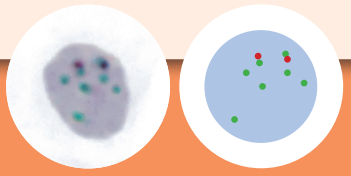
- Count: 2 green and 2 red signals.

ERBB2 non-amplified cell



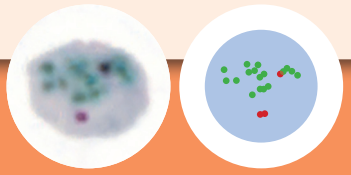
- Count: 2 green and 2 red signals.
  One green signal is split but 2 signals of the same color separated by a distance of ≤ 1 signal diameter, are counted as one.

Cell with low level amplification of ERBB2



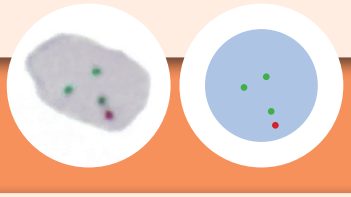
- Count: 7 green and 2 red signals.

Cell with high level amplification of ERBB2



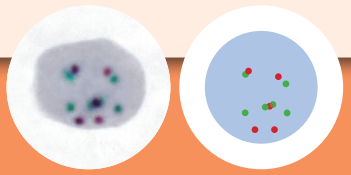
- Green signals overlapping red signals.
  Signal cluster overlapping signal
  Overlapping signals result in brownish staining.

Cell with monosomy of chromosome 17



- Count: 3 green signals and 1 red signal.

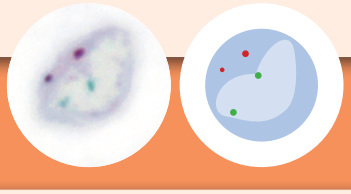
Cell with polysomy of chromosome 17



- Count: 5 green and 5 red signals.

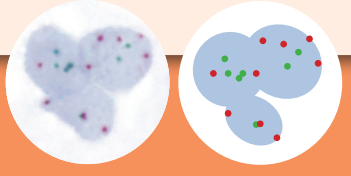
- Artifacts (crush or edge artifacts) that make interpretation difficult should be excluded from counting.
- Do not count if controls are not as expected.
- If > 25% of signals are weak the test cannot be scored.
- The test should be repeated if > 10% of signals occur over cytoplasm.

Over-digested cell



- Over-digestion can be recognized by unstained areas visible inside of the nuclei.
  Over-digested nuclei - Do not count!

Overlapping nuclei



- Nuclei are overlapping. Not all areas of the single nuclei are visible. Exact determination of signals per nucleus is not possible.
  Overlapping nuclei - Do not count!

ZytoDot® 2C Products for CISH analysis

FASTER · IMPROVED SIGNALS · LESS HANDS ON TIME · 2.0 version

ZytoDot® 2C SPEC ERBB2/CEN 17 Probe Kit

The ZytoDot® 2C SPEC ERBB2/CEN 17 Probe Kit contains all necessary reagents to perform user-friendly and successful CISH experiments.

- Heat Pretreatment Solution EDTA
- Pepsin Solution
- ZytoDot® 2C SPEC ERBB2/CEN 17 Probe
- Wash Buffer SSC
- 20x Wash Buffer TBS
- Anti-DIG/DNP-Mix
- HRP/AP-Polymer-Mix
- AP-Red Solution A
- AP-Red Solution B
- HRP-Green Solution A
- HRP-Green Solution B
- Nuclear Blue Solution
- Mounting Solution (alcoholic)

Prod. No.	Product	Label	Tests* (Volume)
C-3032-100	ZytoDot 2C SPEC ERBB2/CEN 17 Probe CE IVD	DIG/DNP	10 (100 µl)
C-3032-400	ZytoDot 2C SPEC ERBB2/CEN 17 Probe CE IVD	DIG/DNP	40 (400 µl)
C-3068-100	ZytoDot 2C SPEC ERBB2/D17S122 Probe CE IVD	DIG/DNP	10 (100 µl)
C-3022-10	ZytoDot 2C SPEC ERBB2/CEN 17 Probe Kit CE IVD Incl. Heat Pretreatment Solution EDTA, 150 ml; Pepsin Solution, 1 ml; Probe, 0.1 ml; Wash Buffer SSC, 210 ml; 20x Wash Buffer TBS, 50 ml; Anti-DIG/DNP-Mix, 1 ml; HRP/AP-Polymer-Mix,1 ml; AP-Red Solution A, 0.1 ml; AP-Red Solution B, 4 ml; HRP-Green Solution A, 0.2 ml; HRP-Green Solution B, 4 ml; Nuclear Blue Solution, 4 ml; Mounting Solution (alcoholic), 1 ml	DIG/DNP	10
C-3022-40	ZytoDot 2C SPEC ERBB2/CEN 17 Probe Kit CE IVD Incl. Heat Pretreatment Solution EDTA, 500 ml; Pepsin Solution, 4 ml; Probe, 0.4 ml; Wash Buffer SSC, 560 ml; 20x Wash Buffer TBS, 2x 50 ml; Anti-DIG/DNP-Mix, 4 ml; HRP/AP-Polymer-Mix, 4 ml; AP-Red Solution A, 0.4 ml; AP-Red Solution B, 15 ml; HRP-Green Solution A, 0.8 ml; HRP-Green Solution B, 15 ml; Nuclear Blue Solution, 20 ml; Mounting Solution (alcoholic), 4 ml	DIG/DNP	40
Related Products			
C-3044-10	ZytoDot 2C CISH Implementation Kit CE IVD Incl. Heat Pretreatment Solution EDTA, 150 ml; Pepsin Solution, 1 ml; Wash Buffer SSC, 210 ml; 20x Wash Buffer TBS, 50 ml; Anti-DIG/DNP-Mix, 1 ml; HRP/AP-Polymer-Mix,1 ml; AP-Red Solution A, 0.1 ml; AP-Red Solution B, 4 ml; HRP-Green Solution A, 0.2 ml; HRP-Green Solution B, 4 ml; Nuclear Blue Solution, 4 ml; Mounting Solution (alcoholic), 1 ml		10

** Using 10 µl probe solution per test. CE IVD only available in certain countries. All other countries research use only! Please contact your local dealer for more information.*

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