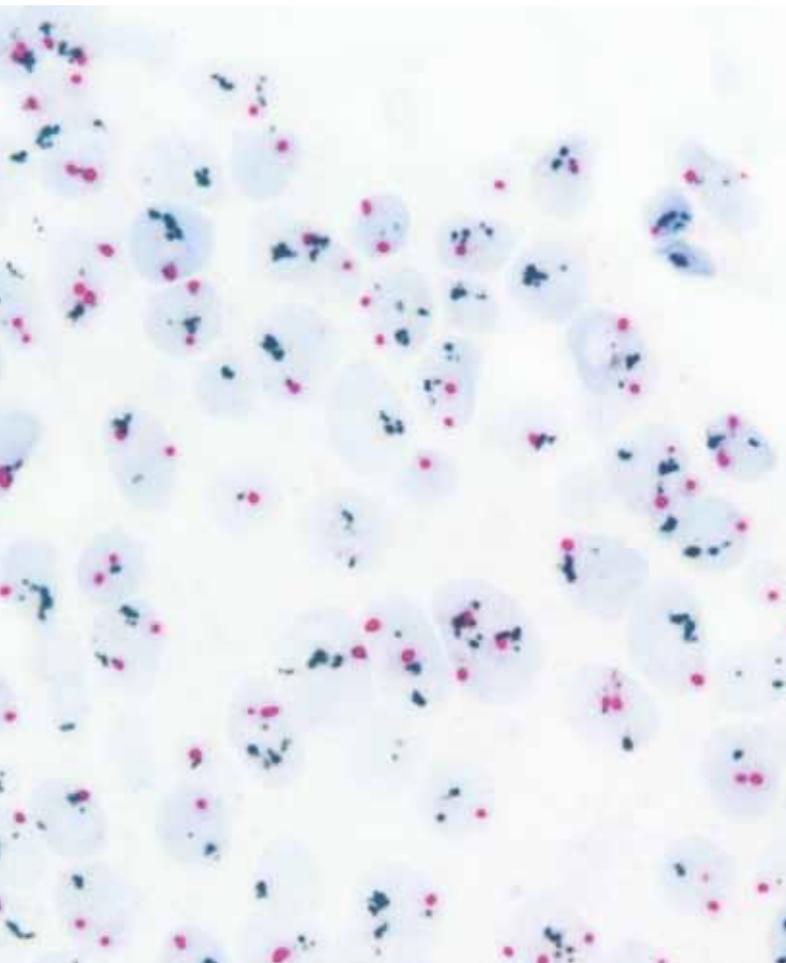


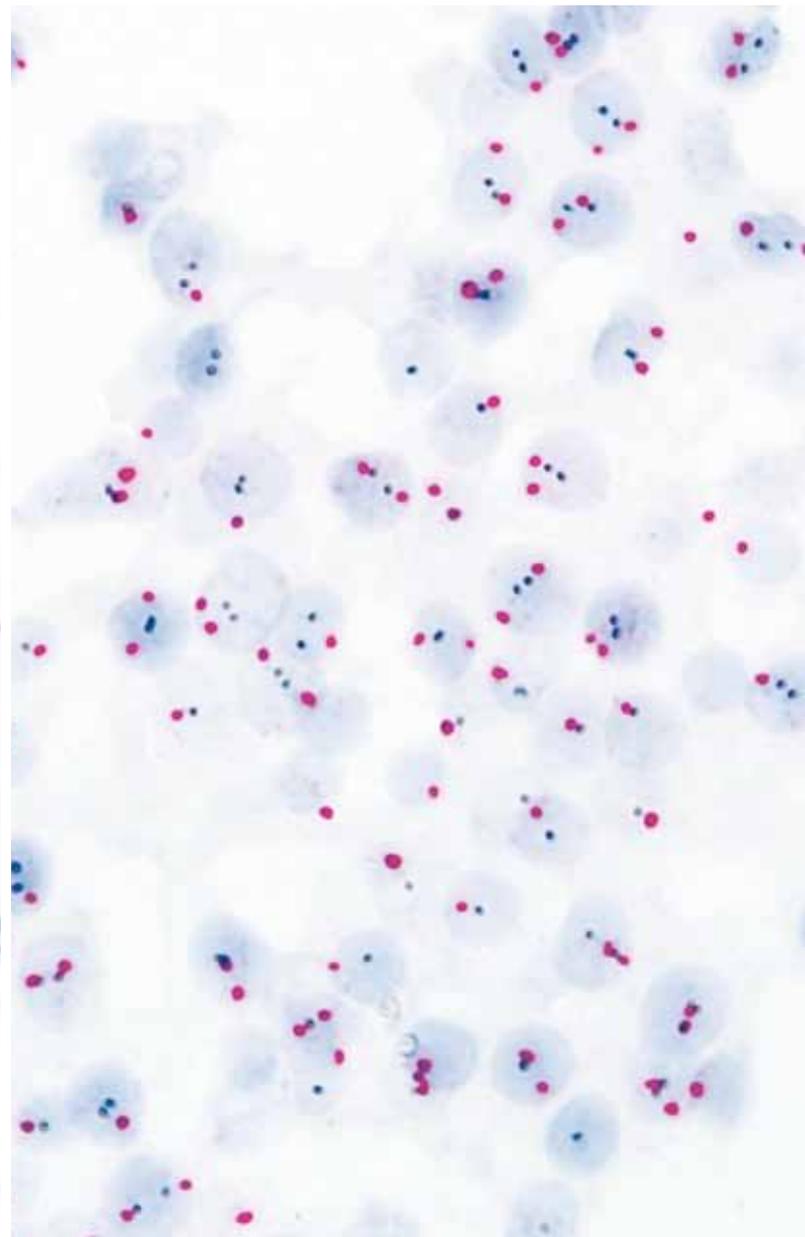
Interpretation Guide

Ventana INFORM HER2 Dual ISH DNA Probe Cocktail Assay



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Front cover images:

HER2 clusters and Chr17 60X, amplified (left)

HER2 and Chr17 60X, non-amplified (right)

This interpretation guide is intended for application with the CE IVD INFORM *HER2* Dual ISH DNA Probe Cocktail assay outside the United States of America.

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Introduction

General Description of Ventana INFORM *HER2* Dual ISH DNA Probe Cocktail assay

The INFORM *HER2* Dual ISH DNA Probe Cocktail is designed to quantitatively detect amplification by light microscopy of the *HER2* gene via two colour chromogenic *in situ* hybridisation (ISH) in formalin-fixed, paraffin-embedded human breast and gastric cancer, including the gastroesophageal junction, following staining on Ventana automated slide stainers using light microscopy. The INFORM *HER2* Dual ISH DNA Probe Cocktail is indicated as an aid in the assessment of patients for whom Herceptin (trastuzumab) treatment is being considered.

The INFORM *HER2* Dual ISH DNA Probe Cocktail assay is designed to determine *HER2* gene status by detecting: (1) *HER2* copies via silver *in situ* hybridisation (SISH) and (2) Chromosome 17 (Chr17) copies via chromogenic Red *in situ* hybridisation (Red ISH) on a single slide. Before interpreting results, staining of internal positive control nuclei must be evaluated by a qualified reader experienced in the microscopic interpretation of breast and gastric carcinoma specimens, ISH procedures, and the recognition of single and amplified *HER2* copies (which may require microscopic examination using objectives as high as 40X to 60X).

Purpose of this Interpretation Guide

This guide is intended to provide pathologists with a tool to facilitate *HER2* gene status determination via interpretation of *HER2* and Chr17 staining patterns using the INFORM *HER2* Dual ISH DNA Probe Cocktail. The following cases illustrate the variety of staining patterns that may be observed in breast and gastric specimens following staining with the INFORM *HER2* Dual ISH DNA Probe Cocktail (Figures 2-11).

These photomicrographs allow a new user to become familiar with the spectrum of staining patterns. These include single copy staining of *HER2* and Chr17, multiple copies and clusters of *HER2* staining, Chr17 polysomy, as well as artifact staining that may be encountered. Additionally, the images aid in the determination of slide adequacy, enumeration of copy numbers using the scoring algorithm, and assay troubleshooting.

Any staining performed in the end user's laboratory should be interpreted within the context of the internal positive control nuclei that are present in each clinical case. For further information, see the package inserts online (www.ventanamed.com), the Quality Control section, and the Slide Adequacy section of this guide.

Images contained in this Interpretation Guide were obtained using the INFORM *HER2* Dual ISH DNA Probe Cocktail assay, which was developed and validated on Ventana automated instruments, under the direction of Ventana Medical Systems, Inc.

What You Can Expect to See Regarding Performance of the *HER2* Dual ISH DNA Probe Cocktail in your Laboratory

For the INFORM *HER2* Dual ISH DNA Probe Cocktail, the recommendation is that tissue be fixed in 10% neutral buffered formalin (NBF) for six to forty-eight hours, paraffin embedded and sectioned at approximately four microns¹. Recent studies suggest that the majority of inconclusive *HER2* gene results relate to pre-analytic factors including "under" and "over" fixation² and delay to fixation³. While strict implementation of fixation condition is possible², it is difficult to precisely control tissue fixation time in reference laboratories receiving samples from multiples sources. To compensate for tissue variations, such as variable pre-analytical factors, this assay has been developed with certain selectable protocol steps, including those within the pre-treatment, hybridisation, as well as detection chemistries. These options may enable further optimisation of the assay, as needed for specific specimens. Note that a certain percentage of slides (not more than 6%) still may need to be re-stained due to slide drying and/or other artifacts⁴. The slide drying artifact is easy to recognize (see Troubleshooting section for further discussion on selectable protocol options, troubleshooting, and staining artifacts in the assay). In addition, some fixatives are not recommended for ISH-based assays (including Bouin's and AFA). See Pre-analytical consideration section for discussion of fixatives for the INFORM *HER2* Dual ISH DNA Probe Cocktail assay.

Identification of Appropriate Staining Pattern

HER2 Gene Status

In humans, the *HER2* gene, located on Chr17, encodes the *HER2* protein. Amplification of the *HER2* gene occurs in approximately 15 to 25 percent of breast cancers, and is associated with aggressive tumor behavior.⁵⁻⁹ In many clinical studies, amplification and/or *HER2* overexpression has been shown to be associated with a poor clinical outcome for women with invasive breast cancer and correlated with several negative prognostic variables, including estrogen receptor (ER) negative status, high S-phase fraction, positive nodal status, mutated p53, and high nuclear grade.¹⁰⁻¹¹ Additionally recent studies indicate that *HER2* is overexpressed in a percentage of gastric cancers.¹⁵ In this interpretation guide examples of INFORM *HER2* Dual ISH DNA Probe Cocktail staining are shown for breast and gastric cancer (Figures 2 and 3). All instructions on pre-analytical considerations, enumeration and troubleshooting addressed in this guide apply to both breast and gastric cancer tissue specimens.

Ventana has designed the INFORM *HER2* Dual ISH DNA Probe Cocktail to enable the *HER2* gene and Chr17 centromere to be co-hybridised and visualised via light microscopy on the same slide. Specifically for this assay, *HER2* is detected by a dinitrophenyl (DNP) labeled probe and visualised utilising

ultraView SISH DNP (silver *in situ* hybridisation) Detection Kit. The Chr17 centromere is targeted with a digoxigenin (DIG) labeled probe and detected using *ultraView* Red ISH DIG Detection Kit (Figure 1). Dual ISH staining results in visualisation via light microscopy in which *HER2* appears as discrete black signals (SISH) and Chr17 as red signals (Red ISH) in nuclei of normal cells (serving as internal positive controls for staining) as well as in carcinoma cells. This strategy allows *HER2* gene status determination in the context of its chromosomal state, using standard light microscopy with 20X, 40X, and/or 60x objectives.

HER2 gene status is reported as a function of the ratio of the average number of *HER2* gene copies to the average number of Chr17 copies in nuclei of cells within an invasive breast or gastric carcinoma. *HER2* gene status is classified as non-amplified (*HER2*/Chr17 ratio < 2.0: Figure 2, Case 1) or amplified (*HER2*/Chr17 ratio ≥ 2.0: Figure 2, Case 3). Care must be taken for cases that fall within the 1.8-2.2 ratio range¹. Ventana has developed a quantitative scoring algorithm to determine *HER2* gene status following staining with the INFORM *HER2* Dual ISH DNA Probe Cocktail. This algorithm is discussed in detail in Slide Scoring. Cases representing the dynamic range of *HER2* status are shown in Figures 2-4.

Figure 1.

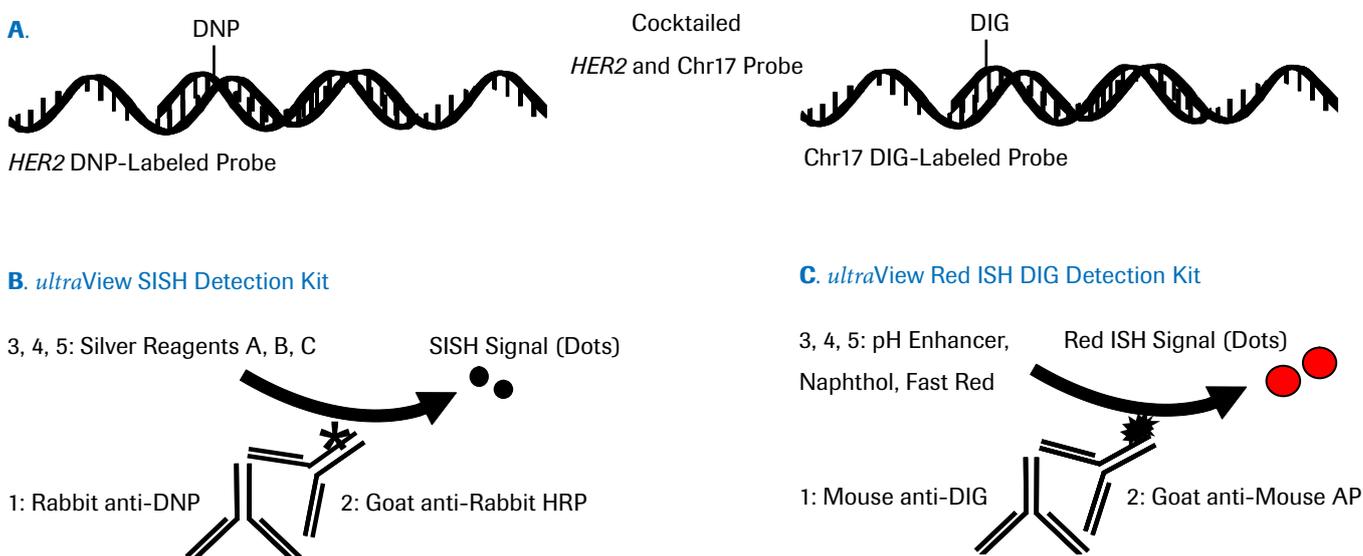
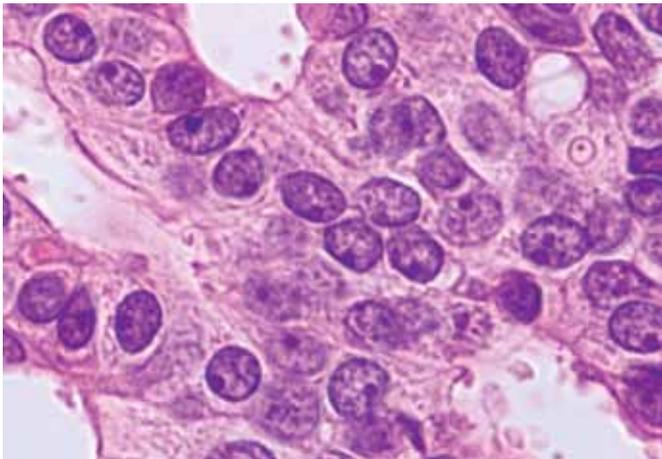
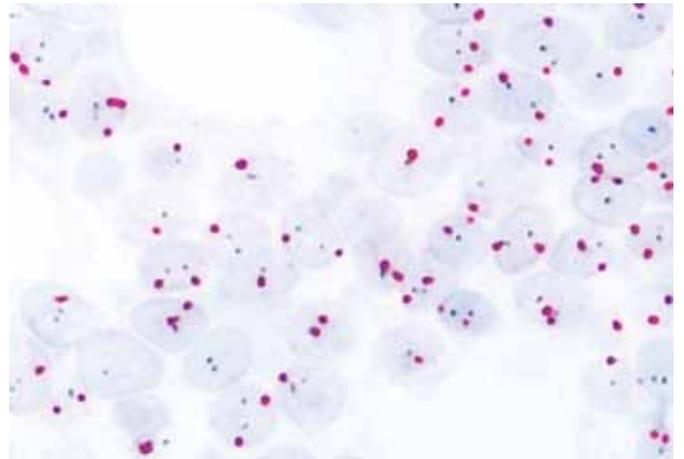


Figure 2. *HER2* gene status representative cases in breast and gastric carcinoma

Case 1. Breast carcinoma is non-amplified for *HER2*

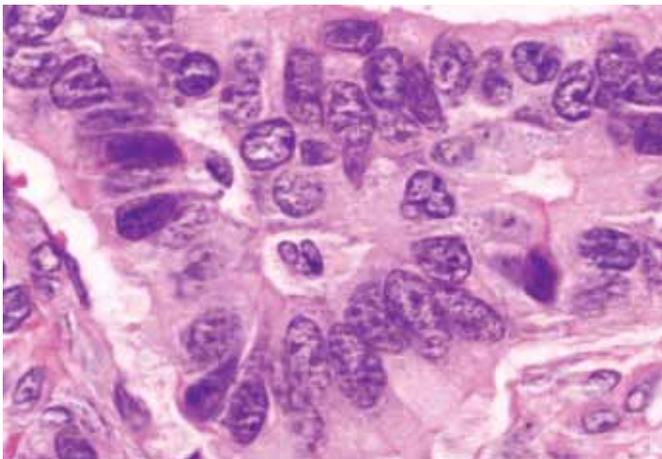


H&E 60X

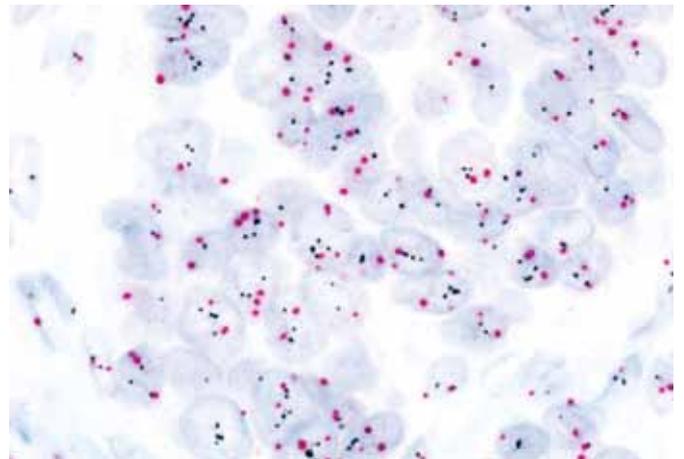


HER2 and Chr17 60X; Distinct SISH and Red ISH Signals are visible as 1-2 copies per tumor cell

Case 2. Breast carcinoma is non-amplified for *HER2*, but contains multiple copies of *HER2* and Chr17



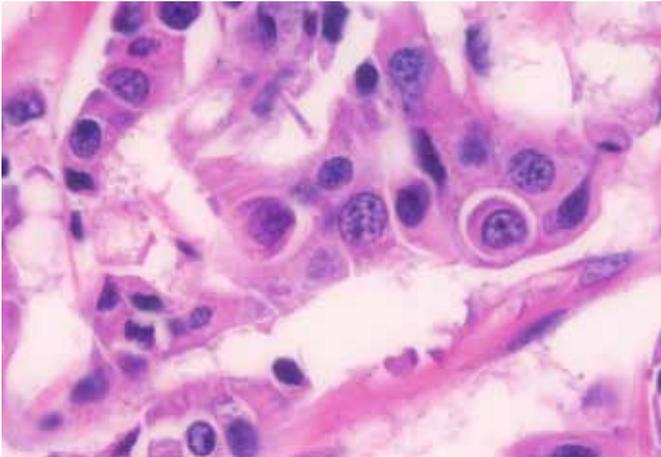
H&E 60X



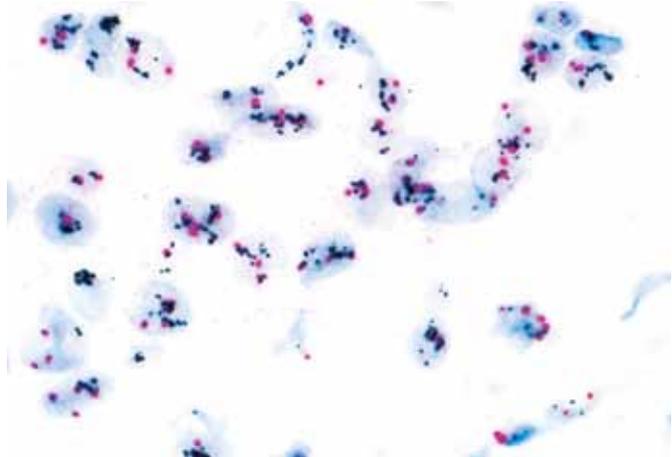
HER2 and Chr17 60X; Greater than 2 SISH Signals are present in the tumor cells, on average.

Figure 2. *HER2* gene status representative cases in breast and gastric carcinoma

Case 3. Breast carcinoma is *HER2* gene amplified with SISH clusters

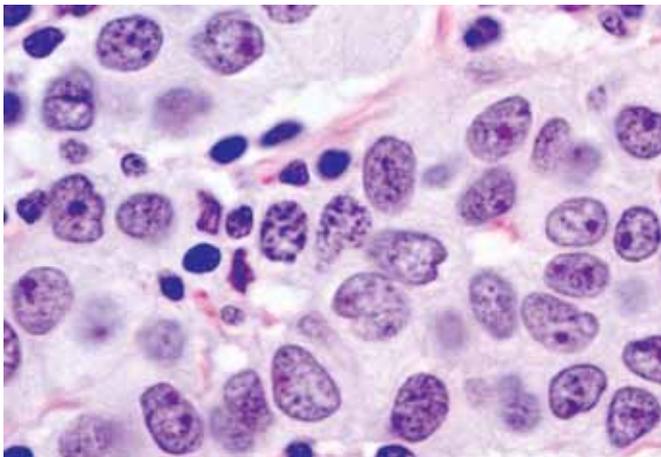


H&E 60X

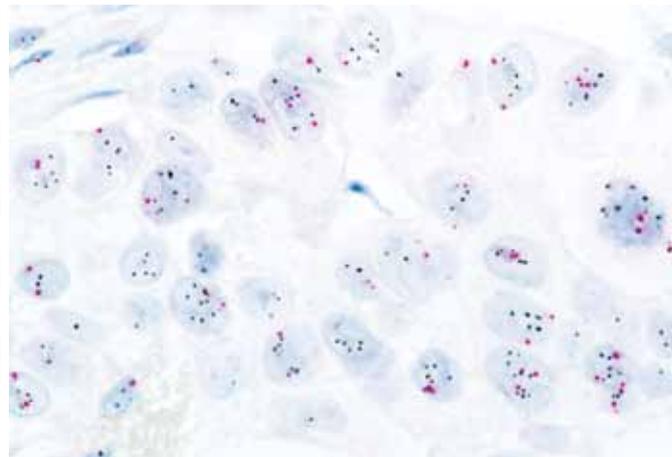


HER2 and Chr17 60X; *HER2* SISH clusters are present and are estimated by the reader. 1-2 copies of the Chr 17 Red ISH signal are present

Case 4. Breast carcinoma is low-level amplified for *HER2*



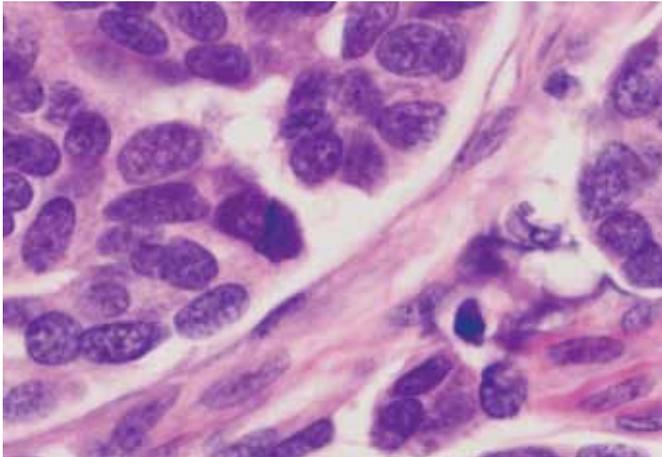
H&E 60X



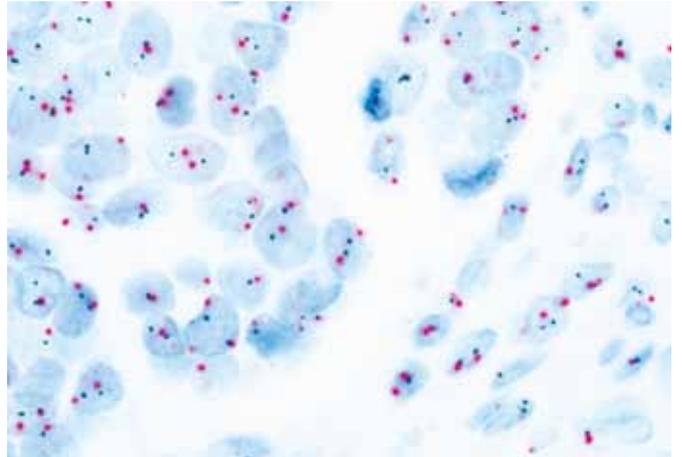
HER2 and Chr17 60X; Greater than 2 *HER2* copies are present, but only 1-2 Chr17 copies, on average. This case is low level amplified

Figure 2. HER2 gene status representative cases in breast and gastric carcinoma

Case 5. Gastric carcinoma is HER2 non-amplified

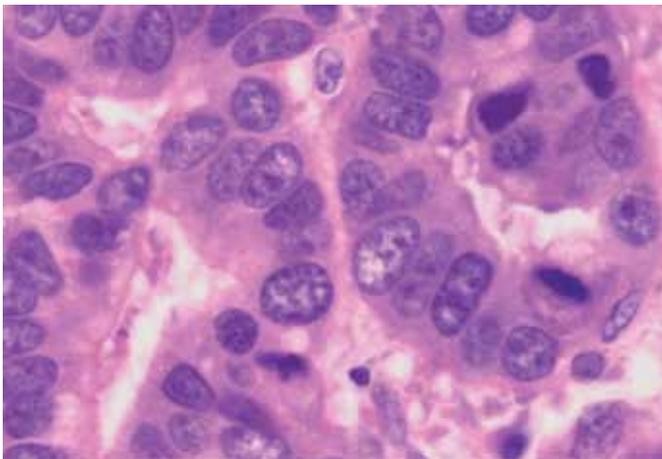


H&E 60X

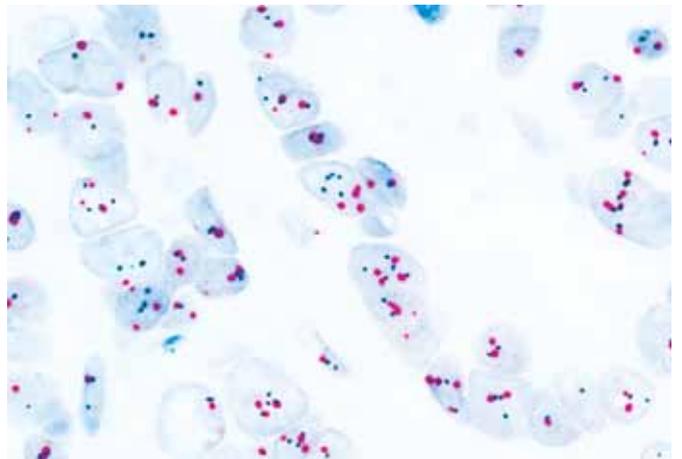


HER2 and Chr17 60X; Single copy HER2, non-amplified HER2, 60X

Case 6. Gastric carcinoma has multiple copies of HER2 and CHR17

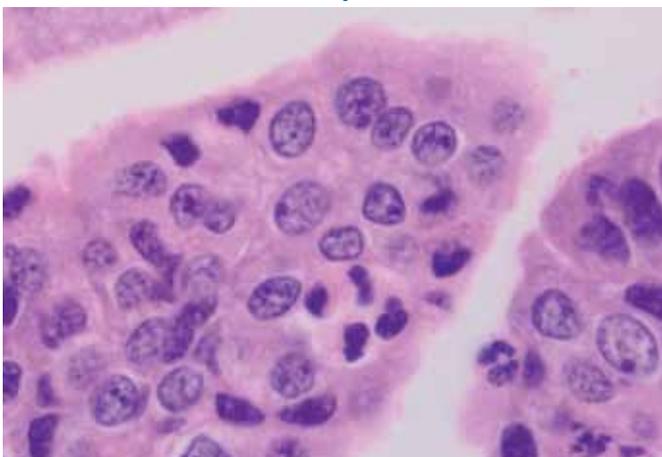


H&E 60X

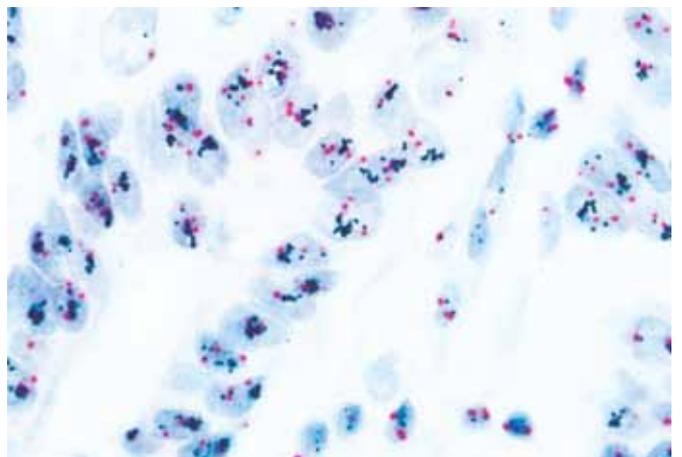


HER2 and Chr17 60X; Greater than 2 HER2 copies and Chromosome 17 are present

Case 7. Gastric carcinoma amplified with SISH clusters



H&E 60X



HER2 and Chr17 60X; HER2 SISH clusters are present and are estimated by the reader. 1-2 copies of the Chr 17 Red ISH signal are present

Signal Visualisation and Enumeration

ISH signals are visualised as single copies, multiple copies and clusters (Figure 3 and Table 1). Single copies in the normal cells are used as a reference to enumerate the signals in the carcinoma nuclei.

Single Copy

A discrete signal is counted as a single copy of *HER2* or Chr17. Discrete single signals visualised in the internal positive control nuclei represent the size of a single copy in carcinoma cells (Figure 2-4). It is important to note that the discrete signals representing a single copy of either *HER2* or Chr17 may appear smaller or larger in some patient samples compared to others. In addition, SISH signals (black) are typically smaller in size and more discrete in appearance than Red ISH signals (red), due to differences in target sizes and detection chemistries. Therefore, it is important to use the single signals visualised in the internal positive control nuclei (the physiologic control) adjacent to the target area (the pathologic lesion) as a reference for relative signal size for SISH signals. Red ISH signals may appear larger than SISH signals, are sometimes elongated in shape, and may vary in size within a target area and across samples. The internal positive control nuclei occur within normal adjacent stromal cells (e.g. fibroblasts/fibrocytes and endothelials) and leukocytes (e.g. lymphocytes and macrophages).

Multiple Copies

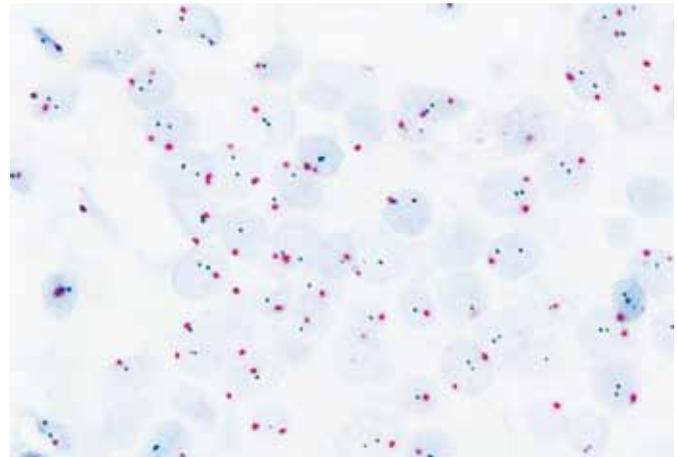
As described above, discrete single black signals (SISH) visualised in the internal positive control nuclei represent single copy size in invasive carcinoma cells. Cases 2, 4, 6 and 9 in Figures 2-4 show a number of nuclei in which multiple discrete copies are visible in the carcinoma nuclei.

Clusters

A cluster is defined as numerous overlapping SISH signals in the nuclei that cannot be individually discerned. As these are difficult to precisely enumerate, the number of *HER2* gene copies in a cluster can only be estimated. For example, a small cluster may be counted as 6 signals, and larger clusters as 12 signals or more. It is possible for a single nucleus to have multiple small clusters, multiple large clusters, a combination of large and small clusters, and/or clusters and single signals. Case 3, 7 and 10 in Figures 2-4 illustrate examples of nuclei in which clusters are visible.

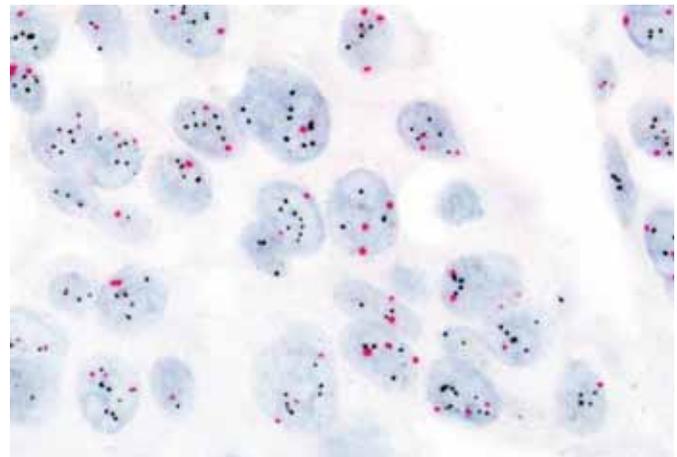
Figure 3. Signal visualisation

Case 8. Breast carcinoma



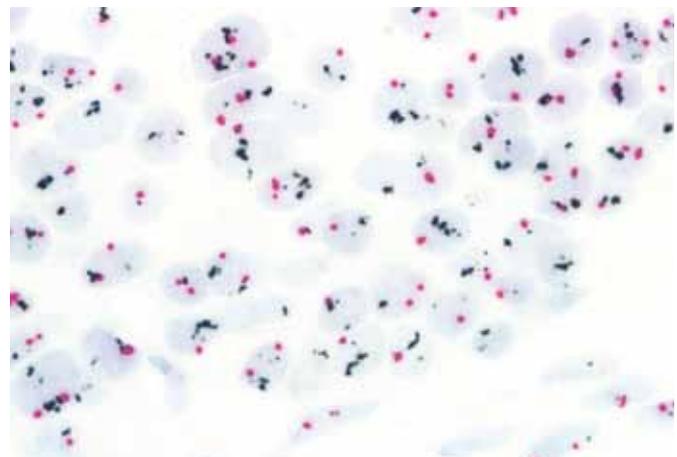
HER2 and Chr17 60X; Single copy *HER2*, non-amplified *HER2*, 60X

Case 9. Breast carcinoma



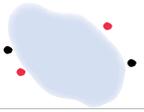
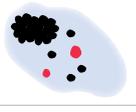
HER2 and Chr17 60X; Multiple single copy *HER2*, low level amplified *HER2*, 60X

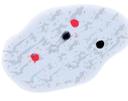
Case 10. Breast carcinoma



HER2 and Chr17 60X; *HER2* clusters, amplified *HER2*, 60X

Table 1. Signal Visualisation

	Do not count if nuclei overlap
	Do not count if no signal is present
	Do not count if only signal of one colour is present
	Do not count if signal is outside the nuclei
	Count as 1 black (<i>HER2</i>) and 1 red (Chr17) signal
	Count as 2 black (<i>HER2</i>) and 2 red (Chr17) signals
	Count as 1 black (<i>HER2</i>) and 2 red (Chr17) signals. The black signal is a "doublet". Count two adjacent signals of same colour only if the distance between the signals is equal or greater than the diameter of a single signal
	Small SISH clusters are estimated by using the size of a single signal as reference. Use stromal cells to estimate signal size (smaller cell on left). For instance, this cluster could be estimated as 6 SISH signals - adding the other 2 single signals yields a total count of 8. Count as 2 red signals. Note on scoring sheet that clusters are present for <i>HER2</i>
	Estimate the large cluster. Here, the cluster can be estimated as 12 black signals - adding the 4 single signals yields a total count of 16. Count red signals as 2 copies of Chromosome 17. Note on scoring sheet that clusters are present for <i>HER2</i>
	A red signal close to a black signal should be counted as one red signal and one black signal. This may require enumeration at 60x objective to discern. Therefore, count as 4 black signals and 2 red signals. If overlapping signals cannot be distinguished, do not count that nucleus

	Cluster of black signal obscuring red signal(s). Higher magnification (60x) may be utilised in attempts to confirm presence or absence of red signal(s); otherwise do not count: always count nuclei with clear red signals. Note the presence of SISH clusters on the score sheet. Nuclei with visible and higher numbers of red signal should be scored in nuclei with SISH clusters
	If background SISH "dust" occurs in the nuclei, only count if specific black (SISH) signals are clearly distinguishable from background
	Red haze may be observed and should not be mistaken for signal. Red signal may vary in intensity but is always discrete. The image shows 2 discrete Red (Chr17) signals and 2 black (<i>HER2</i>) signals

Slide Adequacy must be verified before enumerating the slide

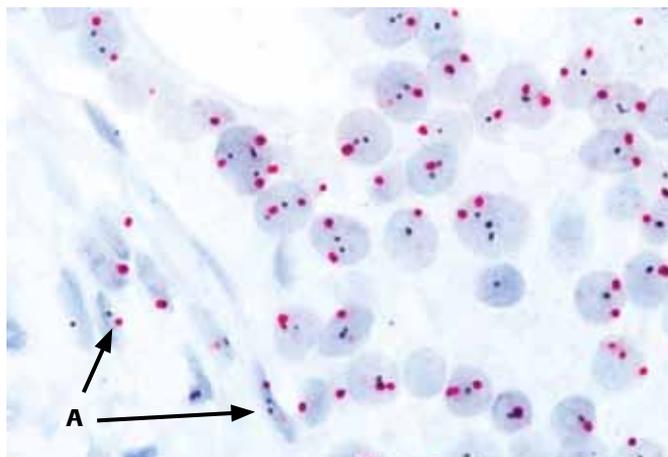
Before enumerating *HER2* and Chr17 signals to determine *HER2* gene status, it is critical to determine whether the invasive target area (the lesional tissue) is adequately stained and satisfies the criteria described below. If the target is inadequate for enumeration, the user should refer to the Troubleshooting section and evaluate appropriate conditions to follow for repeat staining the slide.

Criterion 1. Internal positive control staining must be present

HER2 and Chr17 signals in non-neoplastic nuclei (one to two copies per cell that are visible as distinct "single copy" staining) act as internal, physiologic, "same slide" positive controls and should be visible using 20X, 40X, or 60X objectives. This distinct nuclear staining may be located in the normal cells in and/or around the target area, including: stromal fibroblasts, endothelial cells, lymphocytes and benign cells (See Fig. 4). Due to truncation artifacts in the plane of sectioning, it is usually not possible to visualise single *HER2* or Chr17 signals in all cells on the slide, nor in all regions of the tissue. However, accurate enumeration requires that single-copy signals are visible in normal cells within and/or adjacent to the target area.

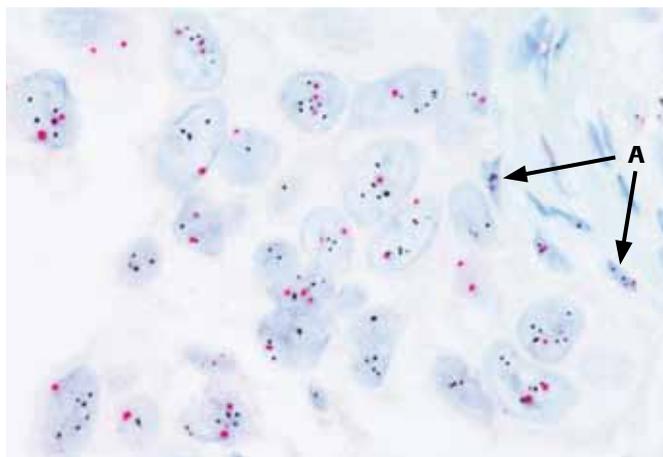
Figure 4. Normal staining in internal positive control nuclei

Case 11. Breast carcinoma



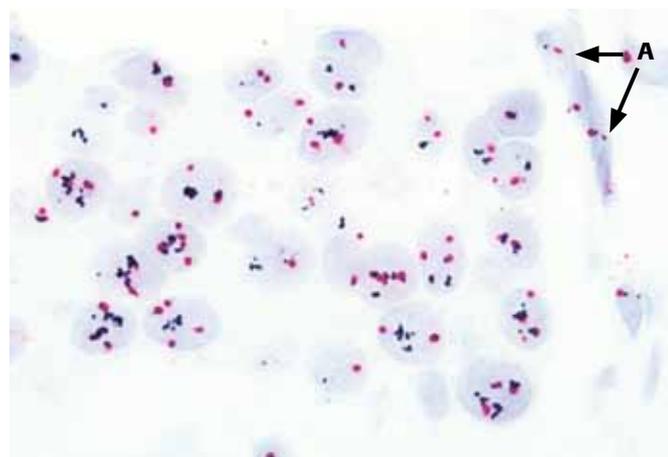
HER2 and Chr17 60X; Non-amplified for *HER2*
A: Internal positive control nuclei

Case 12. Breast carcinoma



HER2 and Chr17 60X; multiple copies of *HER2*
A: Internal positive control nuclei

Case 13. Breast carcinoma



HER2 and Chr17 60X; *HER2* amplified with SISH clusters
A: Internal positive control nuclei

Figure 4 contains examples of cases with appropriate internal positive control staining.

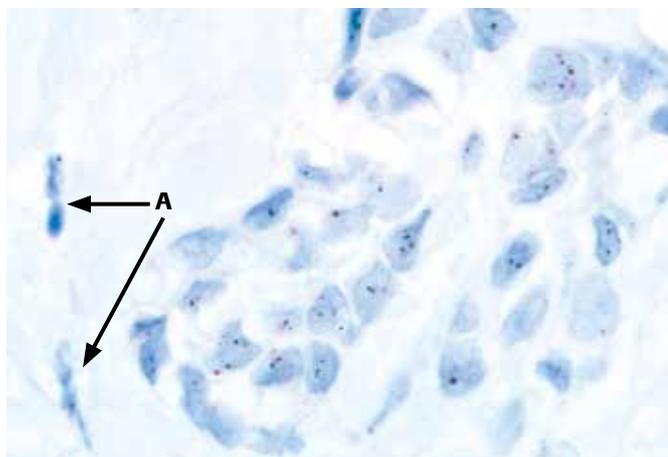
Figure 5 shows examples of cases that are inadequate due to a lack of either SISH or Red ISH staining in both the positive control nuclei and tumor nuclei. Such cases must be repeated before enumeration.

Figure 6 shows a case which is inadequate due to absent staining in the positive control nuclei. Therefore this case cannot be enumerated and must be repeated.

See the Troubleshooting section for repeat staining samples that are inadequate for enumeration.

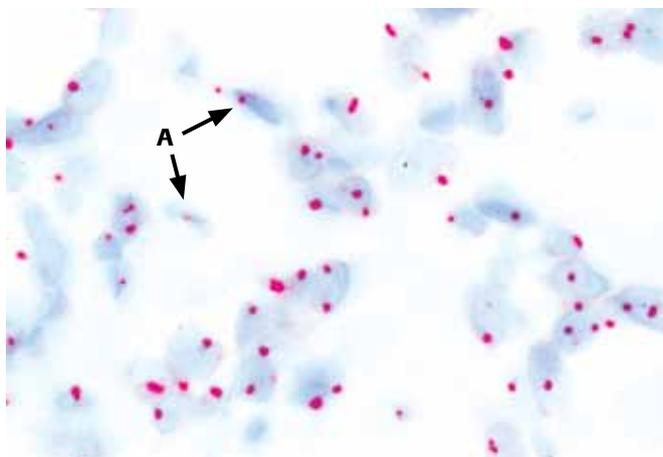
Figure 5. Inadequate due to lack of staining for either SISH or Red ISH

Case 14. Breast carcinoma



HER2 and Chr17, 60X, inadequate due to weak/absent Red ISH staining in positive control nuclei and tumor nuclei. A: Internal positive control nuclei

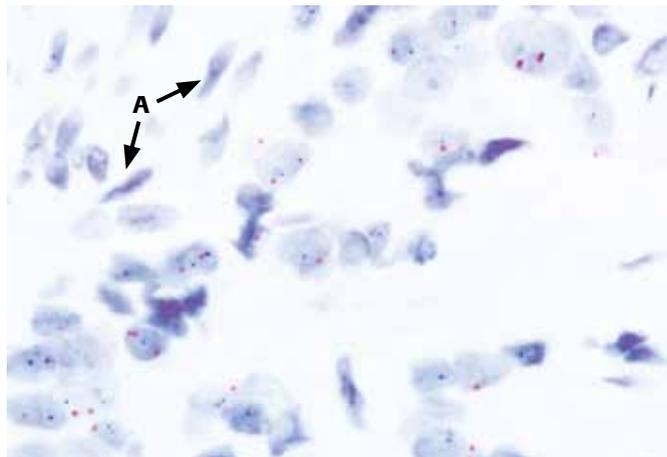
Case 15. Breast carcinoma



HER2 and Chr17, 60X, inadequate due to lack of SISH staining in positive control nuclei and tumor nuclei. A: Internal positive control nuclei

Figure 6. Inadequate due to lack of staining in positive control nuclei

Case 16. Breast carcinoma



60X, inadequate due to lack of staining for both SISH and Red ISH in positive control nuclei. A: Internal positive control nuclei exhibit weak/absent staining for both SISH and Red ISH, although the tumor cells are stained with both probes. This case is inadequate for enumeration

Criterion 2. Staining within the invasive breast or gastric carcinoma cells in the target area must be enumerable

Using 20X, 40X, and/or 60X objectives, the invasive breast or gastric carcinoma in the target area must exhibit an enumerable field of *HER2* (SISH) and Chr17 (Red ISH) signals. Due to truncation in the plane of sectioning, it is likely that not every carcinoma nucleus will contain signals. However, it is important that the target area contains an acceptable region that is enumerable. If a particular target area is deemed too weak to enumerate, it is often possible to enumerate a different target area on the same slide. If all larger areas exhibit inadequate staining, then the slide is considered inadequate and cannot be enumerated.

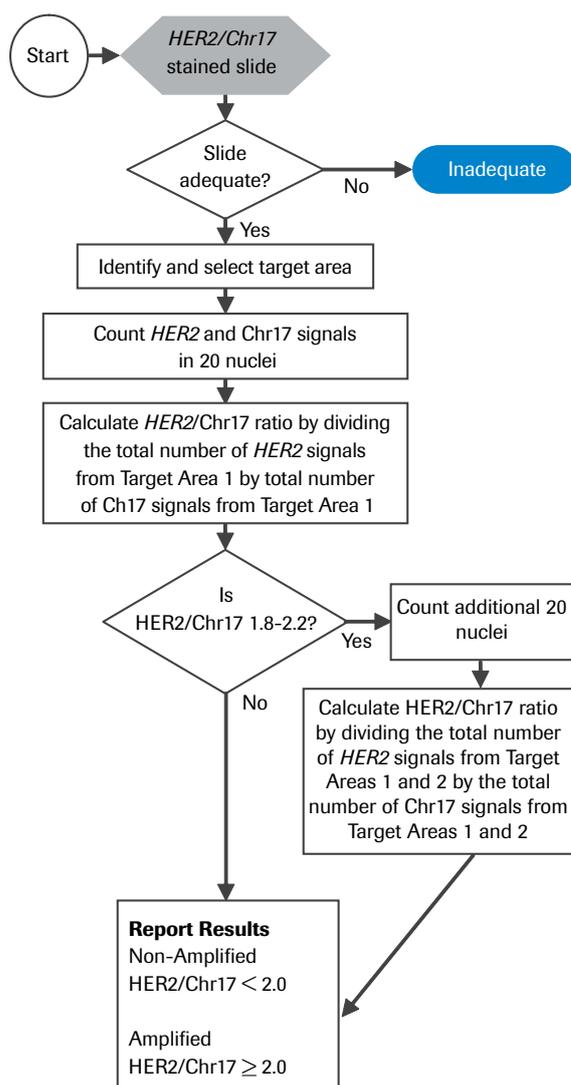
Criterion 3. Background staining must not interfere with enumeration

Finally, any background staining resulting from either SISH or Red ISH detection systems will need to be evaluated to determine if it interferes with enumeration of the specific SISH or Red ISH signals. SISH background typically appears as SISH “dust” that is distinguishable from the specific signal (Figure 21). Red background may appear as red haze that is fainter in intensity compared to the specific signal. (Figure 20 and 22). See Troubleshooting section for more information on repeat staining cases that may have background staining that interfere with enumeration.

Slide Scoring

Ventana has developed a quantitative scoring algorithm that maximizes precision and efficiency to determine *HER2* gene status. Once an adequate target area is identified, the reader records the scores for *HER2* and Chr17 copy numbers that are present in 20 representative nuclei. If the resulting *HER2* / Chr17 ratio falls within 1.8-2.2, the reader is recommended to score an additional 20 nuclei and the resulting ratio is calculated from the total 40 nuclei. *HER2* gene status is reported as non-amplified ($HER2/Chr17 < 2.0$) or amplified ($HER2/Chr17 > 2.0$). A flow diagram of the scoring algorithm is shown below.

Figure 7. Scoring algorithm flow diagram



Summary of scoring

Once a target area that exhibits adequate staining is located, only representative carcinoma cells that contain both SISH (*HER2*) and Red ISH (Chr17) signals should be enumerated. Signal enumeration should not be performed in areas that contain: weak or absent SISH or Red ISH signals, absent internal control cell staining, compressed or overlapping nuclei, nuclei with excessive Red ISH or SISH background staining or necrosis. Additionally, signal enumeration should not be performed in nuclei that are not representative of the general population of invasive carcinoma nuclei in the target area. For example, abnormally large nuclei (2-fold or greater in size relative to other carcinoma nuclei in the field) and small nuclei (approximately half the size of other carcinoma nuclei) should not be enumerated. Finally, in target areas that are genetically heterogeneous for *HER2* copy numbers, count only nuclei that are representative of the population of invasive carcinoma nuclei with the highest average number of signals. Heterogeneity is discussed in greater detail in Additional Observations.

To score a slide stained with the INFORM *HER2* Dual ISH DNA Probe Cocktail:

1. Examine H&E stained slide to locate areas containing invasive breast or gastric carcinoma
2. Examine *HER2* Dual ISH stained slide corresponding to the H&E, and identify an invasive breast or gastric carcinoma target area where:
 - The majority of cells in the selected area display hybridisation signals for both SISH and Red ISH that are not obscured by non-specific background staining.
 - There are internal positive control cells in each area adjacent to the tumor area to be scored (Note: internal positive control cell staining is not applicable for xenografts, as xenografts are comprised of human tumor cell lines grown in mice; therefore, the non-tumor cells are mouse cells containing mouse *HER2* and Chr17 sequences which will not be detected by either probe.
3. Count 20 representative nuclei within the invasive region per specimen, that meet the following requirements:
 - The area is considered adequate for enumeration if one to two copies of *HER2* and Chr17 signals are present in various normal cells including: stromal fibroblasts, endothelial cells, lymphocytes and other non-neoplastic cells.
 - Compare nuclei not significantly greater or less than median diameter with overall tumor nuclei.
 - Select cells that have minimal or no overlap with other cells. If Red ISH and SISH signals appear overlapping, the reader may have to examine the signals at 60x magnification to discern.
 - Do not count nuclei that are severely over-digested or “bubbled”, or are found in areas containing non-specific staining on the slide that could interfere with enumeration. See Troubleshooting for images of these types of nuclei. Also see discussion on Pre-Analytical Factors.
 - Score *HER2* and Chr17 signals detectable in 20 representative nuclei.
 - Note the presence of *HER2* clusters, and estimate the number of copies based on the size of a single copy. Note that higher number of Red ISH signals visible in nuclei with SISH clusters should be counted.
4. Determine the sum of *HER2* and Chr17 copy numbers and calculate the resulting ratio.

If the ratio falls within 1.8-2.2, an additional 20 nuclei must be counted and the resulting ratio is based on the total of 40 nuclei.

Examples of *HER2* and Chr17 Staining Patterns in Clinical Cases

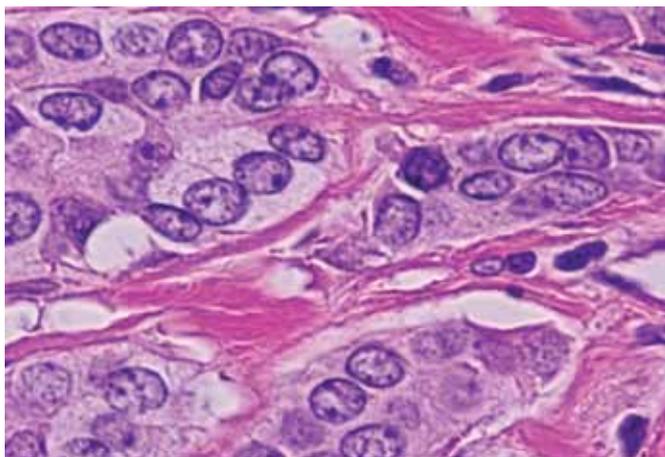
The following photographs (Figures 8-11) are provided to illustrate a variety of staining patterns that may result from staining breast or gastric carcinoma cases with the INFORM *HER2* Dual ISH DNA Probe Cocktail. The intended use of these photographs is to allow the new user of this test to

become familiar with the spectrum of staining patterns that may be encountered. Any staining performed in the end user's laboratory should be interpreted within the context of the internal positive control nuclei or controls run with the clinical cases at the time of evaluation (See Quality Control section).

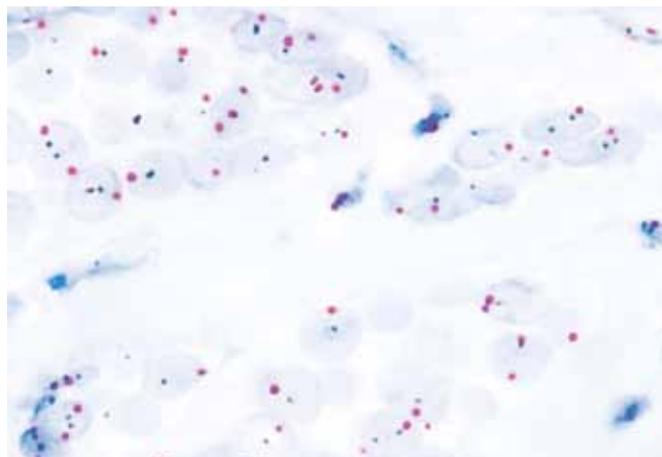
Typical dynamic range

Figure 8. Non-amplified, multiple copies and amplified cases in breast carcinoma

Case 17. Breast carcinoma

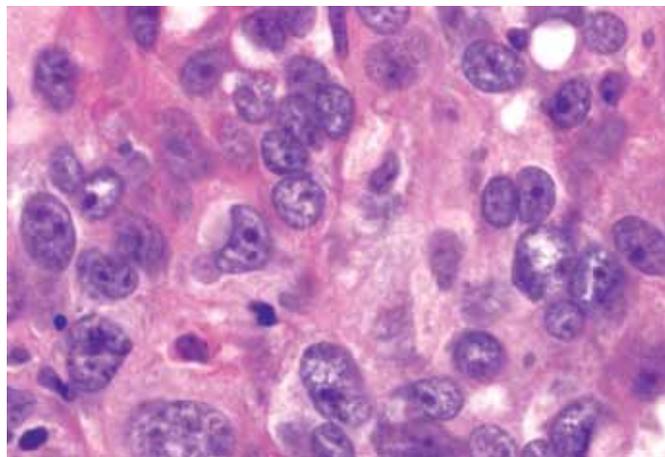


H&E 60X

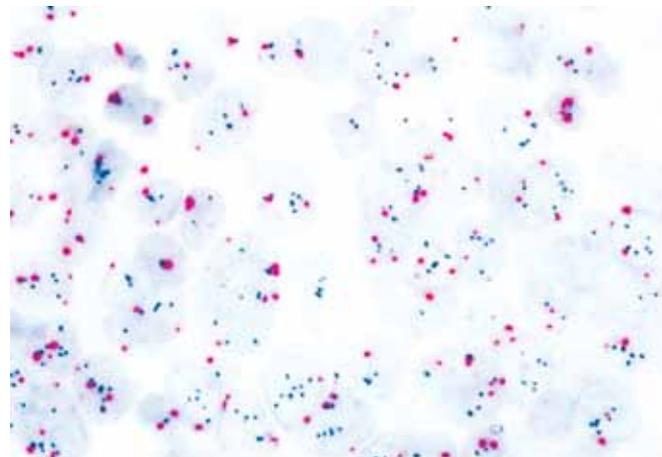


HER2 and Chr17 60X; Single copy *HER2*, non-amplified *HER2*, 60X

Case 18. Breast carcinoma



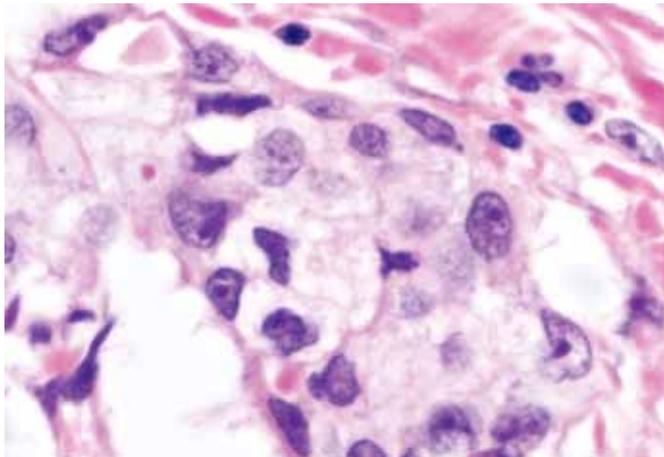
H&E 60X



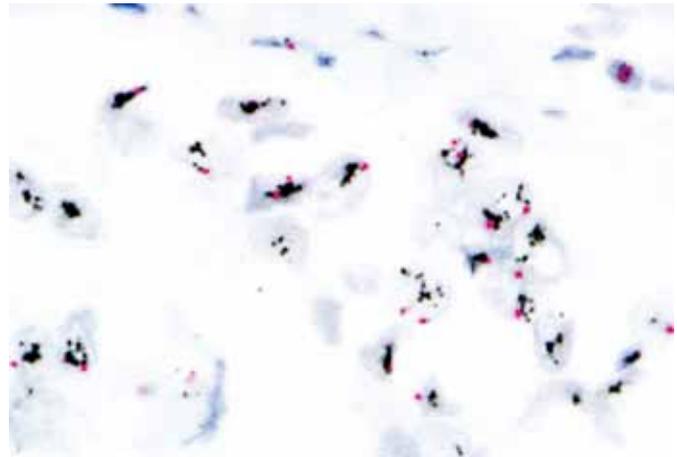
HER2 and Chr17 60X; Multiple single copy *HER2*, 60X. Multiple discrete copies of *HER2* and Chr17 that must be enumerated with care.

Figure 8. Continued

Case 19. Breast carcinoma



H&E 60X



HER2 and Chr17 60X; HER2 clusters, amplified HER2

Additional observations

Observations other than the typical dynamic range (Figure 8) regarding *HER2* or Chr17 staining may be clinically significant and should be noted as comments on the pathologist's report.

Heterogeneity

Tissue samples may contain breast or gastric carcinoma nuclei that are genetically heterogeneous for *HER2* copy numbers. In these cases, there can be a mixture of nuclei that are amplified and non-amplified and/or a mixture of nuclei containing various copy numbers (Figure 9).

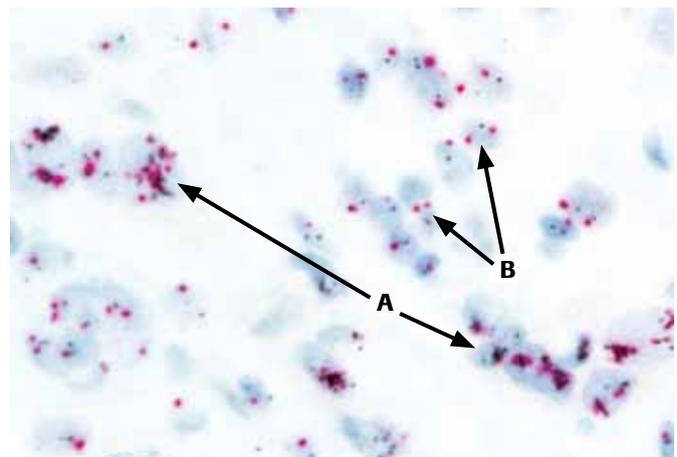
This may be observed among carcinoma cells within the same target area, or between two different target areas within the tissue. It is recommended that the nuclei with the higher numbers of *HER2* copies be chosen for enumeration, but that the heterogeneity in relative copy number be noted on the patient's report.

Invasive breast carcinoma vs ductal carcinoma *in situ*

Clinically, *HER2* and Chr17 copies should be enumerated only in the invasive carcinoma. Staining in ductal carcinoma *in situ* (DCIS) in breast specimens should not be enumerated. It is recommended that the reader refer to the matched H&E slide to determine appropriate target areas to enumerate on the Dual ISH stained slide (Figure 10).

Figure 9. Heterogeneity

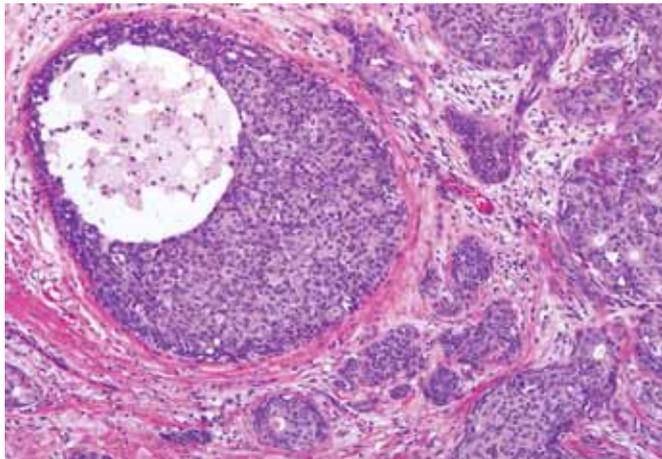
Case 20. Breast carcinoma



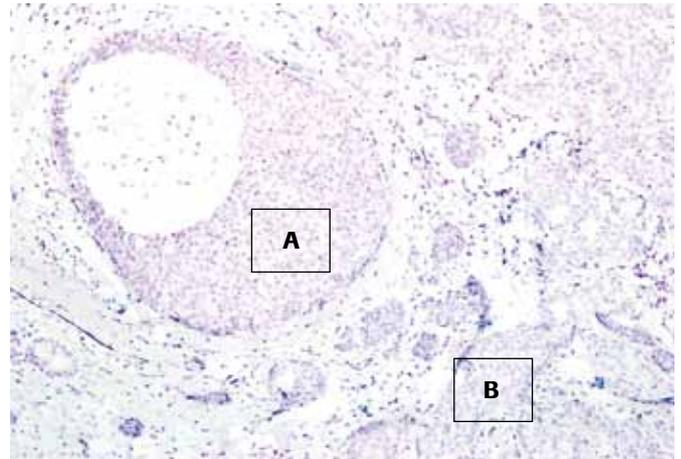
HER2 and Chr17, 60X, heterogeneous for *HER2* copy number within one target area. A: high copy number for *HER2* and apparent polysomy for Chr17. B: low copy number for *HER2*

Figure 10. Invasive versus ductal carcinoma in breast specimens

Case 21. Breast carcinoma

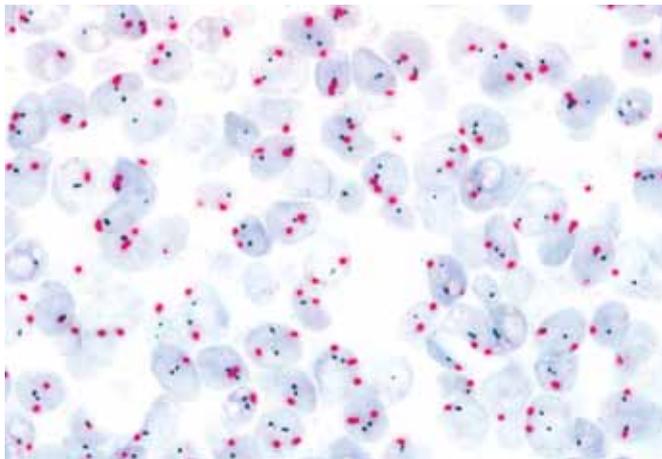


H&E, 10X, ductal carcinoma *in situ* and invasive carcinoma *HER2* and Chr17 60X;



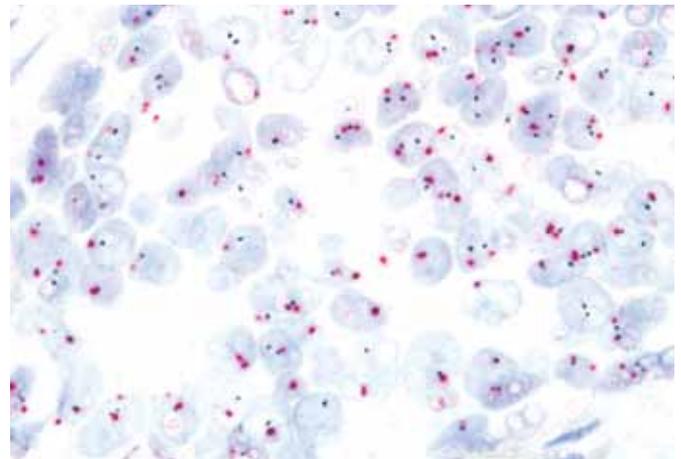
10X, ductal carcinoma *in situ* (A) and invasive carcinoma (B)

(A)



HER2 and Chr17, 60X; ductal carcinoma *in situ*, non-amplified *HER2*

(B)



HER2 and Chr17, 60X; invasive carcinoma, non-amplified *HER2*

Aneusomy of Chr17

Monosomy

Monosomy is a condition in which the carcinoma nuclei contain only one copy of Chr17, on average.

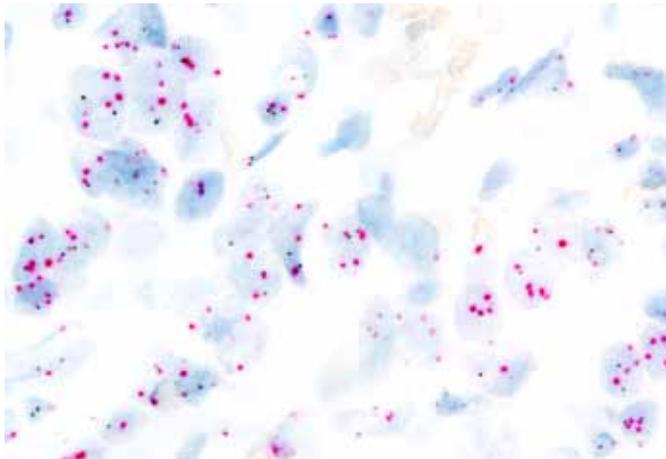
HER2 copy number for Case 19 is approximately 2, but note that Chr17 is polysomic. Thus Chromosome 17 was duplicated but there was also a genetic loss of the *HER2* allele. The case report for Case 19 can note Monoallelic Deletion. Case 20 contains multiple copies of *HER2*, but note coincidental Polysomy Chr17.

Polysomy

Polysomy is a condition in which the carcinoma nuclei contain at least one more chromosome than normal nuclei.¹³ In these polysomic nuclei, there may be three or more copies of Chr17 (Figure 11). This is not to be confused with diploid, dividing nuclei present throughout the tissue that contain three to four copies of Chr17.

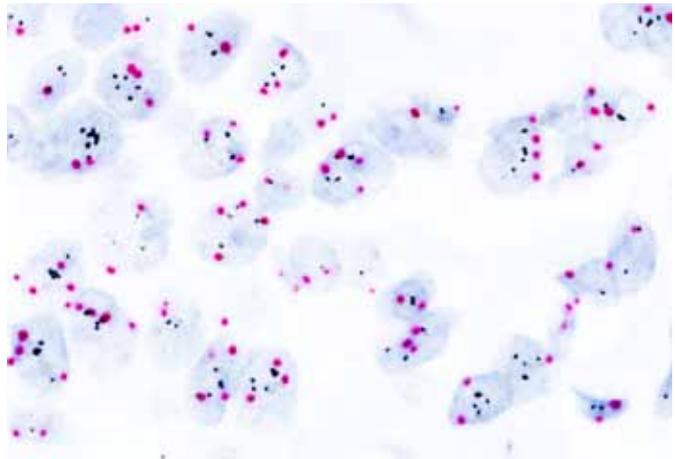
Figure 11. Aneusomy of Chr17

Case 22. Breast carcinoma



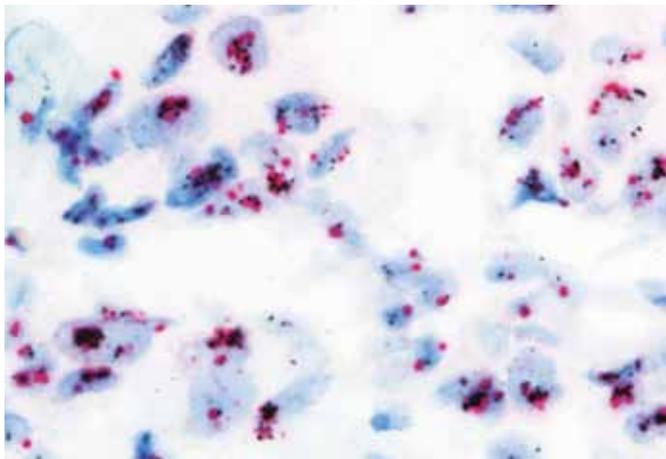
HER2 and Chr17, 60X, Monoallelic Deletion. A small percentage of cases exhibit a loss of the *HER2* gene on one or more of the Chr17, which may also be polysomic. The ratio is therefore < 1.0 and the genetic cause is monoallelic deletion.

Case 23. Breast carcinoma



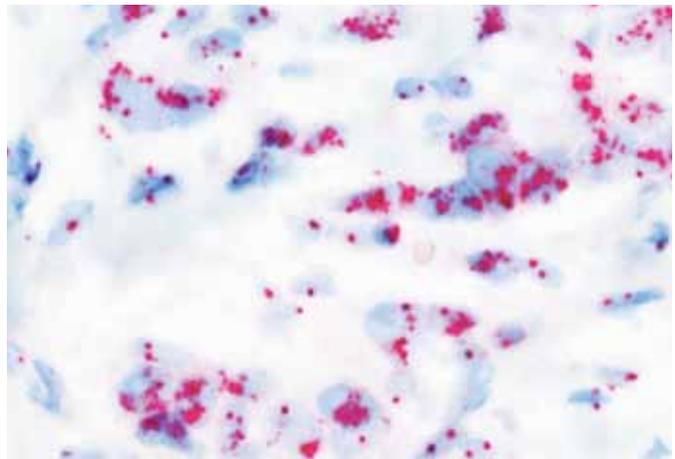
HER2 and Chr17, 60X, multiple discrete copies of *HER2* and polysomy for Chr17. The case must be enumerated with care to determine amplification status.

Case 24. Breast carcinoma



HER2 and Chr17, 60X; amplified *HER2* with pericentromeric amplification of Chr17

Case 24. b

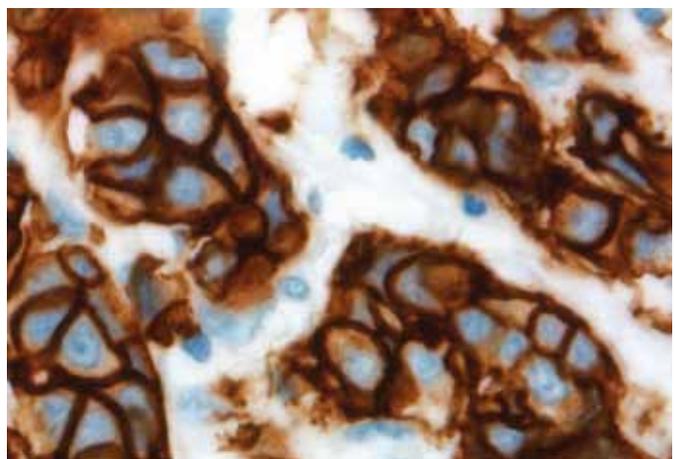


Chr17, 60X; single detection Red ISH staining for Chr17, pericentromeric amplification of Chr17

Pericentromeric amplification of Chromosome 17

Apparent "amplification", clusters, or polysomy of Chr17 (with or without overt *HER2* clusters) have been reported.¹²⁻¹³ In case 24, *HER2* is overtly amplified and Chr17 also exhibits a clustered staining pattern for Red ISH (Figure 11). Even though both *HER2* and Chr17 are clustered, care must be taken not to consider this a *HER2* non-amplified case with ratio of ~ 1.0 . In cases with overt amplification of Chr17, the reader should note the Dual ISH score and staining patterns for both probes, but also refer to IHC in reporting the results. The majority of these *HER2* and Chr17 clustered cases, in our experience, tend to have overexpression of the protein (3+ by IHC staining), see Figure 11, case 24.

Case 24. c



HER2 and Chr17, 60X; *HER2/neu* 4B5 staining, 3+ by IHC, *HER2* overexpressed

Quality Control for ISH Staining

Positive control nuclei are present within each specimen

As discussed in the Slide Adequacy section, normal *HER2* (SISH) and Chr17 Red ISH signals (one to two copies per cell) act as internal positive, "same-slide" controls for each case and must be visible in the sample. Specific *HER2*/Chr17 nuclear staining may be located in various cells including: stromal fibroblasts, endothelial cells, lymphocytes and non-neoplastic breast epithelial cells. However, not all cells will exhibit single gene copy due to biological heterogeneity and the plane of sectioning. Because the *HER2* gene and Chr17 are present in every human cell, there is no true internal negative control.

A positive control may be included with every staining procedure performed, if the user desires. The controls are used to confirm that the reagents and instrument have functioned properly. It is important that optimal staining conditions be established on the control samples prior to running patient samples. Positive controls can include biopsy specimens prepared in a manner identical to the patient specimens to be tested. These specimens may be useful as they serve as quality controls for all steps of the procedure, from the pre-analytical specimen preparation to the staining process. Use of a specimen prepared differently from the test specimens will provide a control for the reagents, instrument and procedures but not for fixation and specimen processing.

HER2 dual ISH 3-in-1 xenograft slides

HER2 Dual ISH 3-in-1 Xenograft Slides are intended to be used for initial assay installation and/or troubleshooting activities in conjunction with the *HER2* Dual ISH DNA Probe Cocktail on Ventana BenchMark series instruments.

HER2 Dual ISH 3-in-1 Xenograft Slides contain three distinct xenograft cores that are formalin-fixed and embedded in a single paraffin block (Figure 12). The cell lines (Calu-3, ZR-75-1 and MCF-7) have been selected based upon their molecular characterization of *HER2* gene copy number and protein levels (from published literature), and represent the dynamic range of *HER2* copies observed in clinical samples. Note that the xenografts contain host mouse cells interspersed with the human carcinoma cells. These cells will not specifically stain, as human *HER2* and Chr17 are not detectable in mouse cells.

Xenograft slides can be used in troubleshooting, if instrument or reagent issues are suspected.

Figure 12. Schematic layout of *HER2* Dual ISH 3-in-1 xenograft slide

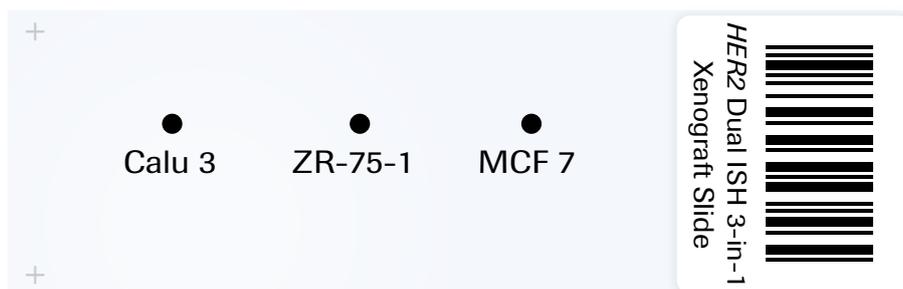
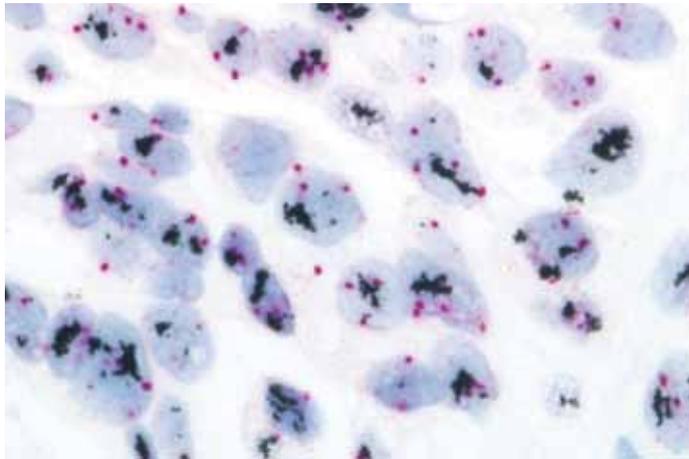
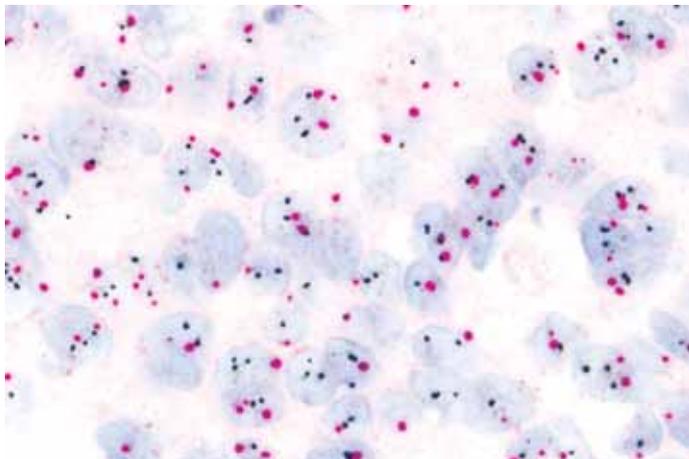


Figure 13. Characteristic staining pattern of *HER2* Dual ISH 3-in-1 Xenograft Slides Cell Lines

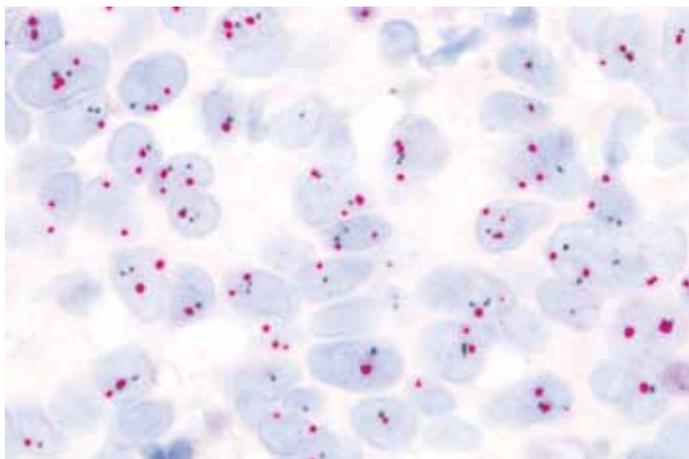
Calu-3
(amplified for *HER2*)



ZR-75-1
(~3 copies of *HER2*)



MCF 7
(~2 copies of *HER2*)



Pre-Analytical Considerations

Pre-analytical sample preparation is an essential factor to consider before using any *in situ* hybridisation assay¹⁻³. For the Ventana INFORM *HER2* Dual ISH DNA Probe Cocktail assay, the recommendation is that the tissue be fixed in 10% neutral buffered formalin (NBF) for six to forty-eight hours (consistent with ASCO/CAP guidelines¹), paraffin embedded and sectioned at approximately four microns. The INFORM *HER2* Dual ISH DNA Probe Cocktail assay has been developed with additional pre-treatment options that may aid in optimising the assay in different laboratories and for subsequent troubleshooting of particular tissues/slides exhibiting sub-optimal staining. It is recommended that each laboratory perform nominal runs on representative control samples that have been prepared under the identical conditions as the clinical samples to be tested. This will aid in optimising the specific staining conditions for individual laboratories that may vary in their exact specimen preparation procedures. Specimens that are pre-analytically prepared using conditions that are not recommended by Ventana may never stain appropriately with the assay.

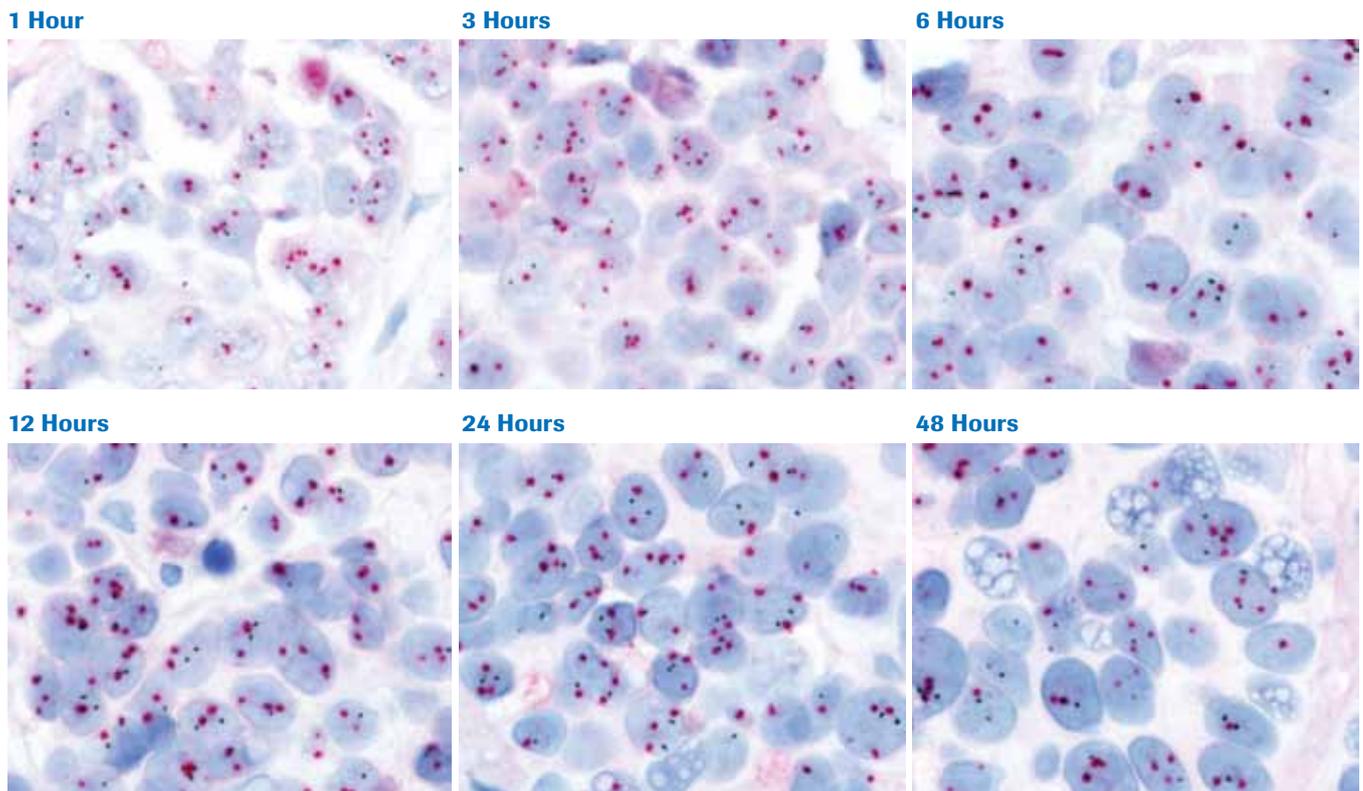
Aside from the Ventana assays, recent studies have found that the majority of "inconclusive" *HER2* gene results by FISH relate to pre-analytic factors including "under" and "over" fixation², and delay to fixation³. Strict implementation of fixation procedures (e.g., a dedicated processor to ensure a minimum of 6 hours fixation) resulted in a 64% reduction in "inconclusive" cases from 10.8% failures to 3.4%².

Fixation

For biopsy specimens that have been fixed in 10% NBF, paraffin embedded and sectioned at approximately 4 µm, Ventana ISH Protease 3 is recommended for sixteen minutes (i.e., the condition for nominal runs) (Figure 14.a). Specimens fixed in zinc formalin or alcoholic formalin also are suitable specimen types, and specimens fixed in Prefer also exhibit single copy detection with this assay; however, tissue morphology may be affected at later time points when fixing with Prefer. Using MCF7 xenograft tumors fixed under different conditions, Ventana has determined that under-fixation (< 6 hours) results in signal loss as observed in hematoxylin counterstaining (Figure 14.b) and increased noise. Additionally, it is not recommended that tissues fixed with AFA or Bouin's fixatives be used with this assay. Specimens fixed for times > 6 hours with these fixatives result in weak or absent staining, (Figure 14.c)¹⁴.

It should be noted that biological heterogeneity and differences in sample preparation also may affect signal intensity (i.e., the size of the SIS and Red ISH signals). Thus, signal intensity may vary from case to case or by probe within cases. For each slide, the stromal cells adjacent to and/or within the target area serve as a reference for signal size and the presence of 1-2 copies of *HER2* and Chr17 staining in them should be used to determine slide adequacy. For additional information on ISH fixation, refer to the CAP/ASCO Guideline Recommendations for *HER2* Testing.¹

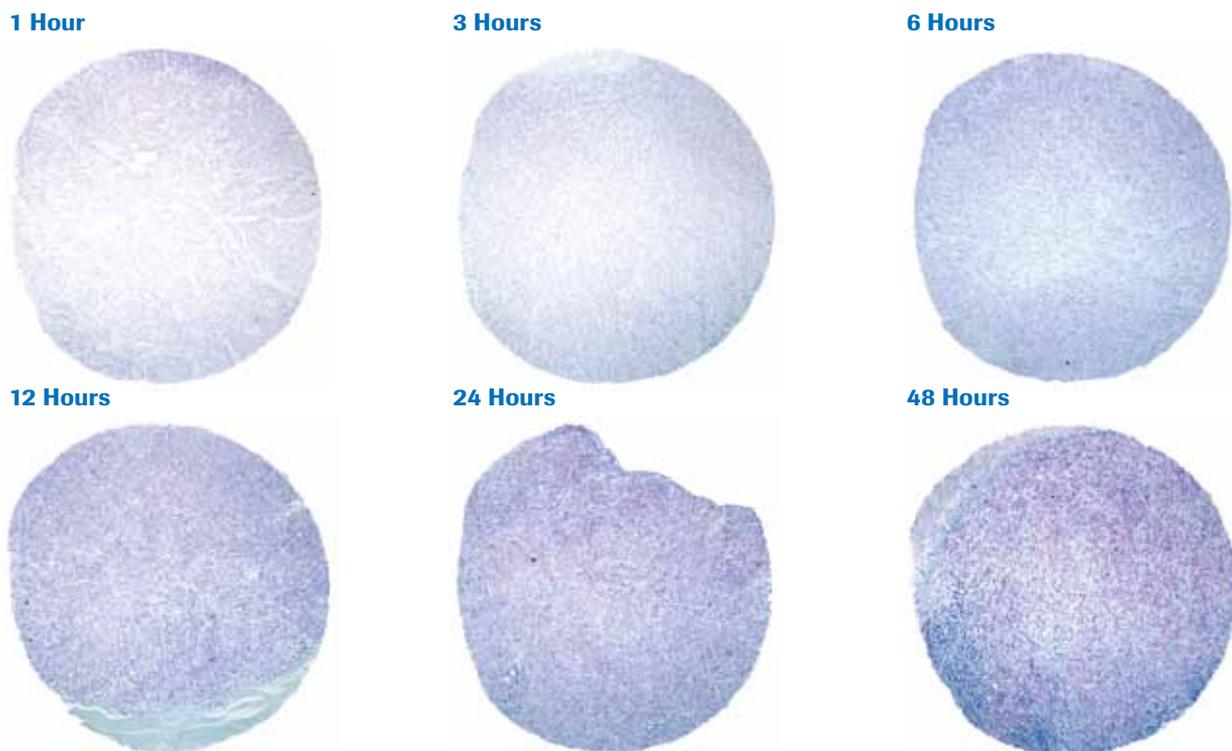
Figure 14.a. Effects of 10% NBF fixation time on SISH and Red ISH staining



MCF7 xenograft core, 10% NBF, 60X.

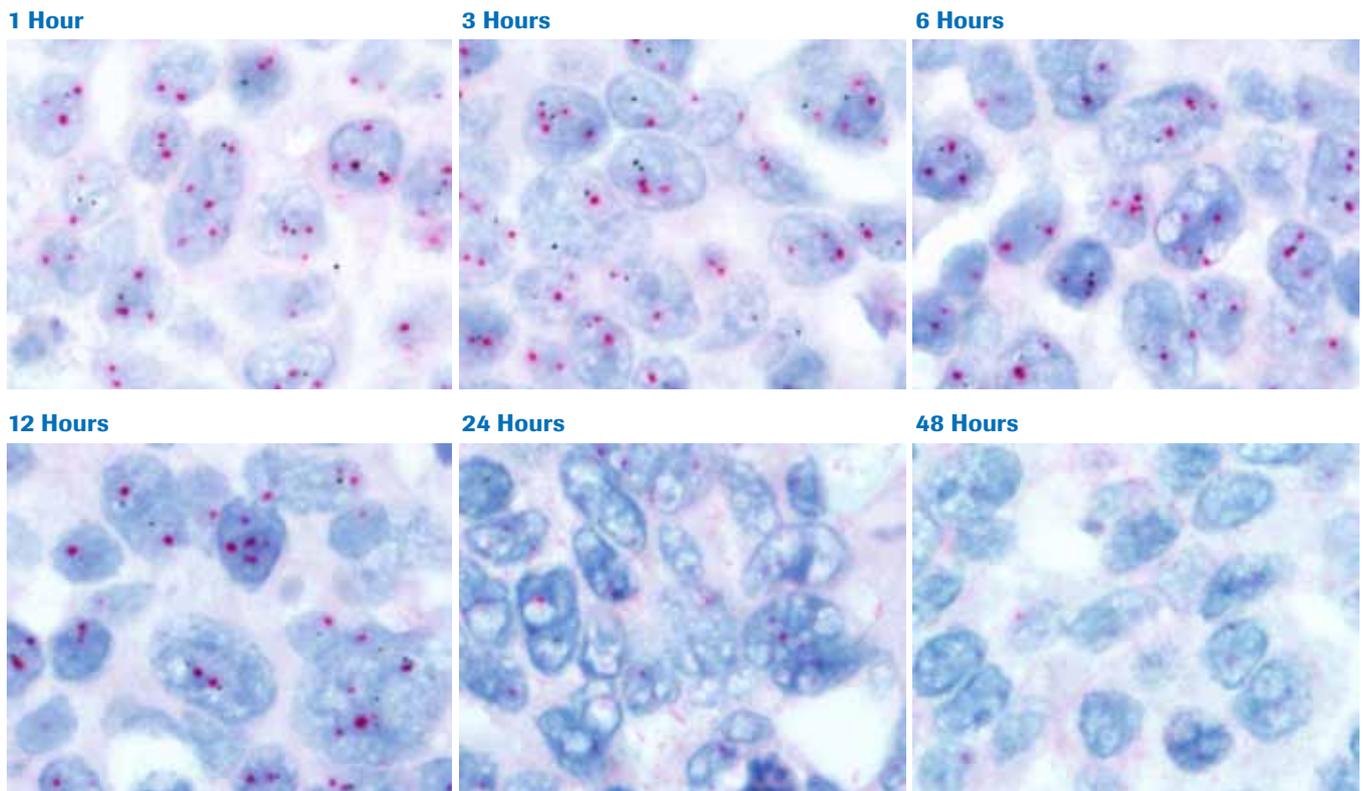
Underfixed 10% NBF tissue (1-6 hours) will not exhibit optimal staining and will show poor, “overdigested” morphology (Figure 14.a). This can easily be observed at low power where lighter counterstain is evident at short fixation times (Figure 14.b). The recommended fixation time for 10% NBF is 6-48 hours.

Figure 14.b. Effects of 10% NBF fixation time on hematoxylin counterstain



MCF7 xenograft core, 10% NBF, 4X.

Figure 14.c. Effects of AFA fixation time on SISH and Red ISH staining



MCF7 xenograft core, AFA, 60X.

AFA and Bouin's fixed tissue only yielded acceptable staining results at early time points (~3 hours)(Figure 14.c). Note this trend is reversed compared to tissue fixed with 10% NBF.

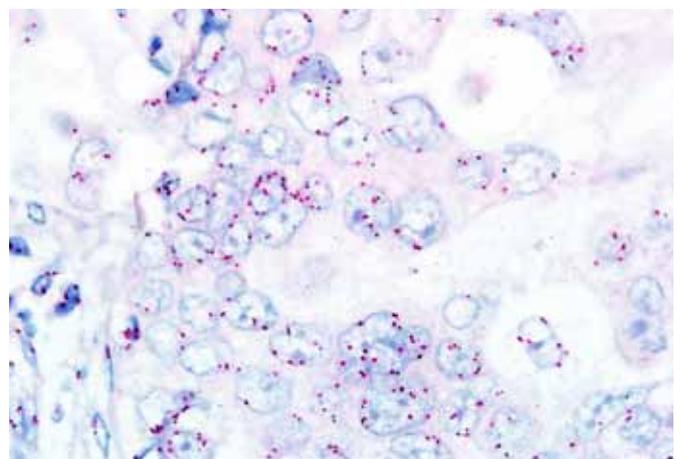
Sample Thickness

Sections thicker than 4µm may require stronger protease pretreatment such as Ventana ISH Protease 3 for ≥ 20 minutes, or Ventana ISH Protease 2 for ≥ 4 minutes. These longer protease incubations are effective at unmasking DNA in thicker samples. Thinner sections may require gentler protease treatments (e.g., Protease 3 for 4 minutes) if excessive nuclear background staining or nuclear over-digestion is observed (See Troubleshooting section). In addition, sections thicker than 4 µm may exhibit more "nuclear bubbling" than thinner sections due to excess paraffin in the tissue (Figure 16). These may need to be deparaffinised in alcohol and xylene baths prior to staining on the instrument, or the user can select "extended depar" (Figure 24).

As illustrated in Figure 15, nuclear bubbling may also occur due to under-fixation (e.g., 1-3 hours with formalin) which is a less discrete, "fuzzier" nuclear bubble. This may be remedied at 3 hours with changed cell conditioning/protease treatment, but at 1 hour is probably beyond remedy.

Figure 15. Example of "nuclear bubbling"

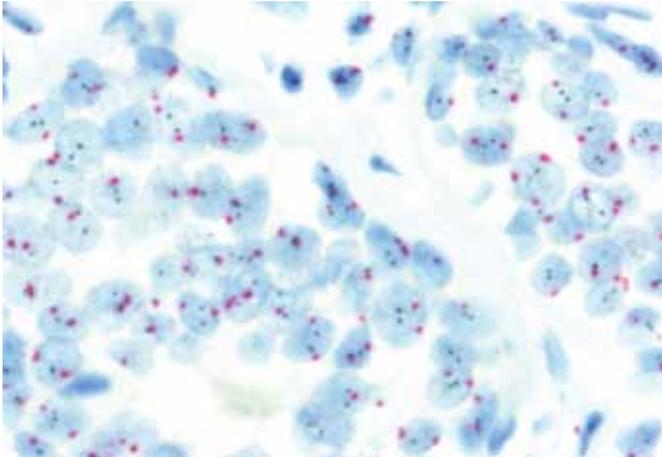
Case 25. Breast carcinoma



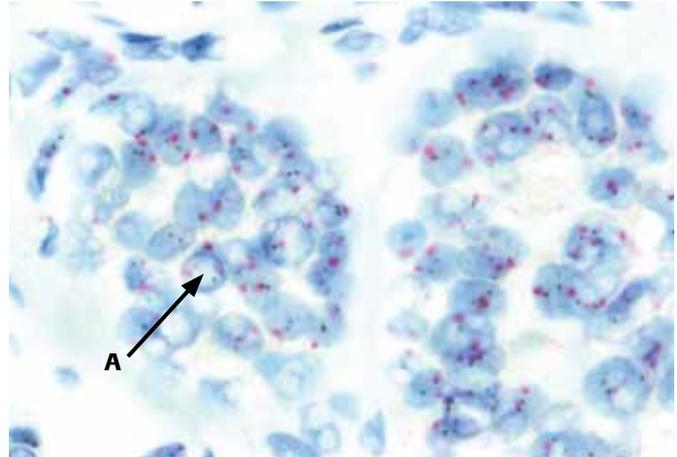
HER2 and Chr17, 60X, "nuclear bubbling"

Figure 16. Effect of tissue thickness on staining

Case 26. Breast carcinoma



HER2 and Chr17, 60X, recommended section thickness of 4µm



HER2 and Chr17, 60X, 2 x recommended thickness (8 µm section) A: nuclear bubbling. Note that "nuclear bubbling" increases in the thick section due to excess paraffin.

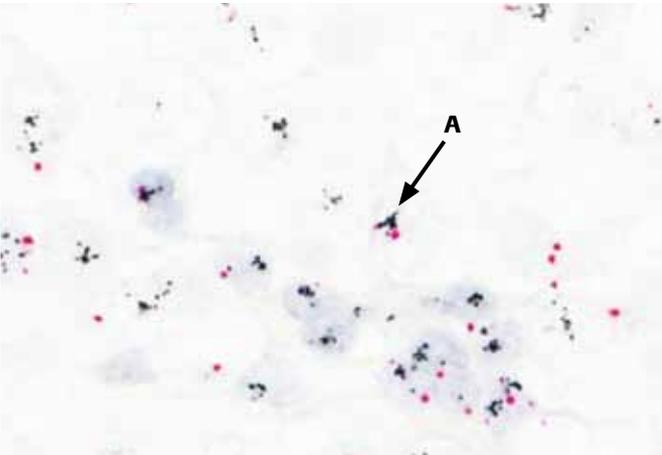
Other considerations

Type of specimen

It is important to consider the type of specimen to be tested in the assay (for example, tissue obtained from a core needle biopsy vs. an excisional biopsy). Optimising the ISH pre-treatment and/or detection conditions on each specimen type may be necessary if differences in staining quality are observed.

Figure 17. Poor nuclear morphology due to insufficient fixation and subsequent protease overdigestion

Case 27. Breast carcinoma



HER2 and Chr17, 60X, overdigested. A: overdigested nuclei

Overall sample quality

Staining may be sub-optimal in tissue containing areas of crushed artifact, necrosis or in tissue of overall poor morphology. It is recommended that H&E staining be evaluated from each case to assess overall specimen morphology.

The balance between nuclear morphology and signal intensity.

In specimens prepared differently from the recommended procedure, it is often possible to obtain adequate staining by simply optimising the ISH Protease treatment. However, the use of ISH Protease 2 or 3 for extended amounts of time may negatively affect nuclear morphology and yield weak nuclear counterstain. Each reader should be aware of this and balance signal intensity with nuclear morphology and counterstain. Furthermore, HER2 or Chr17 staining may decrease in intensity if too harsh a protease treatment is used. For samples that exhibit weak counterstain, increasing Hematoxylin II staining time from the recommended eight minutes to twelve minutes may enhance the intensity of the counterstain.

Poor nuclear morphology due to protease overdigestion (Figure 17) is usually due to under-fixation (< 6 hours). This can result in a loss of signal due to digestion of the nuclear material. Standardizing fixation times in line with what is recommended by ASCO/CAP⁴ is known to improve failure rates of *in situ* hybridisation.²

Troubleshooting

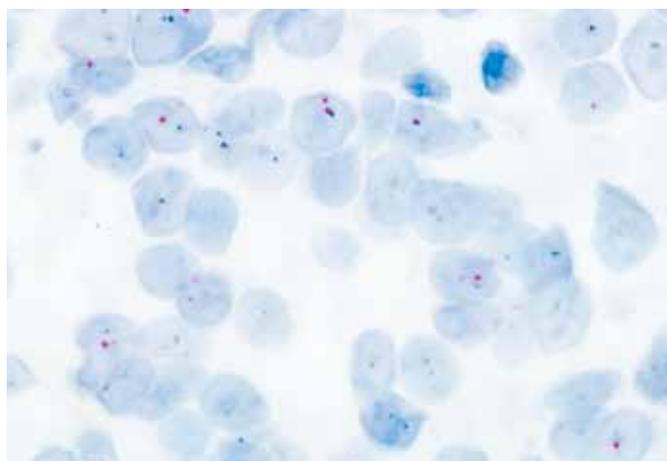
This section discusses potential sub-optimal staining results and how they may be mitigated by manipulation of selectables in the software procedure. The software selectables are further described in Table 3.

Weak or absent staining

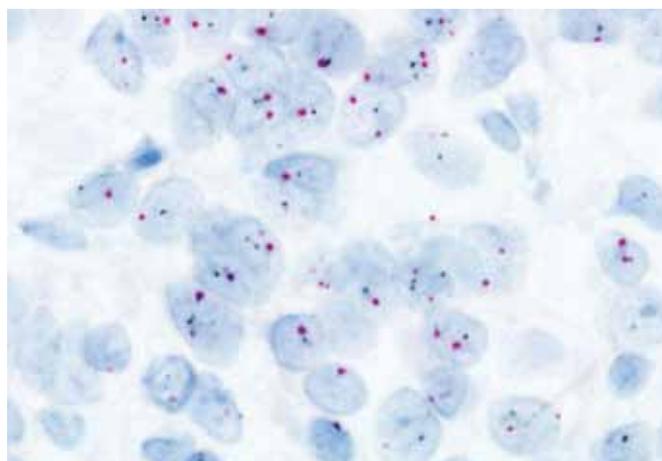
- Cases that completely lack or exhibit weak staining for both *HER2* and Chr17 to such an extent as to prevent cell signal enumeration likely indicate a problem(s) with pre-analytical specimen preparation (Figures 14a-c). Ensure that appropriate fixation time, type and section thickness are used. If nuclei are intact, increase the pre-treatment conditions (i.e., longer incubation times).
- If a case has stained appropriately for one probe but not for the other (e.g., *HER2* stains appropriately but Chr17 exhibits weak or absent staining), this may indicate a potential problem with the reagents involved with the failed detection. In this case, ensure dispensers are not clogged and that bulk containers were filled adequately for the run. Also ensure that the proper dehydration process was followed as alcohol baths, acetone and extended xylene incubations will dissolve the Red ISH signal. If SISH signals are weak or fading, ensure that a compatible mounting medium has been used to preserve signals (see Table 4).
- If these factors are ruled out, then the pre-treatment conditions of protease incubation time and/or cell condition times that are selectable within the software procedure can be increased as these tend to yield the most effective methods for rescuing weak staining (Figure 18). Longer pre-treatment conditions should be performed only if nuclei appear intact and the counterstain is detectable in carcinoma cells. If nuclei appear over-digested and have lost signal due to this effect, then decrease protease incubation time (e.g., protease 3 for 8 or 12 minutes).
- If staining is still inadequate, (or if one detection system displays background staining while the other does not), manipulation of the various detection systems incubation times are recommended. For instance, signal strength can be increased by the use of Silver Chromogen for eight or twelve minutes, or by increasing the SISH multimer incubation time. The Red ISH multimer and/or Red Chromogen incubation times also can be increased, if needed (Figure 19). Increasing detection component incubation times is effective for controlling signal size/intensity but may also contribute to increased background staining. (See Table 3 for explanation of selectable parameters in the software and their effect on staining quality). Decreasing incubation times can mitigate non-specific background staining.

Figure 18. Example of successful repeat staining, increasing detection system incubation time

Case 28. Breast carcinoma



HER2 and Chr17, 60X, weak staining



HER2 and Chr17, 60X, increased Red Chromogen incubation time and Silver Chromogen incubation time

Consistent lack of enumerable staining in internal positive cells

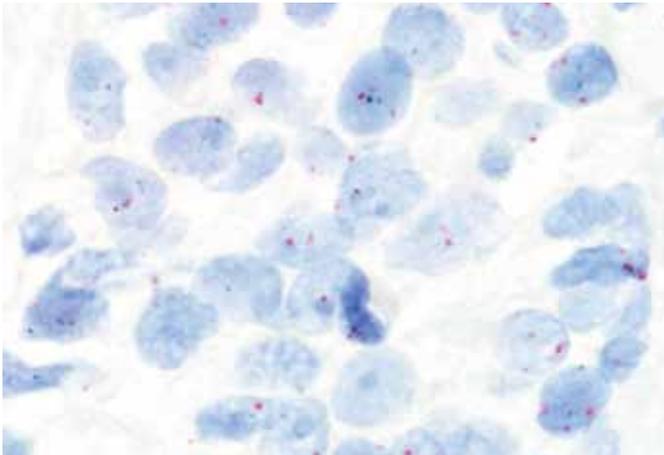
Every human biopsy specimen contains normal cells that serve as internal positive controls (See Slide Adequacy section). The presence of appropriate staining in these normal cells (the "same slide" control) indicates positive staining. Numerous staining failures in which there is total lack of staining, including within these internal positive control nuclei, may indicate an instrument or reagent issue. In cases where an instrument or reagent issue is suspected, it is recommended that positive control specimens or *HER2* 3-in-1 Dual ISH Xenograft Slides are run for troubleshooting purposes.

Non-specific and background staining

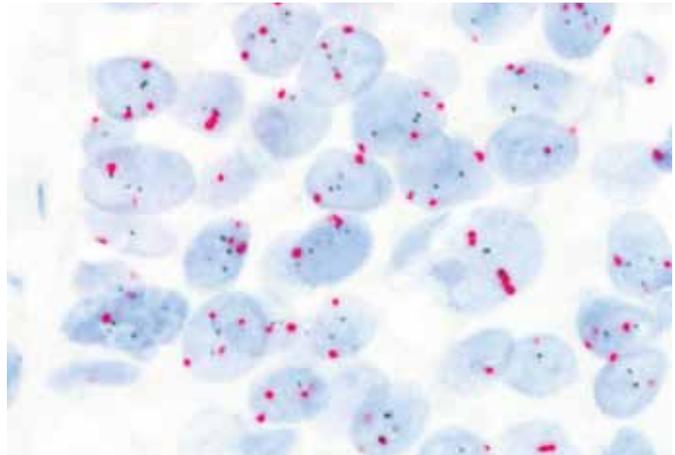
Some cases will exhibit acceptable levels of Red or SISH background staining that still enable enumeration of the specific signals. However, if the slides exhibit excessive background staining that interferes with enumeration of the specific signals (i.e., nuclear dust or haze) (Figures 21 and 22), the use of a gentler protease treatment is recommended (e.g., ISH Protease 3 for 4 minutes, instead of 8 minutes). If the SISH signal is strong and nuclei also contain Red ISH signals that are much fainter in intensity than the specific Red signals (Figure 20), increasing the stringency wash temperature to 76°C mitigates the non-specific red background (red haze). The latter red haze is particularly notable in under fixed tissues.

Figure 19. Example of successful repeat staining, after increased protease treatment

Case 29. Breast carcinoma



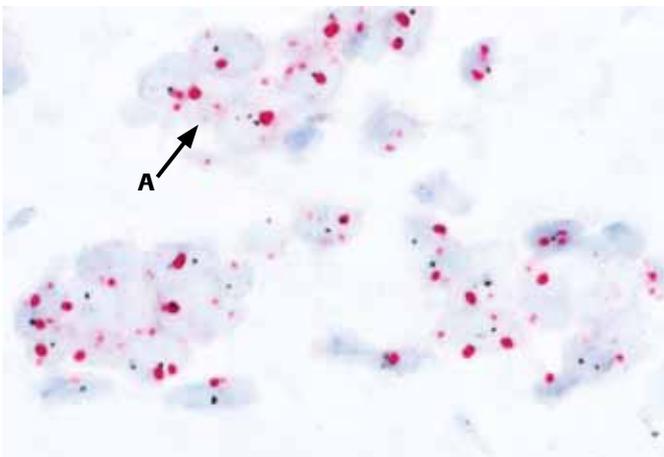
HER2 and Chr17, 60X; inadequate staining



HER2 and Chr17, 60X; increased protease incubation "unmasks" target DNA

Figure 20. Nonspecific Red Background

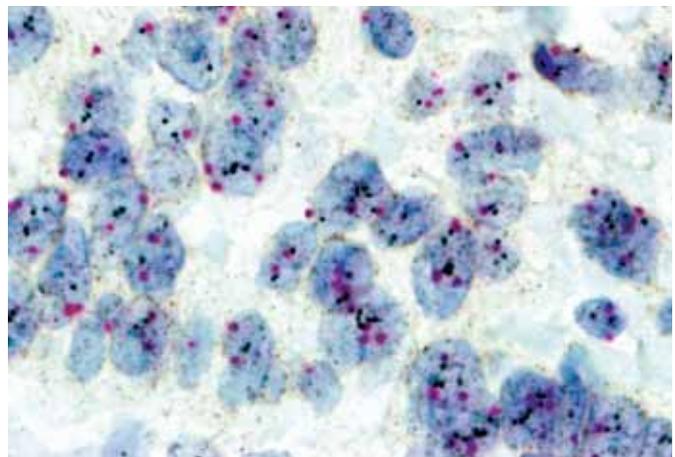
Case 30.



HER2 and Chr17, 60X, non-specific Red haze/background (A)

Figure 21. SISH Dust

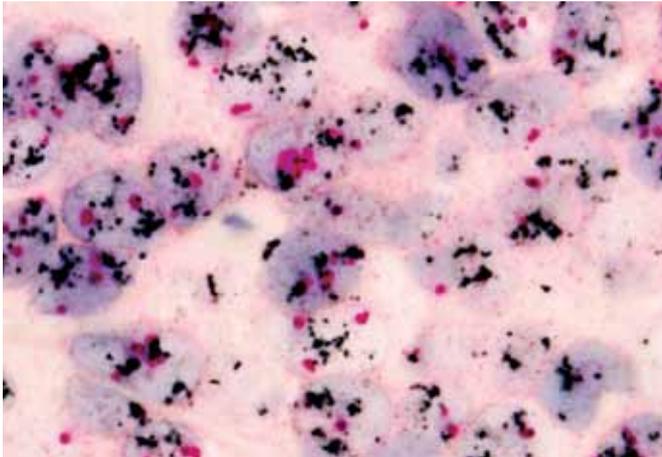
Case 31.



HER2 and Chr17, 60X, SISH dust

Figure 22: Red Haze

Case 32. Breast carcinoma



HER2 and Chr17, 60X, red haze

Slide drying and “speckling” artifacts

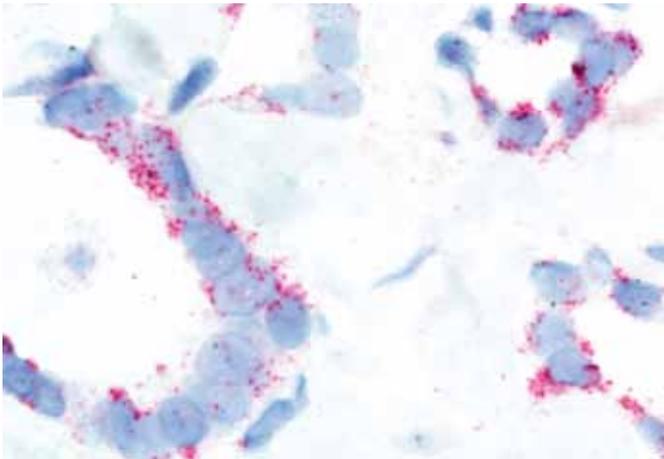
If a case exhibits uninterpretable staining, with brown, black or dark red background throughout the tissue, the slide must be re-run (Figure 23). If staining is still unacceptable, ensure the slides are SuperFrost Plus. If excess Silver or Red is deposited on the slide, making it difficult to enumerate the nuclear signal due to “speckling” throughout the tissue (Figure 23), the slide must also be re-run, unless areas free of the debris can be located. Check that the instrument and dispensers are functioning properly and that the bulk reagents are adequately filled. Also make sure that the dispensers have not been stored without their caps on, as silver acetate is known to oxidize over time. As further guidance, Table 2 helps troubleshoot some of the issues described above. For additional information, refer to the Step By Step Procedure section in the automated slide stainer Operator’s Manual or contact your local representative.

Table 2. Description of suboptimal staining and suggested mitigations for the assay.

If weak or absent staining is encountered	Procedural change(s) to achieve desirable staining
Absent or Weak Red Staining	<ol style="list-style-type: none"> 1. Ensure reagent dispensers are functioning properly (i.e., not clogged or empty) and bulk solutions are filled. If staining is still weak or absent, proceed to 2 below 2. Ensure alcohol baths and extended xylene washes are not used to dehydrate stained slides, as this will degrade Red ISH signals. If staining is still weak or absent, proceed to 3 below. 3. Ensure fixation type, time and thickness is appropriate for ISH-based assays 4. Increase pre-treatment to Protease 3 to ≥ 20 min or Protease 2 for 4 min or greater if nuclear morphology is intact 5. Increase Red ISH Multimer incubation time to ≥ 32 min 6. Increase Red Chromogen incubation time to 12 min
Absent or Weak SISH Staining	<ol style="list-style-type: none"> 1. Ensure reagent dispensers are functioning properly (i.e., not clogged or empty) and bulk solutions are filled. If staining is still weak or absent, proceed to 2 below 2. Ensure fixation type, time and thickness is appropriate for ISH-based assays 3. Ensure use of SISH compatible mounting media (see Table 4) to preserve SISH signal. If staining is still weak or absent, proceed to 3 below 4. Increase pre-treatment to Protease 3 to ≥ 20 min or Protease 2 for 4 min or greater if nuclear morphology is intact 5. Increase SISH Multimer time to ≥ 24 6. Increase silver Chromogen incubation time to 8 minutes.
If background staining is encountered	Procedural change(s) to achieve desirable staining
Nonspecific Red ISH Background	<ol style="list-style-type: none"> 1. Ensure that SuperFrost Plus slides are used and specimen is fixed and sectioned appropriately for ISH-based assays. 2. If Red ISH background is discernible from specific Red ISH signal, enumerate the slide but do not count non-specific Red ISH signals. 3. If Red ISH background in the nucleus interferes with enumeration, repeat the staining using 76 C stringency wash temperature. Decreasing protease time also mitigates Red background.
Nonspecific SISH Background	<ol style="list-style-type: none"> 1. Ensure that SuperFrost Plus slides are used and specimen is fixed and sectioned appropriately for ISH-based assays. 2. If SISH background is discernible from specific SISH signal, enumerate the slide but do not count non-specific signals. 3. If SISH background in the nucleus interferes with enumeration, repeat the staining with lower protease treatment.
Dried slide	<ol style="list-style-type: none"> 1. If drying artifact interferes with enumeration, repeat the staining.
Speckling	<ol style="list-style-type: none"> 1. If speckling artifact interferes with enumeration, repeat the staining.
Bubbling	<ol style="list-style-type: none"> 1. If bubbling interferes with enumeration, ensure pre-analytical procedures are as recommended. 2. For thick sections, deparaffinisation off-line may be required.

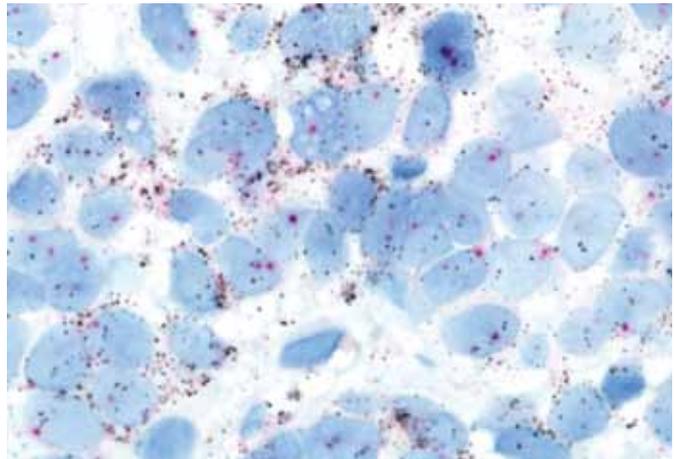
Figure 23: Drying and speckling

Case 33. Breast carcinoma



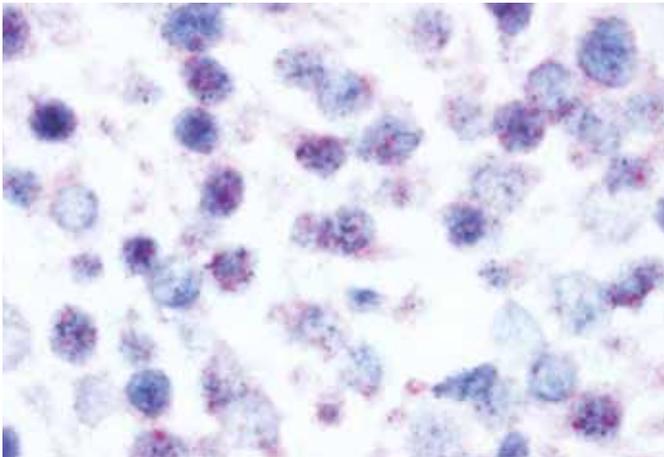
HER2 and Chr17, 60X, Red "Speckling"

Case 34. Breast carcinoma



HER2 and Chr17, 60X, SISH "Speckling"

Case 35. Breast carcinoma



HER2 and Chr17, 60X, Dried Slide

Table 3. Modification of selectable software staining procedure steps and their impact(s) on the assay

Selectable procedure step	Range of the selectable Step software staining	Effect of manipulating the selection
Deparaffinisation	Depar or Extended Depar	Extended depar can be used to mitigate excess paraffin in the specimen
Cell Conditioning	Three cycles, from 4 to 16 minutes each	Increase in SISH and Red ISH signal staining if times are increased, Decrease in signal intensity if times and/or cycle numbers are decreased
ISH-Protease 2	4 to 32 min	Increase in SISH and Red ISH signal staining, can affect morphology at extended times
ISH-Protease 3	4 to 32 min	Increase in SISH and Red ISH signal staining with increased time but can affect morphology, decrease in background with decreased time
Denaturation	12 to 32 min	Increase in SISH and Red ISH signal staining when time is increased but may also affect background
Hybridisation	1 to 12 hours	Increase in SISH and Red ISH signal staining with increased incubation but also contributes to slide drying
Stringency Wash Temp.	72°C to 76°C	Reduction of non-specific Red ISH signals at 76°C
SISH Multimer	12 to 60 min	Stronger SISH signals when time is increased but also can contribute to background SISH dust
Silver Chromogen	4 to 12 min	Stronger SISH signals when time is increased but also can contribute to background SISH dust
Red ISH Multimer	12 to 60 min	Stronger Red ISH signals and large Red ISH signal size at increased times but can also contribute to Red ISH background
Red Chromogen	4 to 12 min	Stronger Red ISH signals and larger Red ISH signal size at increased time but also contributes to Red ISH background
Counterstain	Hematoxylin II 4 to 32 min	Darken counterstain at longer times
Post Counterstain	Bluing Reagent 4 to 32 min	Darken counterstain at longer times

*Note that for the Ultra platform, the user should also select 63° C for 20 minutes in the baking step.

Fading of SISH signal

Oxidation (indicated by a colour change from black to orange), fading and/or disappearance of SISH signals may be due to the use of certain brands of mounting media. The following mounting media are known to cause oxidation: EUKITT (EMS), Entellan (Merck), Entellan NEW (Merck), HSR

(Sysmex) and Malinol (Muto Chemical). Refer to Table 4 for mounting media compatibility. If the mounting media used is not included in this list, it is recommended to validate the compatibility of the media with the SISH detection prior to use with patient samples. Internal studies have shown that fading of SISH signals typically occur within a few hours to one week.

Weak or absent counterstain

For cases that lack or exhibit weak nuclear counterstain (making it difficult to discern nuclear boundaries), repeat the staining of the affected slide(s) and either increase the counterstain time or use a gentler protease treatment (i.e., ISH Protease 3 for 4 or 8 min.) Weak counterstaining can indicate that the specimen has not been prepared as recommended (i.e., over-digestion due to under-fixation). Also refer to Pre-Analytical Considerations section for more information on the balance between nuclear morphology and signal intensity.

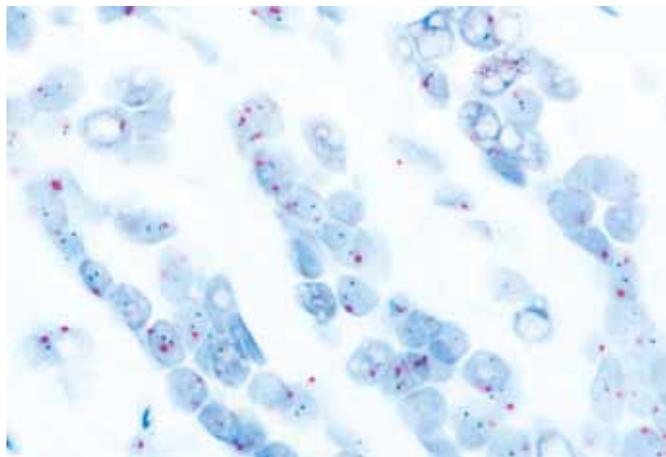
Nuclear bubbling

As mentioned in the Pre-Analytical section, some nuclei may appear “bubbled”. This can happen at relatively low levels throughout many different cases and is typically due to excess paraffin in the tissue, and/or differences in pre-analytical treatments, and will not affect enumeration. Especially in thicker sections (>6 um), a greater frequency of bubbled nuclei may occur. For these thick sections, it is known that deparaffinising the sections in alcohol and xylene baths prior to placing them on the instrument for staining is effective at removing the excess paraffin and reducing nuclear bubbling. The selection of "Extended Depar" in the staining procedure also mitigates bubbling due to excess paraffin (Table 3 and Figure 24).

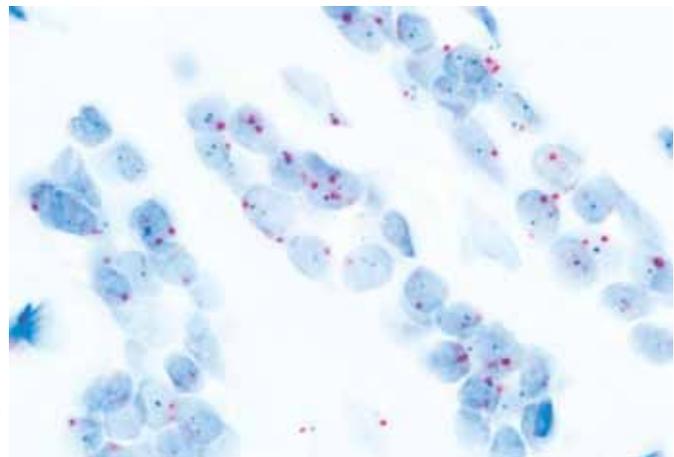
If a condition persists across multiple cases and repeated attempts to correct the condition(s) have failed, contact your local representative.

Figure 24. Nuclear bubbling due to excess paraffin

Case 36. Breast carcinoma



Without extended depar selected



With extended depar selected

Table 4. Various Manufacturer’s Mounting Media and their compatibility with SISH

Mounting Media	Manufacturer	Type (Xylene, alcohol, aqueous)	Result (Fading = F: No fading = N)
Eukitt	EMS	Xylene	F
Entellan New	Merck	Xylene	F
Entellan	Merck	Xylene	F
HSR	Symex	Xylene	F
Malinol	Muto Chemical	Xylene	F
Cytoseal XYL	Richard Allan Scientific	Xylene	N
Softmount	WAKO	Lemasol A	N
Paramount	Protaqs Quartett: Dako	Xylene	N
DPX	BDH: Raymond Lamb	Xylene	N
Cytoseal 60	Richard Allan Scientific	Xylene	N
Permout	Fisher	Xylene	N
Histomount	Raymond Lamb	Xylene	N
Ultramount	Dako	Xylene	N
Thermo EZ Mount	Thermo Scientific	Xylene	N
SureMount	Triangle Biomedical Sciences	Xylene	N
Flo-Texx	Lerner Labs	Xylene	N
Mountex	Histolab	Xylene	N
Shandon Consul mount	Thermo Scientific	Xylene	N
MM24	SurgiPath	Xylene	N
Pertex	Cell Path	Xylene	N
MicroMount	SurgiPath	Xylene	N
Diamount	Diapath	Xylene	N
Alcolmount	Diapath	Alcohol	N
BioMount 2	BBInternational	Xylene	N
Acrytol	SurgiPath	Xylene	N
Gel Mount	Biomeda	Aqueous	N
Mount-Quick	Daido Sangyo Co.	Aqueous	N

References

1. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptro 2 Testing in Breast Cancer. Wolff AC, Hammond MEH, Schwartz JN, et al. Arch Phathol Lab Med 2007; 131: 18-43.
2. Implementation of American Society of Clinical Oncology/College of American Pathologists *HER2* Guideline Recommendations in a tertiary care facility increases *HER2* immunohistochemistry and fluorecence in situ hybridisation concordance and decreases the number of inconclusive cases. Middleton LP et al. Arch Pathol Lab Med. 2009 ; 133: 775-780.
3. Delay to formalin fixation effect on breast biomarkers. Khoury T, Sait S, Hwang H, Chandrasekhar R, Wilding G, Tan D, Kulkami S. Mod Phathol. 2009; 22: 1457-1467.
4. Chromogenic in situ hybridisation: a multicenter study comparing silver in situ hybridisation with FISH. Bartlett JM, Campbell FM, Ibrahim M, Wencyk P, Ellis I, Kay E, Connolly Y, O'Grady A, Di Palma S, Starczynski J, Morgan JM, Jasani B, Miller K. Am J Clin Pathol. 2009; 132: 514-520.
5. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Science. 1987; 235:177-182.
6. Independent prognostic factors in breast cancer patients. Narita M, Nakao K, Ogino N, Nakahara M, Onishi A, Tsujimoto M. Am J Surg. 1998; 175:73-75.
7. The relationship between c-erb-2 expression, S-phase fraction, and prognosis in breast cancer. O'Rielly SM, Barnes DM, Camplejohn RS, et al. Br J Cancer 1991; 63:444-446.
8. The effect of *HER-2/neu* overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Pegram MD, Finn RS, Arzoo K, Beryt M, Pietras RJ, Slamon DJ. Oncogene. 1997; 15:537-547.
9. *Her-2/neu* expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. Press MF, Pike MC, Chazin VR, Hung G, Udove JA, Markowicz M, Danyluk J, Godolphin W, Sliwkowski M, Akita R, et al. Cancer Res. 1993; 53:4960-4970.
10. *HER-2/neu* gene amplification characterized by fluorecence in situ hybridisation: poor prognosis in node-negative breast carcinomas. Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR, Flom KJ. J Clin Oncol. 1997; 15:2894-2904.
11. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Säve-Söderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golouh R, Reed R, et al. J Clin Oncol. 1992; 10:1049-1056.
12. Evaluation of *Her-2/neu* status in carcinomas with amplified chromosome 17 centromere locus. Troxell ML, Bangs CD, Lawce HJ, Galperin IB, Baiyee D, West RB, Olson SB, Cherry AM. Am J Clin Pathol. 2006; 126:709-716.
13. Breast cancer and aneusomy 17: implications for carcinogenesis and therapeutic response. Reinholz MM, Bruzek AK, Visscher DW, Lingle WL, Schroeder MJ, Perez EA, Jenkins RB. Lancet Oncol. 2009 Mar; 10:267-277.
14. The effects of tissue fixation alternatives on DNA content: a study on normal colon tissue. Baloglu G, Haholu A, Kucukodaci Z, Yilmaz I, Yildirim S, Baloglu H. Appl Immunohistochem Mol Morphol. 2008; 16:485-492.
15. Assessment of a *HER2* scoring system for gastric cancer: results from a validation study. Hofmann M, Stoss O, Shi D, et al. Histopathology 2008; 52:797-805.

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